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A scanning electron microscope study of the microtopography of *Aphalloides timmi* Reimar, 1970 (Digenea; Cryptogonimidae)[1]

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The SEM investigation of *A. timmi* is based on specimens from the coelomic cavity of the common goby *Pomatoschistus microps* (Kröyer) sampled in an estuarine area (Øra area) in the south-eastern part of Norway.

The body is fusiform and subcylindrical, with a small terminal oral sucker and a reduced sunken ventral sucker situated about one-fourth along the body from the anterior end. The area between the suckers was characterized by transversal corrugations — partly due to body flexure — and distinct tegumental ridges and depressions.

Posteroventrally and anterodorsally the tegument had a cobblestonelike appearance in contrast to the conspicuous longitudinal ridges posterodorsally. On the rim and surroundings of the oral sucker the tegument was irregularly convoluted with three types of distinct structures; domed aciliate papillae, pits, and domed ciliated papillae protruding or not protruding from pits. An aggregation of papillae was present encircling the ventral sucker. The tegument was unspined.

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INTRODUCTION

In the body cavity of the Common goby *Pomatoschistus microps* (Kröyer, 1878), two species of an interesting fluke genus have relatively newly been described. These are *Aphalloides coelomicola* Dollfus, Chabaud and Golvan, 1957 from France and *A. timmi* Reimar, 1970 from the Baltic Sea.

The life cycle is unique in that the 2nd intermediate host also functions as the final for these parasites. This together with retention in some cases of larval structures such as eye spots indicates a progenetic situation and a state of neoteny. The systematic position of these flukes is also puzzling; Dollfus, Chabaud & Golvan (1957) placed them in the family Cryptogonimidae Ciurea, which I consider correct, but Naidenova (1970) placed them in the Heterophyidae Leiper and Yamaguti (1971) in the Opisthorchidae Looss.

In the field of parasitology, scanning electron microscopy (SEM) has been employed for more in-depth characterization and visualization of surface features of helminth morphology for use in taxonomy. As Common gobies infected with *Aphalloides* were found in an estuarine area in Norway, a SEM study of this parasite group is presented here for the first time.

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MATERIAL AND METHODS

The coelomic cavities of 269 Common gobies *Pomatoschistus microps* sampled in September 1977 in an estuarine area (Øra area) at the outlet of the river Glomma, SE Norway, were examined for adult *Aphalloides*. The living adults recovered were identified by comparison with the only two species described in this genus and found to be similar to *A. timmi* in morphological and anatomical measurements. (There were some discrepancies in the female anathomy, but these are believed to be due to an erroneous assumption in Reimar's (1970) paper on the female apparatus, on which I have started a closer examination). The specimens were isolated from the body cavity of the infected fishes and 10 gravid specimens from single infections were taken for SEM investigations.

The flukes were killed in hot (c. 70°C) 10% formaldehyde, dehydrated through an ascending series of ethyl alcohol to critical point drying with CO_2 to minimize distortion by surface tension forces. They were mounted on metal specimen stubs, rotary coated with gold and examined with a Stereoscan, MK 2A (Cambridge Instrument Co.).

RESULTS

Aphalloides timmi was the only fluke species recovered from the body cavity of Common gobies in the Øra area, and adult specimens were recorded in 72 (26.8%) of the 269 fish specimens examined. 1 to 4 adults were found per infected fish. In single infections the parasite was always located between the intestinal canals in the posterior intestinal loop.

Body outline and sucker location is shown in Fig. 1. The body was fusiform and subcylindrical, and with maximum width at the anterior fourth. The small oral sucker was terminal (Figs 1, 2) and the reduced ventral sucker was situated anteriorly in a depression of the body wall (Fig. 2). Anteriorly, large transverse corrugations were prominent in all specimens examined by SEM, especially ventrally, but these were partly due to body flexure, as a slight vertical bending of the flukes was caused by fixation. The circular oral sucker rim was sparsely supplied with non-ciliated papillae (Fig. 3). In one of the specimens examined the oral sucker aperture was partly fused and non-functional as a feeding apparatus (Fig. 4). The closest surroundings of the oral sucker rim were especially richly equipped with presumed sensory structures of different types; papillae- ciliated and non-ciliated, pits, beside tegumental folds often arranged in circles (Fig. 3). Aggregated around the sunken ventral sucker were domed papillae, as seen in Fig. 5 (the genital pore is situated close to and anterior to the ventral sucker within this tegumental depression). The excretory pore is posterior and terminal, and is surrounded by a radial arrangement of surface ridges (Fig. 6).

