Parasit. hung. 17. 1984

Scanning electron microscopic examination of the tegumental surface of some amphistomes (Trematoda: Amphistomida)

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"Scanning electron microscopic examination of the tegumental surface of some amphistomes (Trematoda: Amphistomida) - Sey, O. - Parasit. hung., 17: 45-49. 984.

ABSTRACT. Examination of the surface topography of eighteen species of amphistomes from different vertebrate hosts (fishes, amphibians, reptiles, mammals) revealed three further new types of tegumental papillae. Different types of papillae can occur on the same species and their individual variability was demonstrated on the very same species. Taking the sources of errors into consideration, which impair such examinations, the results show that the tegumental papillae, combined with other characters, might be a useful set of traits in amphistome diagnosis.

KEY WORDS: Scanning electron microscopy (Trematoda: Amphistomida), new papillae, taxonomic value.

The examination of the surface topography of digenetic trematodes by scanning electron microscope (SEM) has thrown new light upon the structure and importance of the tegumental papillae. Although several previous authors (FISCHOEDER, 1903; STILES and GOLDBERGER, 1910; LEIPER, 1910; DOLLFUS, 1950; TANDON, 1955; DINNIK, 1961, 1962, 1964; GRETILLAT, 1964; VELAZQUEZ-MALDONADO, 1976; SEY, 1983) have also observed the presence of these papillae with the aid of light microscope, the limitations of their methodology prevented them from unravelling the exact nature of these formations, and hence their bearing has never been seriously considered.

Of the digenetic trematodes several species have been examined by SEM, and some of the authors (e.g. TANDON and MAITRA, 1981) emphasized the physiological (host-parasite interface relationship) and of the taxonomic (e.g. EDUARDO, 1980a, 1982a) value (type, distribution, occurrence) of the tegumental papillae.

In the case of the amphistomes which are sometimes morphologically similar the usage of the SEM examinations offers promises of the discovery of new sets of character states of diagnostic importance. Most of the many amphistome species studied so far (NOLLEN and NADAKAVUKAREN, 1974; TANDON and MAITRA, 1981, 1982, 1983; EDUARDO, 1980b, 1980c, 1980d, 1980e, 1982a, 1982b, 1983) are with a few exceptions, species living in the rumen. If we accept that the similar adaptation to a similar microenvironment (rumen) resulted in the general occurrence of the papillae (BAKKE, 1978 cit. TANDON and MAITRA, 1982), then species adapted to another microenvironment (intestine) can be supposed to exhibit other types of tegumental papillae.

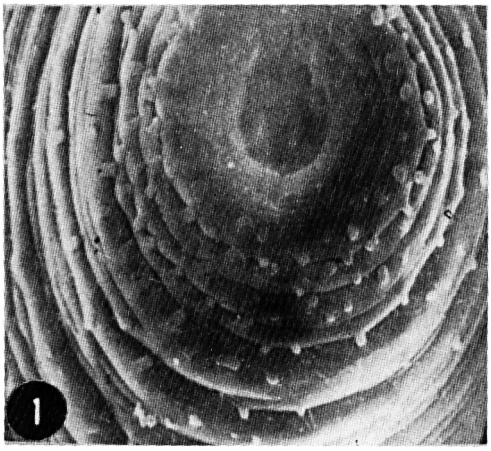
The present paper comprises a selected set of amphistomes of different vertebrate hosts which live in ruminal and intestinal habitats, and examines the pattern and consistancy of their tegumental papillae from the taxonomic point of view.

MATERIAL AND METHODS

The majority part of the test material was collected by the author the rest of them is derived from European, African and overseas amphistome collections from the following hosts: fishes: Spinibarbichthys denticulatus (Neocladorchis multilobularis, Vietnam); Mormyrus cannumae (Basidiodiscus ectorchis, Egypt); Pterodoras granulosus (Dadaytrema oxycephalum, Naturhistorisches Museum, Vienna); amphibians: Rana cyanophlyctis (Diplodiscus mehrai, India); reptiliens: Batagur baska (Stunkardis dilymphosa, India); mammals: Hippopotamus amphibius (Platyamphistoma polycladiformae, Carmyerius cruciformis, Africa); Bos primigenius taurus (Stephanopharynx compactus, Africa; Fischoederius elongatus, India; Homalogaster paloniae, Vietnam); Hippotragus equinus (Stephanopharynx coilos, Africa); Bubalus arnae bubalis (Carmyerius gregarius, Egypt; Fischoederius cobboldi, India); Syncerus caffer (Carmyerius mancupatus, Africa); Boocercus euryceros (Carmyerius schoutetedeni, Musée Royal de l'Afrique Central, Tervuren); Elephas maximus (Hawkesius hawkesi, India); Papio papio (Watsonius deschiensi) and Macacus cynomolgus (Watsonius noci, Muséum National d'Histoire Naturelle, Paris). The species were identified based on their histology, following NASMARK's (1937) terminology. Pouched worms were dissected along the opening of the ventral pouch to bare the genital opening that is surrounded by papillae. For the SEM studies, after thorough washing in saline the flukes were fixed and placed in storage in 70% alcohol till further processing. There was dehydration through an ascending series of alcohol to the critical point drying with CO_2, coated gold-palladium and observed with TESLA B-S 300.

