

**Scanning Electron Microscopy of the Tegument  
of *Paramphistomum microbothrium* Fiscoeder, 1901  
and *Cotylophoron cotylophorum*  
(Fiscoeder 1901) in Egypt**

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**ABSTRACT.** The surface microtopography of *Paramphistomum microbothrium* and *Cotylophoron cotylophorum* was studied by Scanning Electron Microscopy (SEM) to investigate their morphological characteristics. The tegument posterior to the anterior third in *P. microbothrium* has wrinkles or folds encircling the body but in *C. cotylophorum* the tegument in the same area is smooth. *P. microbothrium* has concentric aggregation of dome-shaped papillae located at its anterior third, while the corresponding location in *C. cotylophorum* is with button-like papillae.

Amphistomes are extremely difficult to identify possibly due to the great morphological similarities exhibited by these trematodes (Yamaguti 1958 and Soulsby 1982). Hence, other diagnostic procedures such as the use of scanning electron microscopy (SEM) could add tremendously to the identification of these worms. However, such studies are extremely meager (Eduardo 1980 a,b,c, and Tandon and Maitra 1981, 1982) and the commonest amphistomes in Egypt, *Paramphistomum microbothrium* Fiscoeder 1901 and *Cotylophoron cotylophorum* (Fiscoeder 1901) (Omar 1983) are yet to be investigated by SEM. Hence, the microtopography of both worms has been investigated in the present study to delineate any differential characteristics in their tegument.

### **Materials and Methods**

Adult *P. microbothrium* and *C. cotylophorum* were collected from the rumen and reticulum of freshly slaughtered cattle and were identified according to the methods of

Sey and Abdel-Rahman(1975).The worms were fixed for 2-4 hrs in aqueous 4% glutaraldehyde: 1% osmium tetroxide( 3: 1) . They were washed repeatedly in distilled water and then dehydrated through ethanol and amyle acetate. They were subsequently critically point dried using carbon dioxide. Then fixed to stubs with colloidal carbon and coated with gold palladium in a sputtering device. They were then examined and photographed using a Japanese Scanning Electron Microscop operating at 20 Kv (Hitachi Model 530).

## Results

Adult *P. microbothrium* is 6-11 X 3-4 mm. It is pear-shaped with a terminal oral aperture surrounded by the oral sucker while the acetabulum is subterminally positioned (Fig. 1). The genital pore is ventrally located at the middle of the anterior third of the body and is a slit-like opening on the middle of a smooth oval shaped thickening of the tegument that has a shallow transverse wrinkle at the level of the pore (Fig. 2). It's dorsal and ventral surfaces are covered with circumscribed transverse wrinkles or folds and its anterior third has clearly visible concentric aggregations of dome-shaped papillae (Fig. 3) of variable size and number. They are densely packed near the oral aperture but decrease gradually toward the middle third of body before faiding out. The interior lining of the buccal cavity is also lined with dome-shaped papillae (Fig. 4). Both dorsal and ventral surfaces are each with an intricate pattern of tegumental folding that form microridges which anastomose and interdigitate in a complex pattern (Figs. 5 and 6).

Around the acetabular aperture the tegument has dense folding which radiate irregularly giving the rim of the sucker a cobblestone-like appearance (Fig. 7) and few dome-shaped papillae occur between these folds. The interior of the acetabular aperture is smooth and is devoid of papillae and folds.

Adult *C. cotylophorum* is 5-8 X 2-2.2 mm and has the same location of oral aperture and acetabulum. However, the tegument surrounding its oral aperture and it's anterior end has longitudinal corrugations with small tubercle-like papillae and congregate anteriorly but gradually diminish in number posteriorly before disappearing altogether (Figs. 8 and 9). The oral sucker is encircled by a groove and carry 2-3 rows of button-like papillae (Fig. 10) and the luminal surface of the buccal cavity is with balloon-like projections.

The genital pore (Fig. 11) is ventrally situated at the middle of the anterior third of the body as a rounded depression in the middle of the genital papilla which is a rounded thickened area of the tegument that is irregular in outline and its base is with some ridges and scattered dome-shaped papillae. The tegument just posterior to the anterior end is smooth but has few wrinkles ventrally. At high resolution, however, the ventral and to a lesser extent the dorsal surface show bead-like protuberances (Fig. 12). Few wrinkles also appear on the tegument around the acetabular aperture that has few small dome-shaped papillae (Fig. 13) while the interior of the acetabular aperture is with bead-like protuberances that lie in between irregularly directed fine ridges or folds (Fig. 14).

