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Studies on Intestinal Trematodes in Korea XV. Tegumental Ultrastructures of *Fibricola seoulensis* according to Developmental Stages

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= Abstract =Tegumental ultrastructures of *Fibricola seoulensis* were studied by means of scanning electron microscopy according to their developmental stages. Metacercariae were obtained from the snakes, and the juvenile and adult worms were recoverd from small intestine of albino rats after experimental infection.

Metacercariae revealed their characteristic spoon-shaped, ventrally concave body, and primordial stage of hindbody. External structures of oral sucker, ventral sucker and tribocytic organ were visible. Excretory pore was seen at postero-dorsal portion of subterminal body. Whole body surface was covered with simple, triangular spines. Uni-ciliated type I sensory papillae were distributed sparsely on whole surface but densely around oral and ventral suckers and peripheral margin. Round swellings, type II papillae, were recognizable but confined to lips of two suckers. More differentiated spines, 3-4 pointed ones, were seen in 2-day old juveniles, and primitive forms of the third type papillae appeared in 3-day old ones.

In adults, hindbody had grown remarkably and revealed fine, velvety tegument. Tribocytic organ had stout recurved spines and honeycomb-like internal structures. Whole forebody was covered with serrated spines but hindbody was completely aspinous. But type III (round swellings without cilia) and type IV (with cilia) sensory papillae appeared in hindbody. These papillae were suggested to be tango-, rheo- and/or chemoreceptive in function.

Key Words: ***Fibricola, SEM, Tegument, Development, Spines, Sensory papillae, Tribocytic organ***

INTRODUCTION

Fibricola seoulensis (Trematoda: Diplostomatidae), an intestinal trematode of animals and man in Korea (Seo *et al.* 1964 &1981), is morphologically characterized by its peculiar contour of bisegmented body into fore-and hind-compartments. At metacercarial stage, however, the hindbody appears to be only a primordium and is attached to postero-dorsal surface in a form of short, cylindrical tail (Hong *et al.* 1982). When the metacercaria was infected experimentally to albino rats and mice, it established its habitat in the duodenum without migration through the lung or other tissues, and rapidly grew to become an adult possessing intrauterine eggs on the fifth day after infection (Hong 1982). During the development, the growth of the hindbody was far more remarkable than that of the forebody, therefore, after 14 days of growth, the hindbody became longer than the forebody.

Recently the developmental pattern of trematodes has often been studied by means of scanning electron microscopy; *Clonorchis sinensis* (Fujino *et al.* 1979), *Metagonimus yokogawai (Lee et al.* 1984), *Cryptocotyle lingua* (Kφie 1977), *Diplostomum spathaceum* (Russel-Smith *et* Wells 1982), *Alaria marcianae* (Shoop *et* Corkum 1984). These reports have shown that the pattern of tegumental development is different by different kinds of flukes.

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In *F. seoulensis*, the tegumental structures of adult worms were studied by Seo *et al.* (1984) and the characteristic ultrastructural features of spinous forebody, aspinous hindbody, three types of sensory papillae, and cobblestone-like processes of protoplasmic membrane were described. However, the developmental patterns of surface structures from metacercaria to adult were not studied. The present study was undertaken to observe the tegumental change of *F. seoulensis* during the developmental stages by means of scanning electron microscopy.

MATERIALS AND METHODS

The metacercariae of *F. seoulensis* were obtained from the flesh and viscera of the snake, *Natrix tigrina lateralis,* one of the second intermediate hosts (Hong *et al.*, 1982), by artificial digestion technique. The snakes were purchased from a local collector in Hoengseong-gun, Kangweon-do in June 1984. Albino rats were used as experimental animals and infected with about 200 metacercariae each. They were sacrificed at 1,2,3 and 8th day after infection and various ages of flukes were recovered from the duodenal content of the rats.