Legend to figures

Figs 1-2: Neocladorchis multilobularis (1= anterior end x 138, 2= closer view of papillae x 445)
Fig. 3: Basidiodiscus ectorchis (general view of oral opening x 208)
Figs 4-6: Dadaytrema oxycephalum (4= anterior end x 95, 5-6= closer view of papillae (5 - x 645, 6 - x 700)
Fig. 7: Diplodiscus mehrai (general view of oral opening x 110)
Figs 8-12: Stunkardia dilymphosa (8= general view of oral opening x 167, 9= closer view of papillae around oral opening x 433, 10= general view of genital opening x 93, 11= closer view of papillae around genital opening x 750, 12= papillae along body surface x 83)
Fig. 13: Platyamphistoma polycladiformae (general view of oral opening x 123)
Figs 14-15: Stephanopharynx coilos (14= anterior end x 119, 15= general view of genital opening x 130)
Figs 16-18: Stephanopharynx compactus (16= anterior end x 26, 17= closer view of papillae around anterior end x 875, 18= genital opening x 50)
Figs 19-21: Carmyerius cruciformis (19= general view of oral opening x 90, 20= general view of genital opening x 75, 21= closer view of papillae around genital opening x 500)
Figs 22-23: Carmyerius gregarius (22= general view of oral opening x 195, 23= general view of genital opening x 170)
Figs 24-25: Carmyerius mancupatus (24= general view of oral opening x 72, 25= general view of opening of ventral pouch x 55)
Figs 26-27: Carmyerius schoutedeni (26= general view of oral opening x 55, 27= closer view of papillae around oral opening x 625)
Figs 28-30: Fischoederius cobboldi (28= anterior end x 75, 29= anterior end with genital opening x 36, 30= genital opening and texture of inner surface of ventral pouch x 55)
Figs 31-32: Fischoederius elongatus (31= anterior end x 82, 32= genital opening x 575)
Figs 33-35: Hawkesius hawkesi (33= general view of oral opening x 47, 34-35= closer view of papillae around oral opening (34 - x 87, 35 - x 1000)
Figs 36-38: Homalogaster paloniae (36= papillae around oral opening x 150, 37= general view of genital opening x 40, 38= closer view of papillae around genital opening x 213)