## Discussion

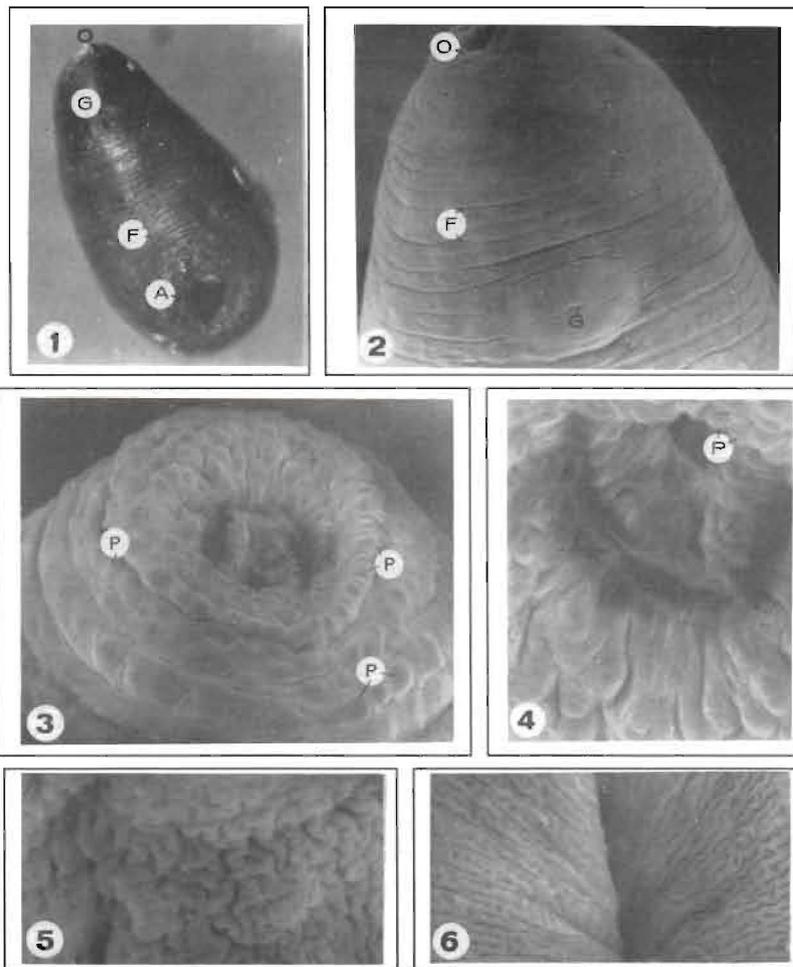
The present study revealed a number of differences in the surface microtopography between *P. microbothrium* and *C. cotylophorum*. The tegument of adult *P. microbothrium*

is transversely folded compared to the smooth one of *C. cotylophorum* while in other studied species of amphistomes it is wrinkled (Tandon and Maitra 1981). Moreover, the former has concentric aggregations of dome-shaped papillae on the dorsal and ventral surfaces of the anterior part while the corresponding location of the latter is occupied by longitudinal corrugations intermingling with small papillae and its oral sucker is provided with button-like papillae. Dome-shaped papillae commonly occur in the tegument of trematodes and a sensory function has been attributed to them (Silk *et al.* 1970, Miller *et al.* 1972, Morris 1973, Bennett 1975, Nadakavukaren and Nollen 1975, Kuntz *et al.* 1976, Sakamoto and Ishii 1977, Bakke 1978, Bakke and Lien 1978, and Tandon and Maitra 1982) and when present near the mouth they have been suggested to be involved in contact reception of food (Eduardo 1980 b,c). Elsewhere in the body surface they might function as pressure sensors for tegument stretches (Bennett 1975).

In the present study, and similar to the observations of Tandon and Maitra (1981) on *Gastrothylax crumenifer*, balloon-like projections were observed on the luminal surface of the buccal cavity of *C. cotylophorum* and such projections, since they are fluidfilled, might also be involved in food absorption. Moreover, similar to the observations of Bakke (1976 a,b) on *Calcophoron papillosum*, dome-shaped papillae were observed in the present study in the surface of the protruding genital papilla of *C. cotylophorum*. Their position might similarly suggest that they may have a specific sensory function during the pairing for sexual reproduction. The genital papilla of the worm was also observed to have an irregular outline together with wrinkles or ridges at its base. Similar structures were described by Tandon and Maitra (1982) in *C. papillosum* and might reflect the strong musculature of the genital sucker of *C. cotylophorum* which was not observed in *P. microbothrium*.