For scanning electron microscopic observation, the metacercariae, juvenile worms and adults were washed three times with 0.85% phosphate-buffered saline and fixed in 2.5% glutaraldehyde (pH 7.2). They were dehydrated in graded ethanols and freeze-dried. Then they were coated with gold in IB-3 ion sputtering apparatus and observed by ISI Korea SS-60 and DS-130 scanning electron microscope (SEM) at accelerating voltages from 10 to 30 kV.

RESULTS

Metacercaria: Body spoon-shaped, concave ventrally and convex dorsally, tapering anteriorly, and 140 μm long and 100 μm wide. Oral sucker subterminal. Ventral sucker apple-shaped, protruded ventrally and mid-median in position. Tribocytic organ, with a longitudinal slit, elevated slightly from tegumental surface, adjacent posteriorly to ventral sucker, and smaller than the ventral sucker (Fig. 1). Dorsal surface discontinuously corrugated in transverse direction and the corrugations formed many circles at the region of the primordium of the hindbody. The hindbody primordium appeared to be a slightly elevated structure at the postero-dorsal portion of the subterminal body. Excretory pore was located in the center of the primordium (Fig. 2).

Cobblestone-like cytoplasmic processes covered both ventral and dorsal surfaces and posteriorly-directed spines were inserted in the cytoplasmic pits. Single-pointed spines, nearly triangular and dorsoventrally flat in shape, distributed densely in the anterior region of the ventral surface. They converted into notched ones and became sparse in density at the level of the posterior margin of the ventral sucker (Fig. 3). However, on the dorsal surface, the single-pointed spines appeared almost evenly along the corrugations on the whole tegument (Fig. 4). These spines appeared as groups of about 4-5 rows on the posterior outer surface of the oral sucker (Fig. 5) and as encirclings on the whole surface of the ventral sucker (Fig. 6). However, the lips of both suckers were devoid of spines. Several groups of single-pointed spines were also seen on the shallow inner surface of the oral sucker (Fig. 5), however, the inner surface of the ventral sucker was devoid of spines.

Two types of sensory papillae, type Ⅰ and Ⅱ, were observed in the tegument of metacercariae. Type Ⅰ papillae consisted of an elongated cilium and a smooth dome, which made a solitary appearance throughout the body surface (Fig. 7). These were abundant around the oral and ventral suckers and on both peripheral margins but were sparsely distributed in other areas of the ventral surface. On the dorsal tegument, they were also distributed sparsely, however, they exhibited bilateral symmetry in their linear longitudinal arrangement. Several numbers of type Ⅰ papillae were located on the funnel portion of the oral and ventral suckers as well as on the spinous surface area of the ventral sucker (Fig. 6 & 7). Smooth tegumental swellings without cilia, *i.e.,* underdifferentiated type Ⅱ sensory papillae, were recognizable but they were confined to the lip protions of the oral and ventral suckers (Fig. 5,6 & 7). Hindbody primordium revealed neither sensory papillae nor spines (Fig. 8), otherwise it had the same tegumental feature as the dorsal surface of the forebody.

Juveniles (Diplostomula): On the first day after infection, diplostomula had a thicker body wall than the metacercariae and the lateral margins curved more ventrally (Fig. 9). Contour of the hindbody was recognizable but only as a protrusion from the posterior portion of the forebody. The protoplasmic processes of the tegument were fine and velvety in texture but without sensory papillae (Fig. 10). The tribocytic organ and the ventral sucker were nearly equal in size and numerous single-pointed spines emerged on their whole surface with outward direction (Fig. 11). Their inner structures were not observed because their openings were retracted. Spines on the body surface and around two suckers were elongated more but their tips were not yet divided into serrated ones. Type Ⅰ and type Ⅱ sensory papillae became more prominent in shape (Fig. 12).