Figs 39-40: Watsonius deschiensi (39= anterior end x 25, 40= general view of genital opening x 35)
Figs 41-42: Watsonius noci (41= part of genital opening x 146, 42= closer view of papillae around genital opening x 1625)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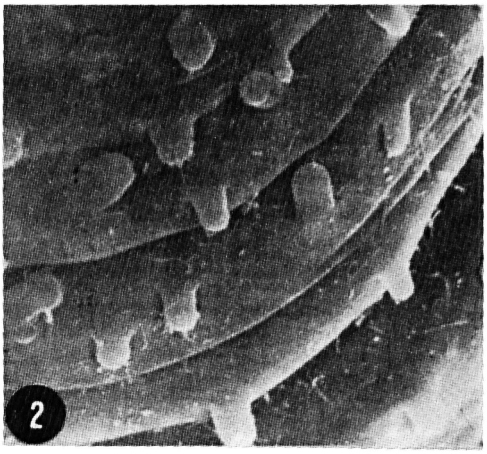
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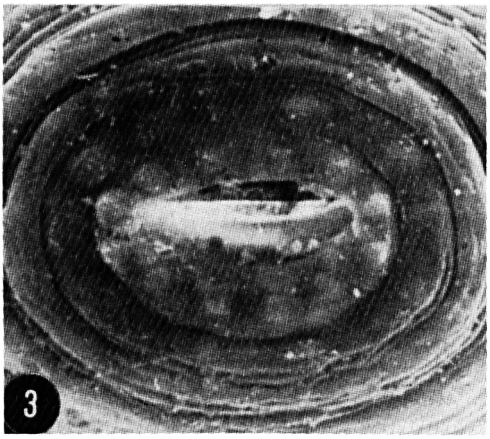
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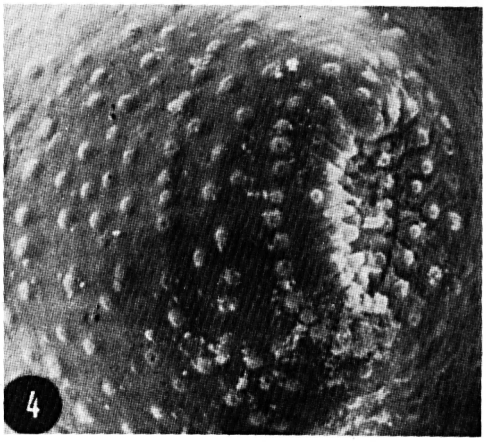
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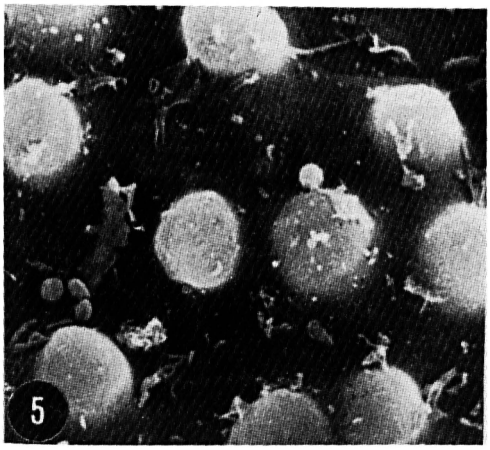
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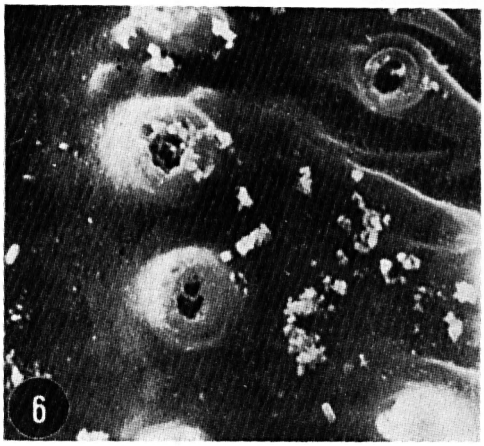
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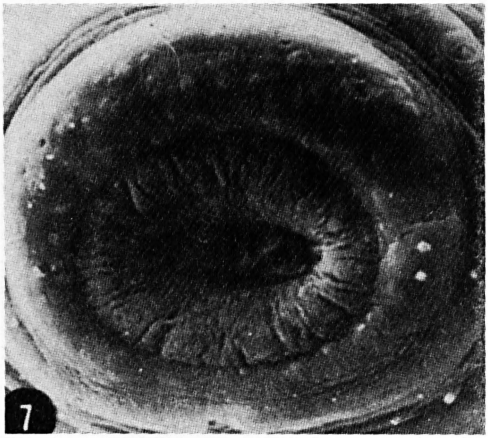
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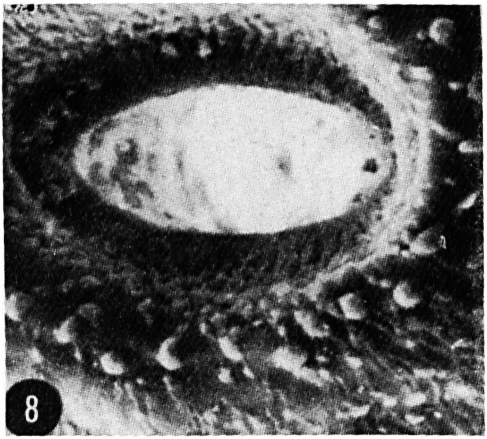
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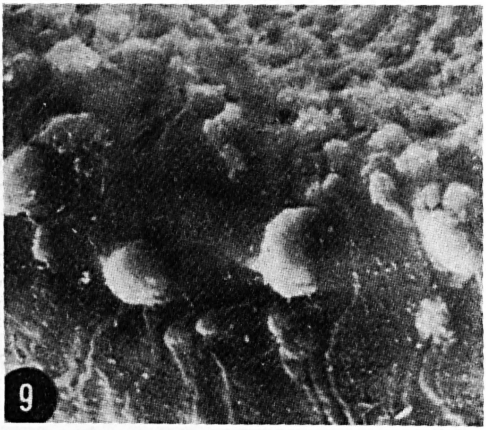