Ventrally, between the delicate and sensorily highly equipped region near the oral sucker (Fig. 3) and the prominent transversal corrugations shown in Fig. 2, is an area with a regularly arranged rhombus-like tegumental pattern which is also equipped, though more sparsely, with ciliated and non-ciliated papillae (Fig. 7). Posteroventrally to the ventral sucker the body had a cobblestonelike appearance (Fig. 8) similar to the dorsal anterior parts of the fluke (Fig. 14). The corrugated tegumental area between the suckers on Fig. 2 contained not only larger folds, but also marked transverse tegumental ridges as seen in Fig. 9 (enlarged in Fig. 11), and transverse tegumental depressions as seen in Fig. 10 (enlarged in Fig. 12). Fig. 13 shows the presence of prominent longitudinal tegumental folds in the posterior dorsal region; this contrasts with the characteristic cobblestoned appearance of the dorsal central and anterior areas shown in Fig. 14.

Three main types of presumed sensory structures were profusely present in the anterior part of the fluke, and occurred with decreasing frequency towards the ventral sucker: non-ciliated domed papillae (Fig. 15), non-ciliated pits (Figs 16a, b) and ciliated papillae (Figs 17a—c). No spines were present on the body.

DISCUSSION

SEM studies provide an additional dimension for the morphological study of flukes, which seems especially important and interesting in the present genus where the architecture of the integument surfaces has been given only incidental attention. As far as I have found, only Reimar (1970) has described *Aphalloides timmi,* and only Dollfus, Chabaud & Golvan (1957), Naidenova (1970) and Maillard (1976) *A. coelomicola,* and all these descriptions are based on light microscopy. These papers contain little information on external microstructure. The unspined adult body surface is reported (Dollfus, Chabaud & Golvan op. cit., Maillard op.cit.), different to the finely-spined metacercarial surface (Naidenova 1970, Maillard 1976). The fusiform, subcylindrical body outline (Fig. 1) is described, as are the anterior transverse corrugations on *A. coelomicola* (Dollfus, Chabaud & Golvan 1957) (see Fig. 2). Figs 9—12 demonstrate that transverse corrugations are not merely a result of body flexure, but also represent specific tegumental structures. The prominent posterodorsal ridges shown in Fig. 13 seem also to be specific tegumental ridges not due to body bending or contractions, and this is supported by the poorly-developed body musculature in these regions, as seen on sectioned specimens (unpublished, see Fig. 1).

The small oral sucker, sometimes fused (Fig.4) and the greatly increased surface area, due to a highly convoluted tegument, and especially prominent in the ventral anterior regions (Figs 3, 7, 9—12), may indicate an absorptive nutritional function of the tegument. Lesions in the body cavity of the infected Common gobies were not observed.

The small sucker sizes (with the ventral sucker depressed into the body wall), delicate tegument (Figs 3, 17) and poor muscular development (unpublished) all seem to represent adaptations to the protected microhabitat.

The well developed, domed papillae were especially numerous in the vicinity of the suckers, but were not arranged in any decipherable pattern (Figs 3, 15). The large number of evenly-spaced ciliated structures anteriorly (Figs 3, 17) indicate the importance of these well-known surface specializations even for adults living in a closed, probably chemically homogeneous and protected environment. Related transmission electron microscope (TEM) observations of these tegumental papillae are necessary to elucidate further their possible function and origin (see Edwards, Nollen & Nadakavukaren 1977).

No infoldings were observed encircling the non-ciliated papillae (Fig. 15) in contrast to those seen on other species (see Bakke 1976), and which in those cases indicate a muscular dependent vertical mobility. The ciliated papillae, however, seem to be capable of muscular activity, as are the protrudent cilia themselves (Figs 17b, c).

The depressions and pits observed were all located anteriorly (Figs 3, 16, 17a), and both non-ciliated (Fig. 16) and ciliated (Fig. 17a) types occurred. Different types of pits have been recorded and different functions suggested for them (see Bakke 1976). The retention and apparent development of these structures on *A. timmi* living in a closed and presumably chemically stable and homogeneous microenvironment, indicate a functional importance still not known. TEM and histochemical tests would be necessary to elucidate further their possible function in the adult, however, neotenic stage.