On the second day, lateral and posterior margins of diplostomulum developed well to give rise to more prominent ventral foldings. The depth of ventral concavity became deeper than that in metacercaria or 1-day old diplostomulum. The cylindrical hindbody grew steadily and reached to the length of 1/3 of the forebody. At this time, a number of primitive stages of the third type sensory papillae appeared on the hindbody tegument (Fig. 13). The tribocytic organ developed to become larger than the ventral sucker but its opening was still retracted and did not show inner structures. Stout spines of the tribocytic organ became more elongated and recurved outwards (Fig. 14). Single-pointed spines differentiated into triple or quadruple-pointed ones on the whole body surface and into double or triple-pointed ones on two suckers (Fig. 15 & 16). The configuration of type Ⅱ papillae was so prominent that they were easily counted; about twenty-one on each lip of the oral and ventral suckers.

On the third day, a diplostomulum was seen to attach to an intestinal villus of the rat host, with spoon-shaped lateral foldings of forebody and roundly contracted hindbody (Fig. 17). In 3rd day worm, the length of the hindbody was about half of the forebody. Its genital atrium appeared to be postero-dorsally adjacent to the excretory pore (Fig. 18).

8-day Adult Worm: The growth of the lateral and posterior margins was so remarkable that the two lateral margins of the worm body conjoined together in a median line, therefore, it was hard to scan the tegumental structures of the ventral surface of the forebody except for the areas near the oral sucker. The hindbody was cylindrical, curved dorsally, and its length and volume were approximated to be equivalent to that of the forebody. The surface of the hindbody was encircled by three or four large transverse wrinklings which again corrugated more finely into many discontinued valleys (Fig. 19 & 20). Spines were absent in the hindbody tegument.

Tegumental spines on the ventral surface of the forebody were retracted within the pits of the protoplasmic membrane, so that only small tips of serrated spines were visible. On the anterior part of the dorsal surface of the forebody, four-or five-pointed spines were arranged radially from the dorsum of the oral sucker and their tips were headed posteriorly (Fig. 21). These serrated spines converted into single-pointed ones at the level of tribocytic organ, gradually became loose in density, and faded out at the junction of the fore-and hindbody (Fig. 22). On the oral and ventral suckers, tips of spines were divided into three or four points (Fig. 23). The tribocytic organ became double the size of the ventral sucker, and revealed honeycomb-like inner structures (Fig. 24) which gradually converted to a labyrinthian appearance in the junctional area with outer spinous portion (Fig. 24). Elongated and recurved stout spines of the tribocytic organ remained as single-pointed ones, however, they enlarged in size (Fig. 25).

Type Ⅰ papillae enlarged in size but there were not observed any changes in their morphology and distribution. Type Ⅱ papillae on the lips of both suckers appeared either solitary or as conjugated groups of two or three in number (Fig. 23). Round swellings of tegument without cilia, type Ⅲ papillae, appeared on the surface of the hindbody (Fig. 26 & 27). Another type of sensory papillae, type Ⅳ, which was previously not observed (Seo *et al.*, 1984), was recognized in this study. This type of papillae was very similar to type Ⅰ but had more rough and bumpy dome and was similar to type Ⅲ in its nature of bulging from the tegument but differed in having a cilium. They were found on the tegument of the hindbody (Fig. 26 & 28). Type Ⅲ and Ⅳ papillae were more abundant around the excretory pore and genital atrium than on other portions of the hindbody.

DISCUSSION

The tegumental surface of *F. seoulensis* was covered with cobblestone-like cytoplasmic processes, spines and sensory papillae through all their developmental stages, but it differentiated more finely and became velvety in appearance by maturity. Such change was also observed in other kinds of trematodes, but detailed comparison of the changing pattern is difficult to perform because of different characteristics in their teguments. As to the reason for such tegumental change, it is regarded as a consequence to suffice the increase of nutriments required (Bennett *et* Threadgold 1975; Fujino *et al.* 1979; Font *et* Wittrock 1980; Lee *et al.* 1984). Especially for hindbody tegument, its differentiation was suggested to be indispensible for nutriment supply during organogenesis of genitalia (See *et al.* 1984; Lee *et al.* 1984).