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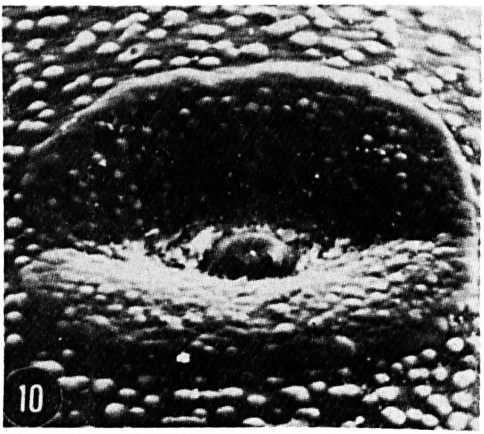
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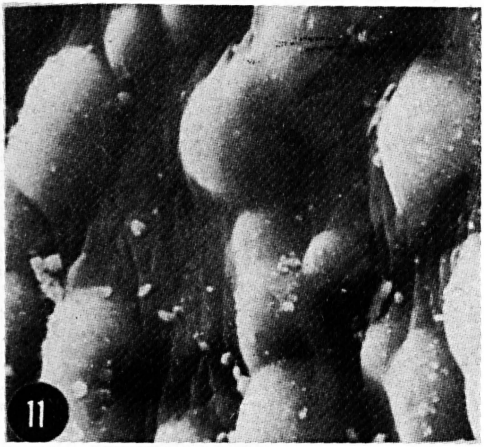
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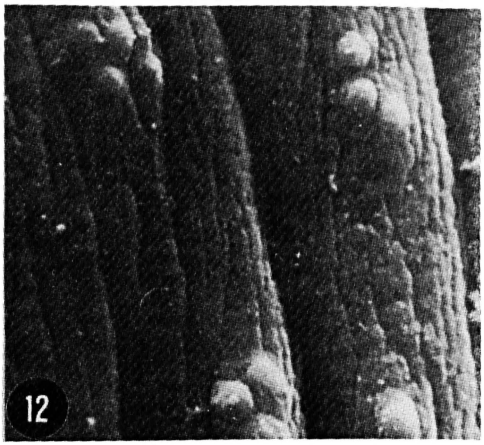
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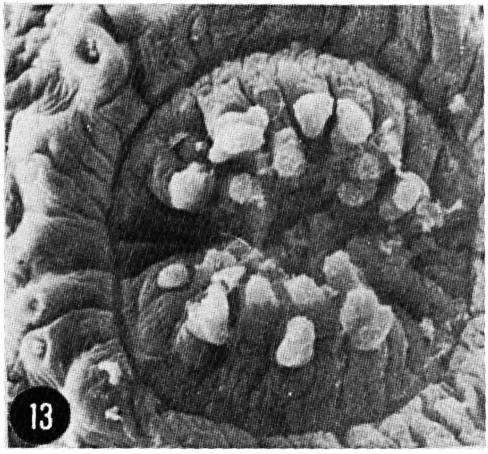
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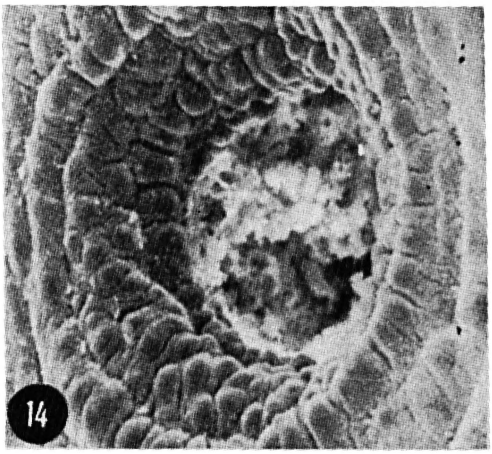
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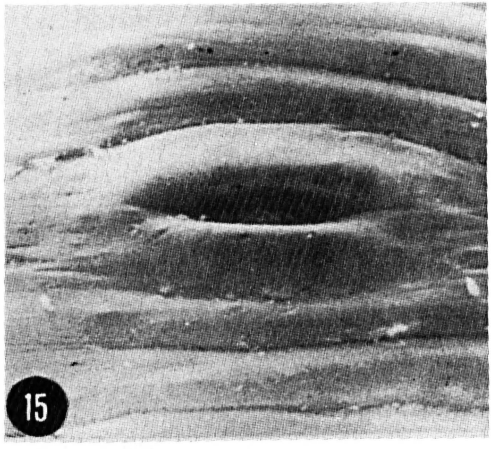
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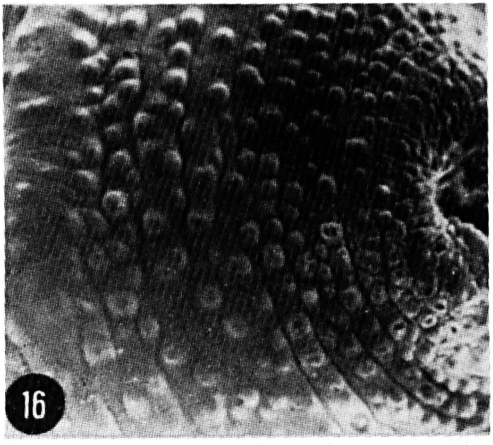
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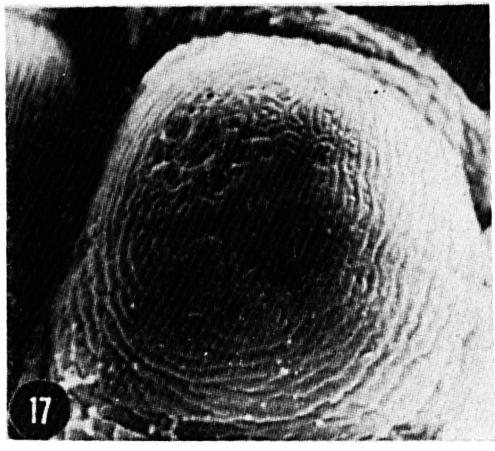
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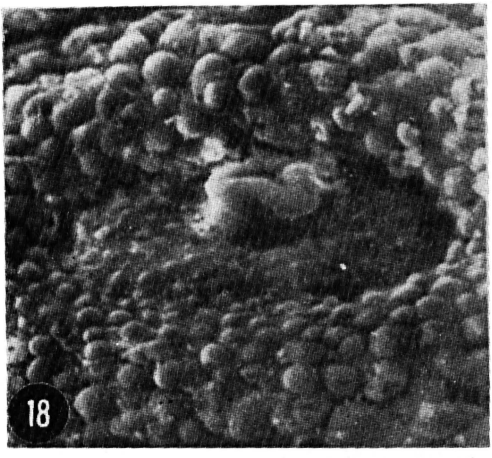
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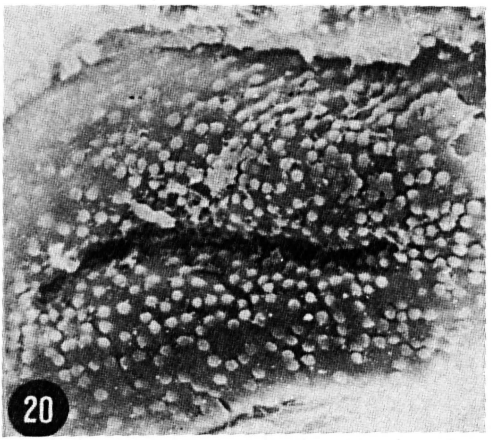
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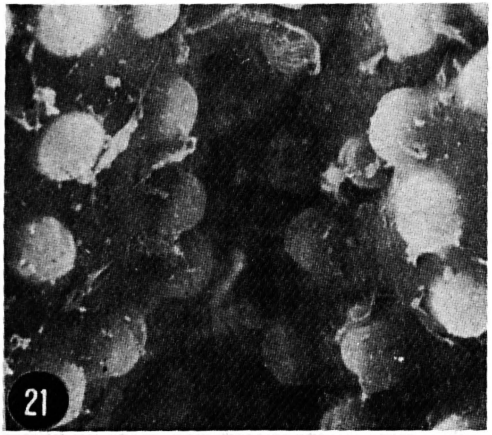