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Figs 1—17. Scanning electron microscope (SEM) pictures of adult *Aphalloides timmi*.

Fig. 1. The whole adult fluke seen from the ventral side (scale bar 0.5 mm).

Fig. 2. The anterior end enlarged (scale bar 0.1 mm).

Fig. 3. The typical appearance of the oral sucker with surroundings (scale bar 10 µm).

Fig. 4. A case with fused oral sucker opening (scale bar 20 µm).

Fig. 5. A micrograph demonstrating the depressed ventral sucker (scale bar 20 µm).

Fig. 6. A micrograph demonstrating the excretory pore opening (scale bar 10 µm).

Fig. 7. The ventral microtopography close to the oral sucker rim (scale bar 5 µm).

Fig. 8. The ventral microtopography posterior to the ventral sucker (scale bar 2µm).

Fig. 9. The ventral microtopography anteriorly in the middle region between the suckers (scale bare 10 µm).

Fig. 10. The ventral microtopography posteriorly in the middle region between the suckers (scale bar 10 µm).

Fig. 11. Enlarged micrograph of the area shown in Fig. 9 (scale bar 2 µm).

Fig. 12. Enlarged micrograph of the area shown in Fig. 10 (scale bar 2 µm).

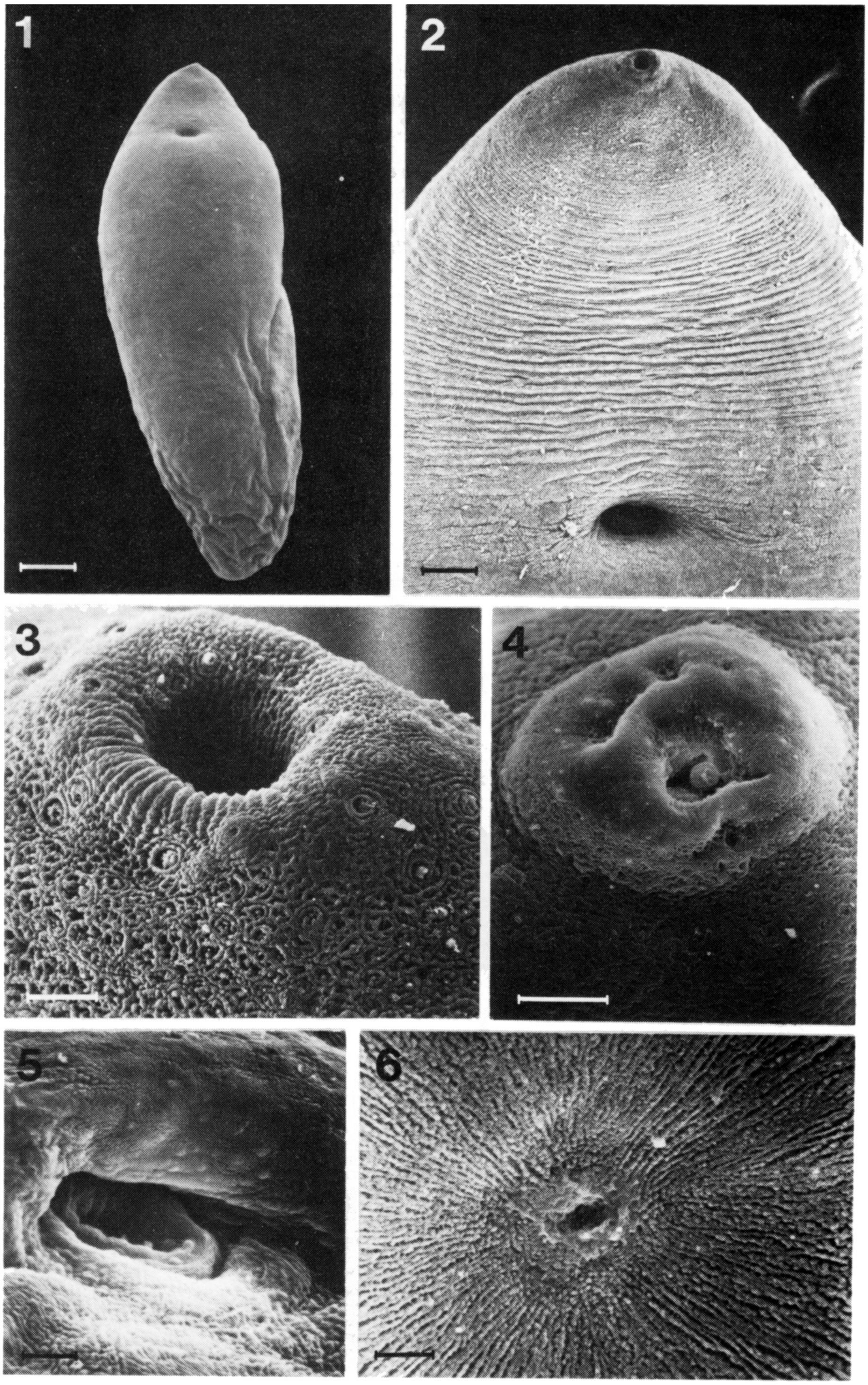
Fig. 13. The tegumental longitudinal folds postero-dorsally (scale bar 10 µm).