Conversion of simple (single-pointed) tegumental spines into serrated ones was one of the most characteristic features during the developmental stages of *F. seoulensis.* It seems a unique feature in various kinds of trematodes; *D. spathaceum* (Russel-Smith *et* Wells 1982), *A. marcianae* (Shoop *et* Corkum 1984), *C. lingua* (Køie 1977), *M. yokogawai* (Lee *et al.* 1984) and *Fasciola hepatica* (Bennett 1975b). In the present study, the simple spines were observed in metacercariae and 1-day old juveniles, and they became serrated ones from the second day after infection. This finding agrees well with the suggestion by the above authors that the simple spines, which headed posteriorly, are convenient in migratory stage while serrated ones are useful for establishment and maintenance of their habitat or niche. In *F. seoulensis*, for example, the serrated spines were confined to the tegument of forebody in this study and host-parasite interface occurred between villus of the host intestine and forebody of the worm (Yoo 1985), so that the above suggestion seems to be more probable.

There was no great change in the contour and distribution of uniciliated type I papillae throughout the developmental stages and the majority of them were found around oral and ventral suckers. Grossly similar ones, type IV sensory papillae, were also recognized in this study. They could be differed from type I papillae in the morphological point that they had more bumpy dome. However, the domed tegument of sensory papillae is generally regarded as an extension of the surrounding tegument, so that it could be different by localities on the body tegument. Such minor difference in external morphology of domed tegument between type I and type IV papillae may not greatly affect their sensory function. The function of ciliated type I papillae has been suggested to be tango- and/or rheoreceptive, when their morphology and distribution in the various digenean trematodes were considered (Erasmus 1967; Morris *et* Threadgold 1967; Bennett 1975a; Fujino *et al.* 1979; Lee *et al.* 1984; Seo *et al.* 1984). On the other hand, Ip *et* Disser (1984) suggested that the ciliated sensillium might be either chemo- and/or mechanoreceptive because this type sensillium extended through the whole thickness of the tegument and had a free sensory cilium.

As in other trematodes such as *C. sinensis* (Fujino *et al.* 1979), *M. yokogawai* (Lee *et al.* 1984), the type II non-ciliated sensory papillae were confined to the lips of the oral and ventral suckers in *F. seoulensis.* From their location, arrangement and morphology, it is inferred that the type II papillae may have tango- and/or pressure-receptive function. The type III papillae, the simple swellings of the cobblestone-like tegumental processes without cilia, appeared only in the hindbody of *F. seoulensis.* They are suggested to be chemoreceptive in function and related to nutriment recognition from the luminal fluid in the host intestine.

The tribocytic organ was one of the most remarkably grown organs during the developmental period of *F. seoulensis.* It revealed stout recurved spines on the outer surface and much elaborative internal structures consisted of honeycomb-like lacunae. Similar reports have been made on the tribocytic organs of other strigeid trematodes; *Cyathocotyle bushiensis* (Erasmus 1967), *Diplostomum phoxini* (Erasmus 1975a & b) and *A. marcianae* (Shoop *et* corkum 1984). Their cytoplasmic processes were reported to be associated with a number of enzymes (Öhman 1965 & 1966; Johnson *et al.* 1971). In the present study, however, no types of sensory papillae to perceive the presence and kinds of nutriments were visible in this organ. Therefore, it is suggested that any other types of chemoreceptors may be present in the tegumental syncytium, because they are needed for the sensation of the chemical nature of the nutrients followed by secretion of suitable lytic enzymes. Thus, the function of tribocytic organ of *F. seoulensis* is suggested to be extracorporeal digestion of food materials, just like the intestinal villi and microtriches of cestodes (Lumsden 1966). The stout recurved spines on the tribocytic organ seem to have a function to anchor on the epithelial layers of intestinal villi and to cause lacerations there and crater formation, when the intestinal pathology in rats and mice due to infection with *F. seoulensis* (Yoo 1985) was considered.