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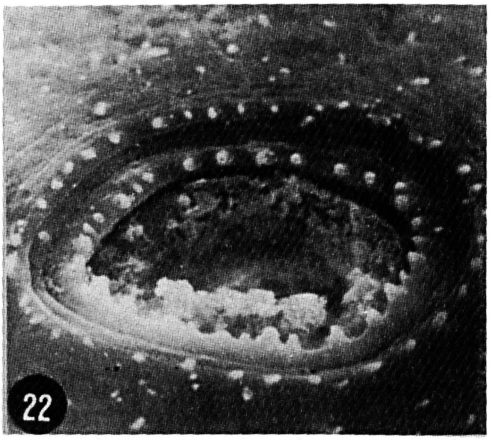
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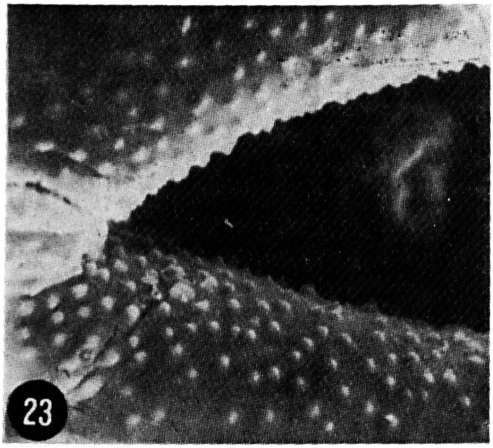
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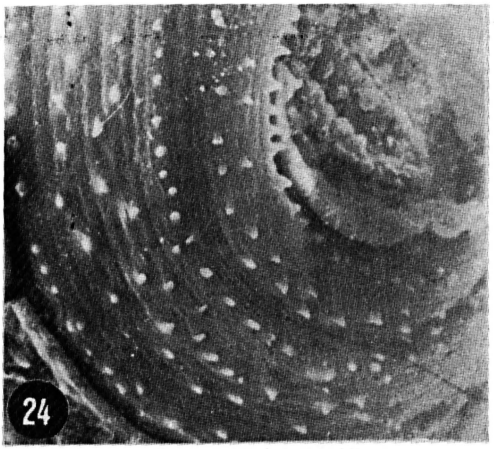
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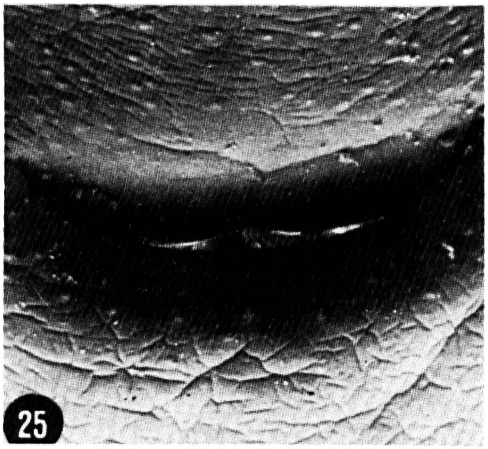
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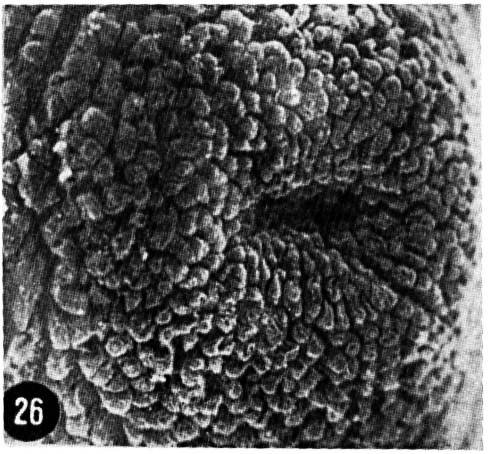
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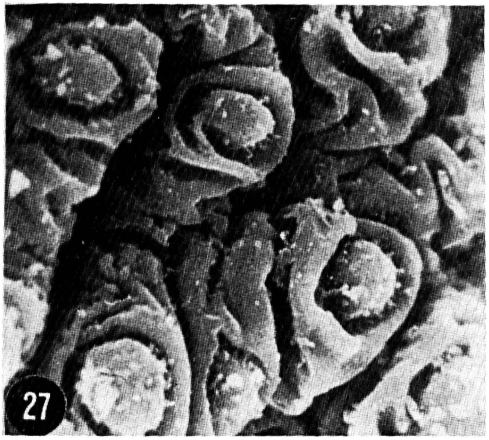
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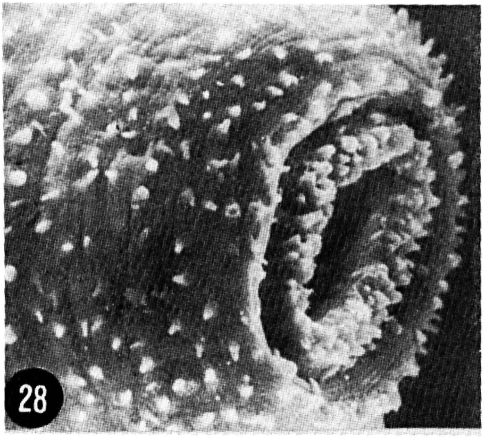
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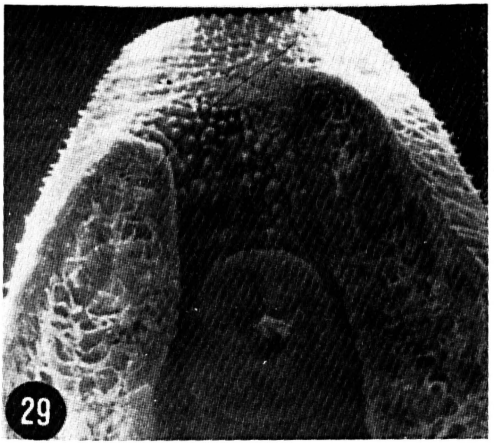
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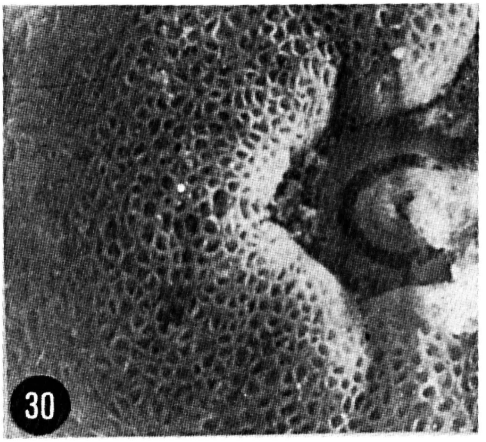
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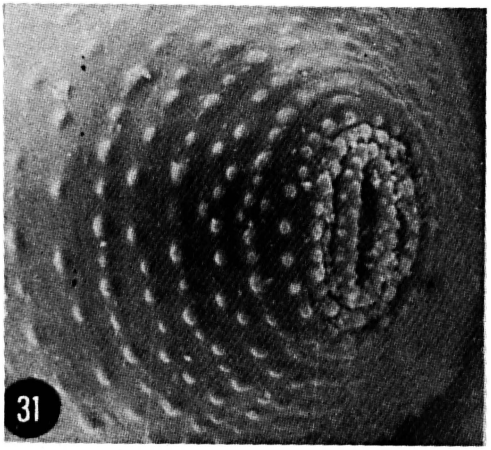
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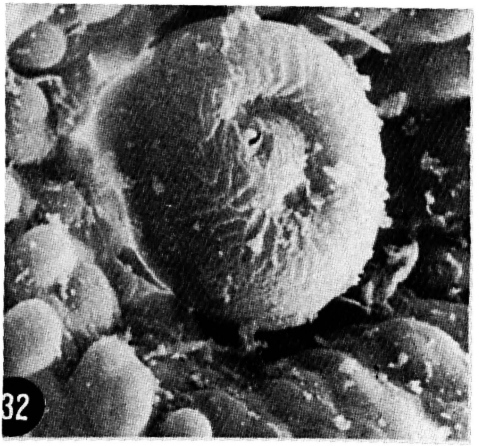