At high resolution the present study revealed that the tegument of *P. microbothrium* contains microridges which anastomose and interdigitate in a complex pattern. Meanwhile, that of *C. cotylophorum* has instead bead-like protuberances. Anastomosing small ridges have also been described in the acetabular tegument towards the interior of the acetabular aperture in *Paramphistomum epiclium* as well as on the genital papilla of *C. papillosum* by Tandon and Maitra (1981, 1982) and also on the surface of the cirrus in *Leeucochloridium sp.* (Bakke 1976 a, 1977). This beaded tegument could well increase the absorptive surface as has been described for other worms (Nadakavukaren and Nollen 1975, Mitchell and Crang 1976, Bakke and Lein 1978, and Tandon and Maitra 1981).

Similar to the observations of Nollen and Nadakavukaren (1974) and of Tandon and Maitra (1982) in other worms, the tegument around the acetabular aperture in *P. microbothrium* was observed to have irregularly radially-directed folds which might reflect the strong radial musculature of the sucker as it is the main adhesive organ of the worm together with the anterior muscular pharynx. On the other hand, the adhesive function of *C. cotylophorum* acetabulum seems to be aided by bead-like protuberances that alternate with irregularly directed fine ridges. Similar suggestions were made by Tandon and Maitra (1981) for another amphistome.



**Fig. 1.** Stereomicrograph of *Paramphistomum microbothrium*, showing oral aperture (O), acetabulum (A), genital pore (G) and circumscribed transverse folds (F).

**Figs. (2-7).** Scanning electron micrographs of *P. microbothrium*.

**Fig 2.** Oral aperture (O), genital pore (G) and transverse folds (F) (x 42)

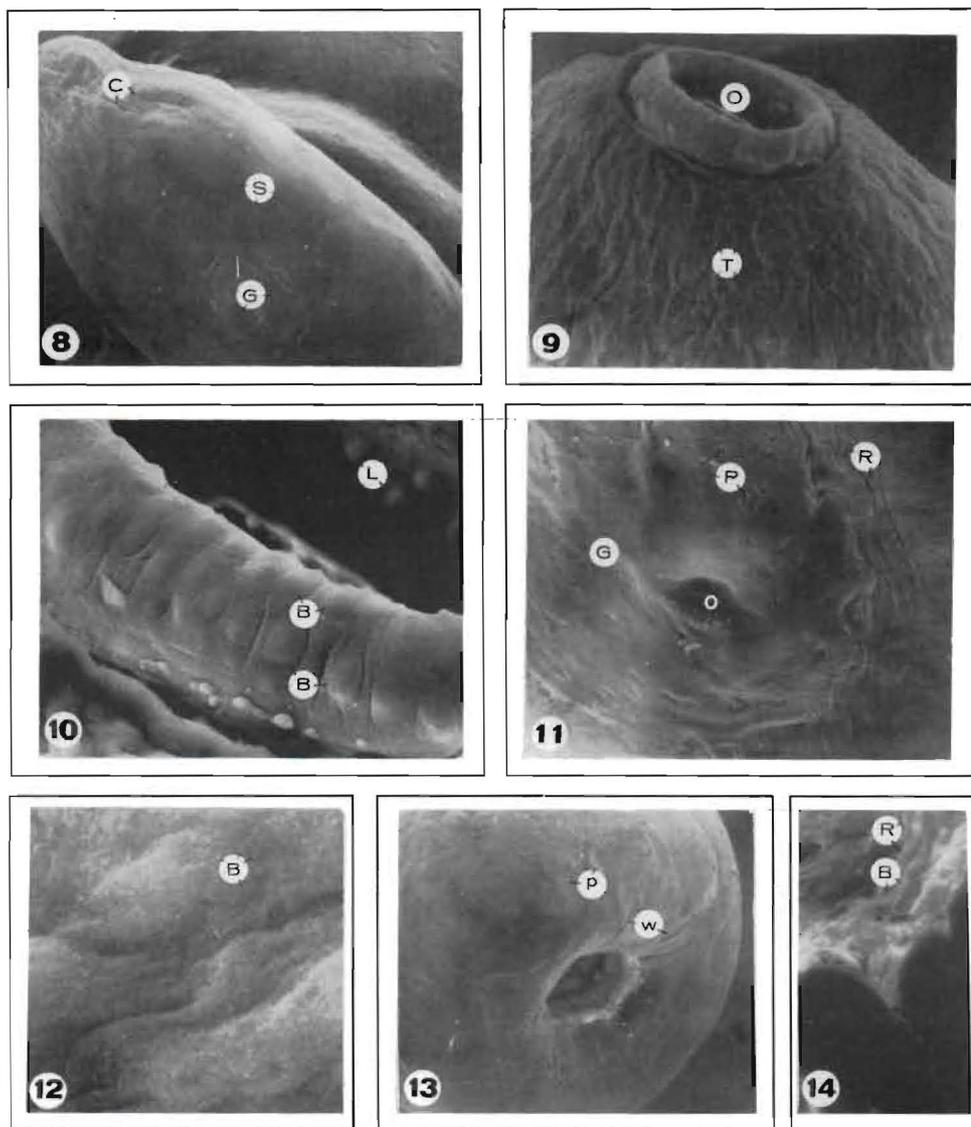
**Fig. 3.** Oral aperture surrounded by concentric aggregation of dome-shaped papillae (P) (x 188)