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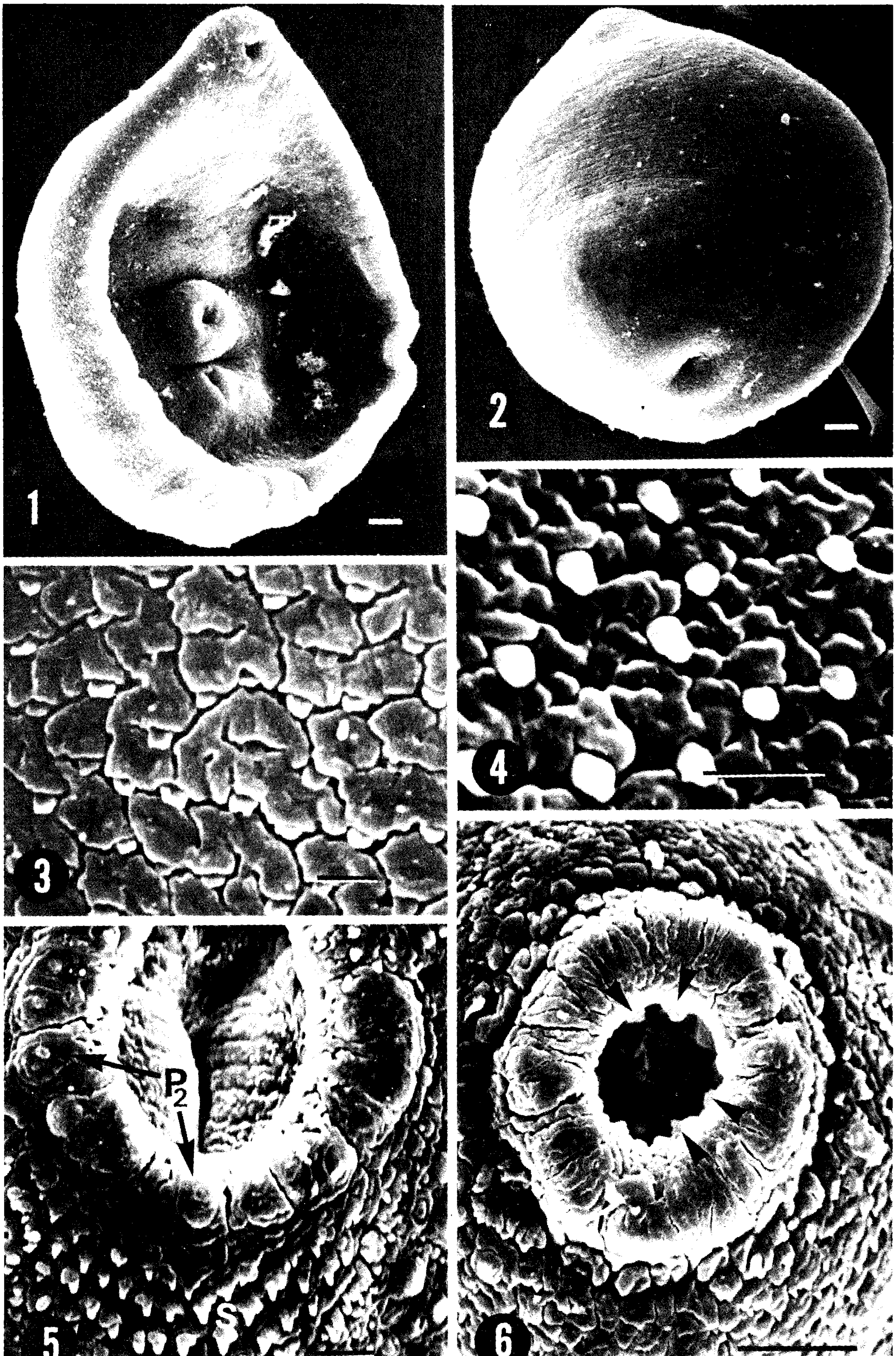