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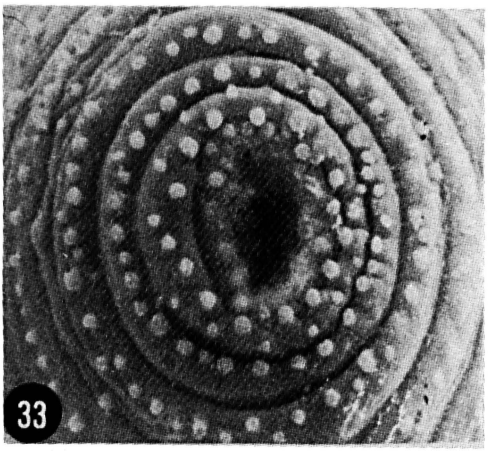
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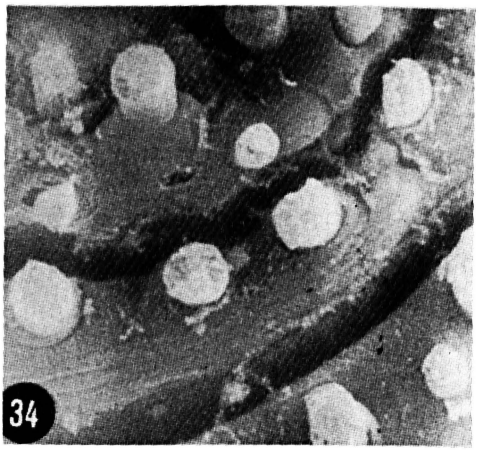
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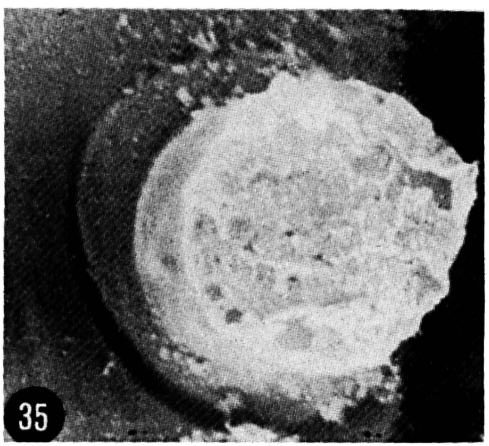
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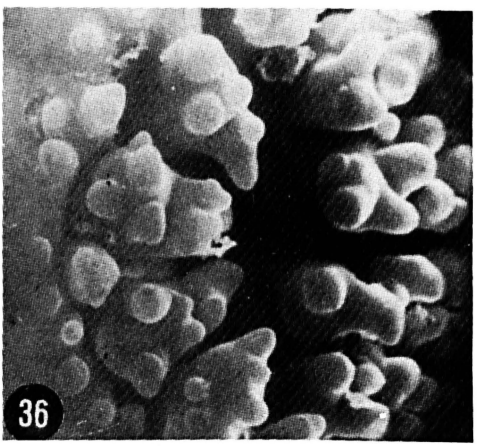
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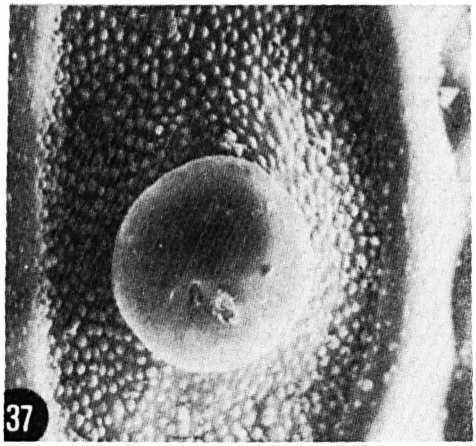
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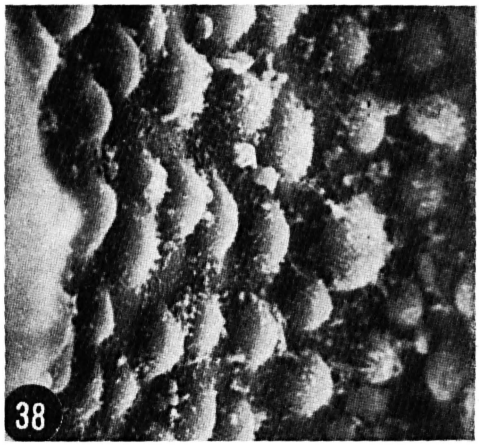
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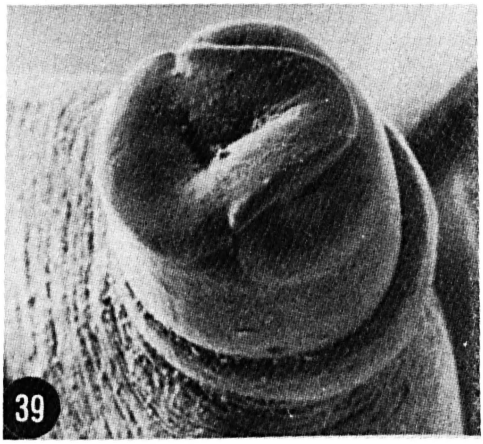
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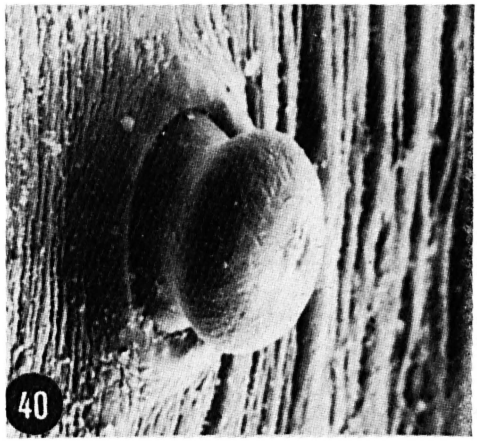
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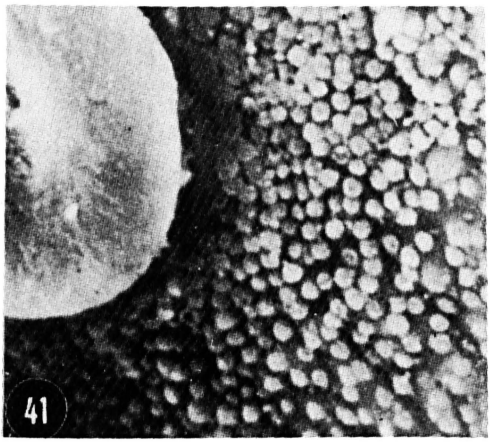
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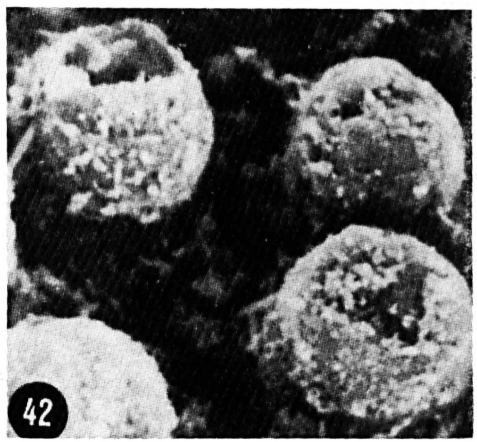
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RESULTS AND DISCUSSION