Fig. 14. The tegumental corrugations anterodorsally (scale bar 5 µm).

Fig. 15. A non-ciliated papilla from the region close to the oral sucker (scale bar 2 µm).

Figs 16a, b. Micrographs showing one pit (a) and two non ciliated pits (b) close together near the oral sucker rim (scale bar 2 µm).

Figs 17a-c. Micrographs showing three (a, b, c) different appearances of the ciliated papillae/pits close to the oral sucker (scale bar 2 µm).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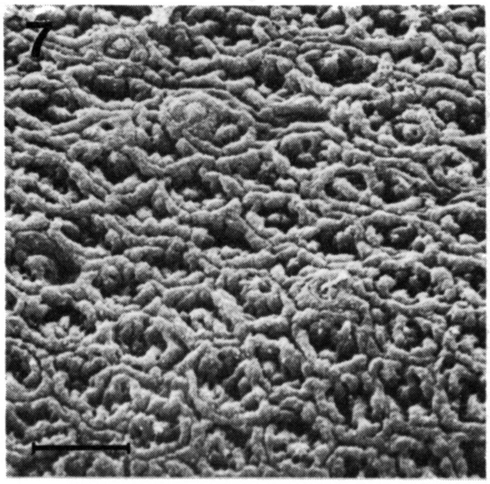