**Fig. 4.** Dome-shaped papillae (P) in the interior lining of the buccal cavity (x 420)

**Fig. 5.** Tegumental folding of dorsal surface forming anastomosing and interdigitating microridges (x 1878)

**Fig. 6.** Ventral surface showing tegumental microridges. (x 1404)

**Fig. 7.** Acetabular aperture showing folding (F). Dome-shaped papillae (P) (x 42)



**Figs. (8-14).** Scanning electron micrographs of *C. cotylophorum*.

**Fig. 8.** Anterior end showing longitudinal corrugations (C), genital papilla (G) and smooth surface (S)(x 42)

**Fig. 9.** Oral aperture (O), and tubercle-like papillae (T)(x 188)

**Fig. 10.** Oral sucker showing transverse divisions each containing 2-3 button-like papillae (B) and balloon-like papillae (L) in the luminal surface of the buccal cavity (x 653)

**Fig. 11.** Genital papilla (G) having genital pore (O), irregular outer ridges (R) and scattered dome-shaped papillae (P) (x 140)

**Fig. 12.** The ventral surface of the tegument showing bead-like protuberances (x 2808).

**Fig. 13.** Acetabulum showing few wrinkles (w) and dome-shaped papillae (P) (x 42)

**Fig. 14.** Bead-like protuberances (B) and fine ridges (R) in the interior of the acetabular aperture (x 289)

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دراسة بإستخدام المجهر الالكتروني الماسح للجليد  
في كل من الديدان المفلطحة نوعي  
*Cotylophoron cotylophorum* Fiscoeder 1901,  
*Paramphistomum microbothrium* Fiscoeder 1901  
في جمهورية مصر العربية

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أظهرت الدراسة التي تمت بإستخدام المجهر الالكتروني الماسح للجليد في كل من *Cotylophoron cotylophorum* و *Paramphistomum microbothrium* وجود اختلافات مورفولوجية بينهم من أهمها أن الجليد في الدودة الاولى تميز بوجود ثنايا عرضية دائرية بينما كان في الدودة الثانية أملس. وقد ظهر في الثلث الاول من الدودة الأولى عدد كثير من الحليمات على شكل قيب مصفوفة دائرياً والتي كانت كثيفة حول الممص الأمامي وتقل في العدد ناحية الثلث الاوسط من الدودة. بينما أظهر نفس المكان في الدودة الثانية تعرجات طولية بينها حليمات دقيقة وزود الممص الفمي بحليمات على شكل أزرار.

كما أظهر جليد دودة *P.microbothrium* عند قوة تكبير عالية وجود ثنايا دقيقة متشعبة ومتشابكة في نظام معقد على السطح الظهري والبطني من الجسم بينما أظهر المكان المشابه في الدودة الأخرى خاصة السطح البطني منه وجود نتوءات دقيقة.

كانت فتحة التناسل في الدودة الأولى تأخذ شكل شق عرضي موجود على جزء متنفخ بيضاوي الشكل بينما كان في دودة *C.cotylophorum* على شكل حفرة دائرية

في وسط جزء متنفخ دائري الشكل يوجد عند قاعدته بعض الثنايا وكذلك بعض الحليمات الصغيرة.

وقد كان حجم الدودة الاولى البالغة  $3,5 \times 8,5$  ملم وكان شكلها كمثري وكان الممص الفمي طرفي بينما الممص الخلفي كان تحت طرفي. وقد أظهر الجليد حول هذا الممص الأخير ثنايا متعرجة كثيرة أعطت حافة الممص شكل فوهة الكهف وكان بين هذه الثنايا بعض الحليمات القبية الشكل وكان هذا الممص من الداخل أملس لا يحتوي على ثنايا أو حليمات.

أما الدودة الثانية فكان حجمها  $2,1 \times 6,5$  مم وكان لها ممص فمي طرفي وممص خلفي تحت طرفي. وكان الجليد حول الممص الخلفي يحتوي على قليل من الثنايا بينها قليل من الحليمات القبية الشكل بينما كان الممص من الداخل يحتوي على بروزات صغيرة موجودة بين ثنايا دقيقة متعرجة.