Examinations aiming at the recognition of surface topography of amphistomes revealed that the presence of the tegumental papillae is one of their characteristics, with a few exceptions. When they are present they are usually situated in five regions of the body surface: around the oral, genital, acetabular openings, as well as around the orifice of the ventral pouch, and sometimes along other regions of the body. These papillae are usually arranged either in several rows or in smaller and bigger groups, loosely or very closely packed. Of the papillae, those around the oral opening and the ones at the anterior extremity, appear to be the most variable.

The tegumental papillae of mammalian amphistome species were studied the most extensively by EDUARDO, in one of his papers (1982a) the anterior papillae were classified and assigned to seven categories. He also emphasized the consistency and uniformity of the papillae characteristic for a given species.

Before comparing our results with those of the previous authors we should point out the source of errors which can impair such examinations: 1) misleading identification can easily occur, especially among species closely related, provided that the gross-morphology, definitive host(s) etc. do not predestine the specific identity because the very specimens subjected to SEM examinations were not fit for identification; 2) as the tegumental papillae are usually minute elements they are subjected to destruction, rather than any other parts of the worms, due to the pre-fixative treatment, condition of storage, age of the samples, etc. Raising this question becomes reasonable when tegumental papillae are not found: whether in primary or secondary condition. Namely, examinations made in this field show that the occurrence of these papillae on the body surface can be said to be typical.

Our examinations, comprising amphistomes living in both ruminal and intestinal habitats, indicate that the majority of the papillae can be assigned to the categories set up by EDUARDO (1982a), but it seems to be justified to designate three other types: 1) crateryform papilla (Figs 4-6), 2) column-like papilla (Figs 1-2) and 3) short and stumpy papilla without hair-like processes (Figs 33-34, 22, 24).

Accordingly, papillae found on the body surface of the species examined belong to the following types:

1) Dome to conical non-ciliated papilla
   a) papilla hardly elevated from tegumental surface (Figs 3, 7)
   b) papilla elevated regularly (Figs 4-5, 8-11, 14, 16-18, 19-21, 23,25, 31-32, 37, 38)
2) Dome to conical ciliated papilla (Figs 26-27)
3) Short and stumpy papilla covered with hair-like processes (Fig. 12)
4) Short and stumpy papilla, sitting on a tegumental elevation (Figs 12, 33-35)
5) Long non-branching and non-ciliated papilla (Figs 22, 24, 28-30)
6) Long papilla with bulb-like branches (Figs 13, 36)
7) Crateryform papilla (Figs 6, 41-42)
8) Column-like papilla (Figs 1-2)
9) Papilla absent (Figs 15, 39-40)

Contrary to EDUARDO's (1982a) finding, different types of papillae were observed on the very same species (Figs 4-6, 8-12).

As to the consistency of the tegumental papillae our observations somewhat deviate from EDUARDO's (1982a) results. This is illustrated by the examples of the following two species. EDUARDO (1980b) regarded Stephanopharynx coilos as a synonym of S. compactus. Examining these species with SEM one could demonstrate differences in arrangement mainly in the papillae existing around the genital opening. It has been found that there are no papillae around the genital opening of S. coilos (Fig. 15) but the genital opening of S. compactus is papillated (Fig. 18). These features are at the same time, correlated with the presence (S. coilos) and the absence (S. compactus) of the papillae along the inner surface of the pharyngeal cavity, which is the most important specific trait distinguishing the two species in question.

The genus Hawkesius is monotypic with H. hawkesi as its only species, a parasite of the Indian elephant. Hence there is no doubt about its specific identity for example because of its definitive host, et our findings remarkably differ from those of EDUARDO's (1980b), indicating either the existence of individual variability or the role of unfavourable conditions, effected on the wast of these papillae.

The amphistomes parasitizing in the alimentary truct exhibit basically a similar distribution and types of papillae as those of the species living in the rumen. Different types of papillae can occur, however, even on the same species. Their individual variability could be demonstrated, hence it is the task of further examinations to detect the nature and extent of the variability which can give us a clue to the appreciation of these papillae, combined with other traits, in amphistome diagnosis.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Prof. Dr. P. KÁSA (Central Laboratory, Medical School, Szeged) for the SEM facilities and to Z. FARKAS for technical assistance.

SEY, O.: Néhány amfisztomum faj (Trematoda: Amphistomida) tegumentális papilláinak pásztázó elektronmikroszkópos vizsgálata

A szerző tizennyolc, különféle gerincesből (hal, kétéltű, hüllő, emlős) származó amfisztomum faj tegumentális papilláit vizsgálta pásztázó elektronmikroszkópos felvételeken. Három új papilla-típust állapított meg; kimutatta a papillák variabilitását és különféle papillák ugyanazon mételyfajon való előfordulását. Felhívta a figyelmet az ilyen jellegű vizsgálatok hibaforrásaira, amelyeket ha figyelembe veszünk és minimálisra csökkentünk, a tegumentális papillák, más bélyegekkel kiegészítve, eredményesen felhasználhatók az amfisztómum-diagnózisnál.

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Received: 15 July, 1983

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