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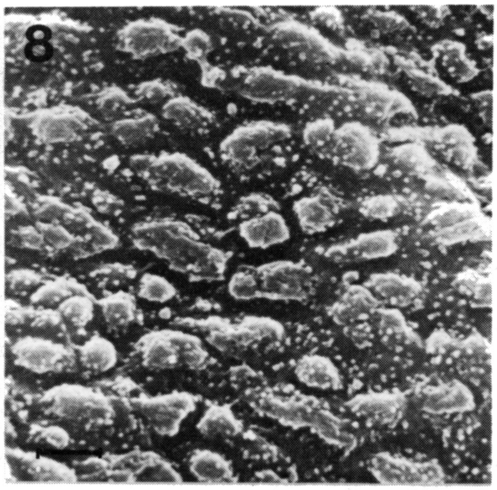
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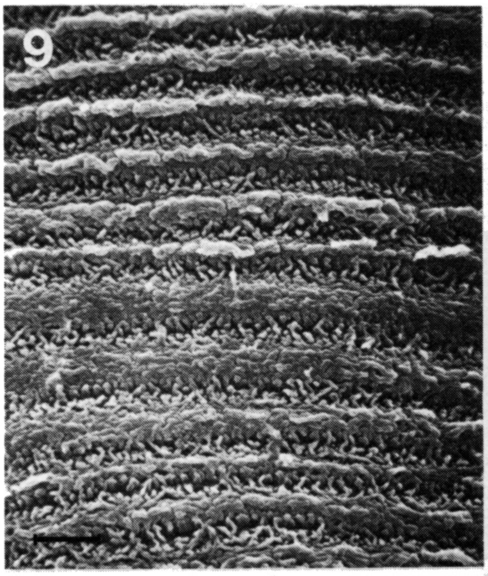
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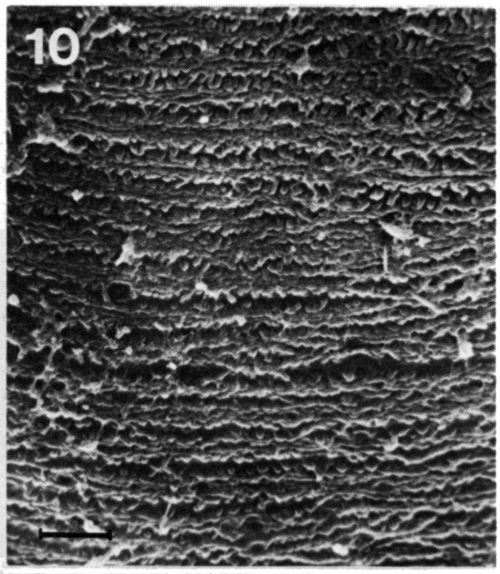
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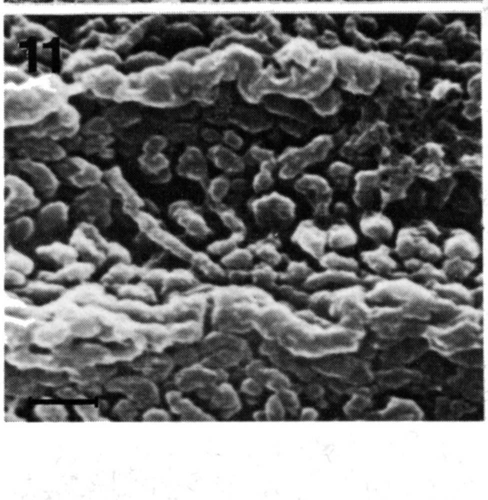
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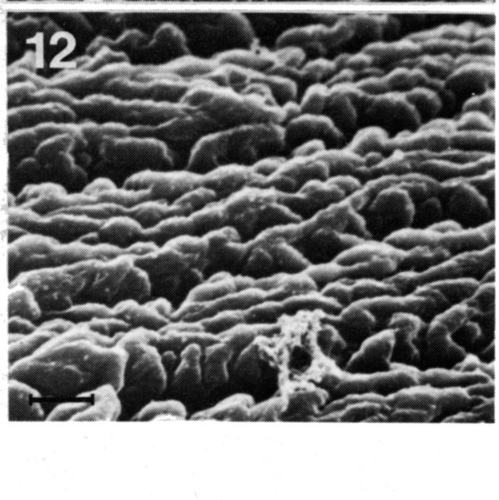
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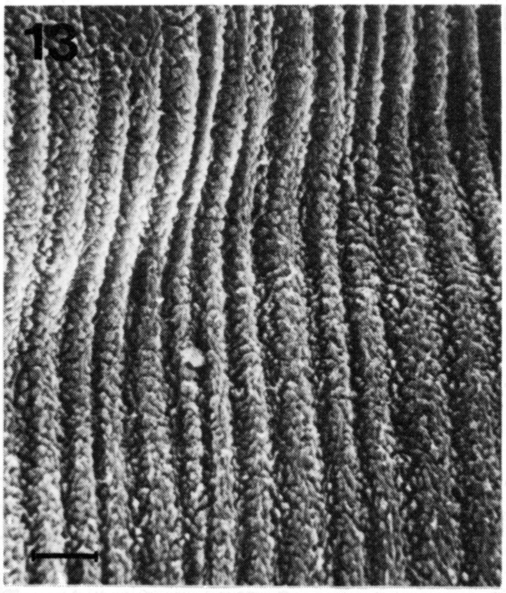
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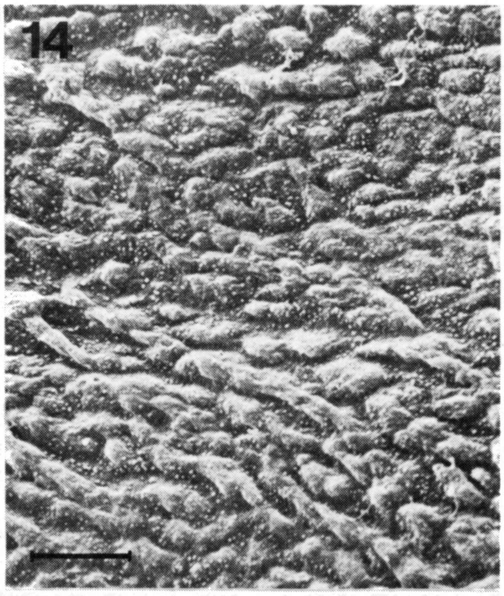
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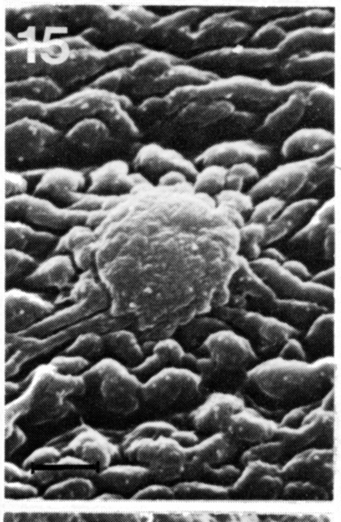
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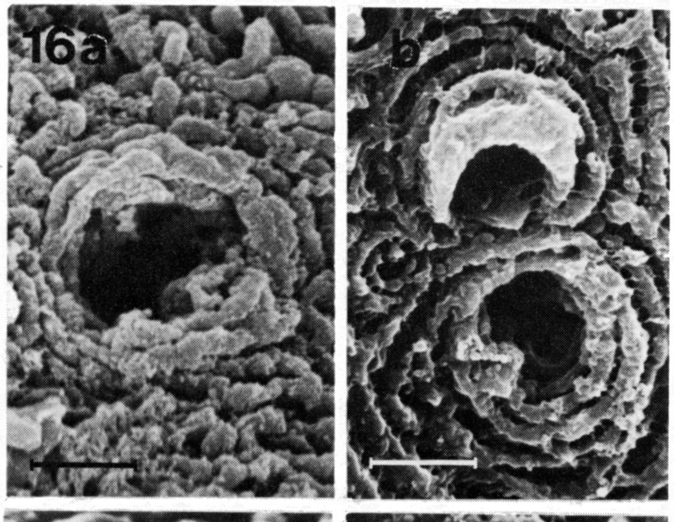
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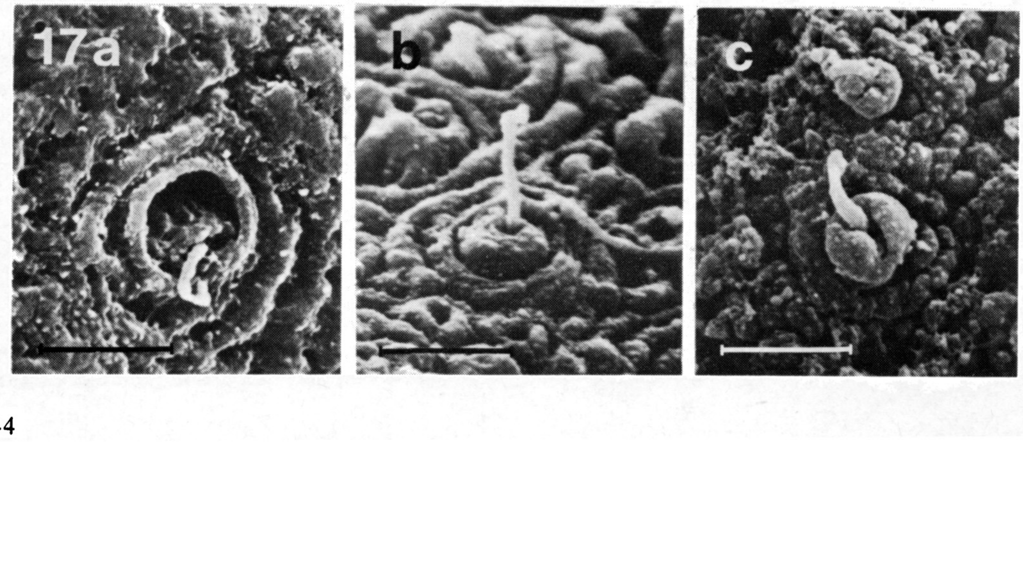
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