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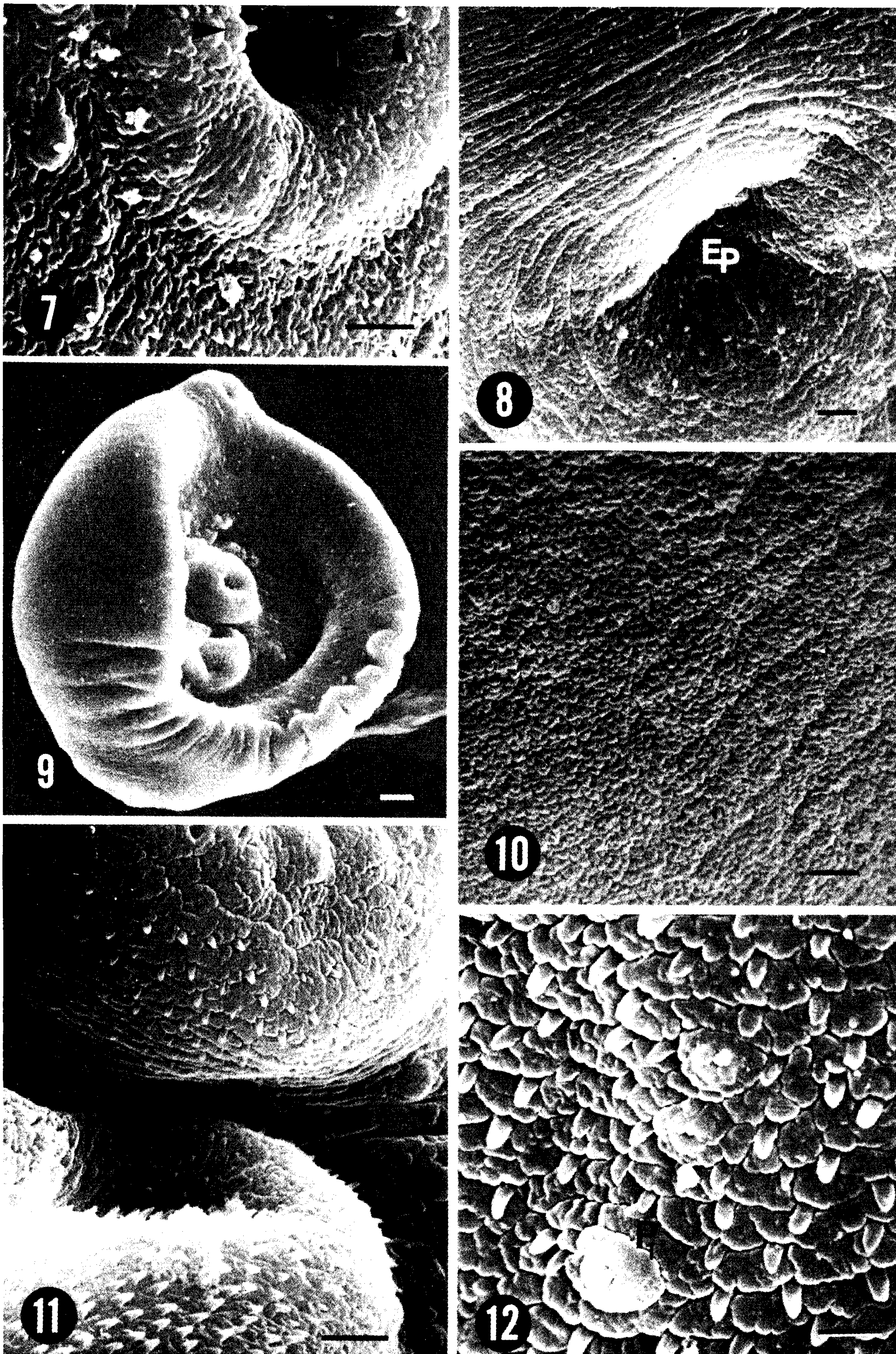
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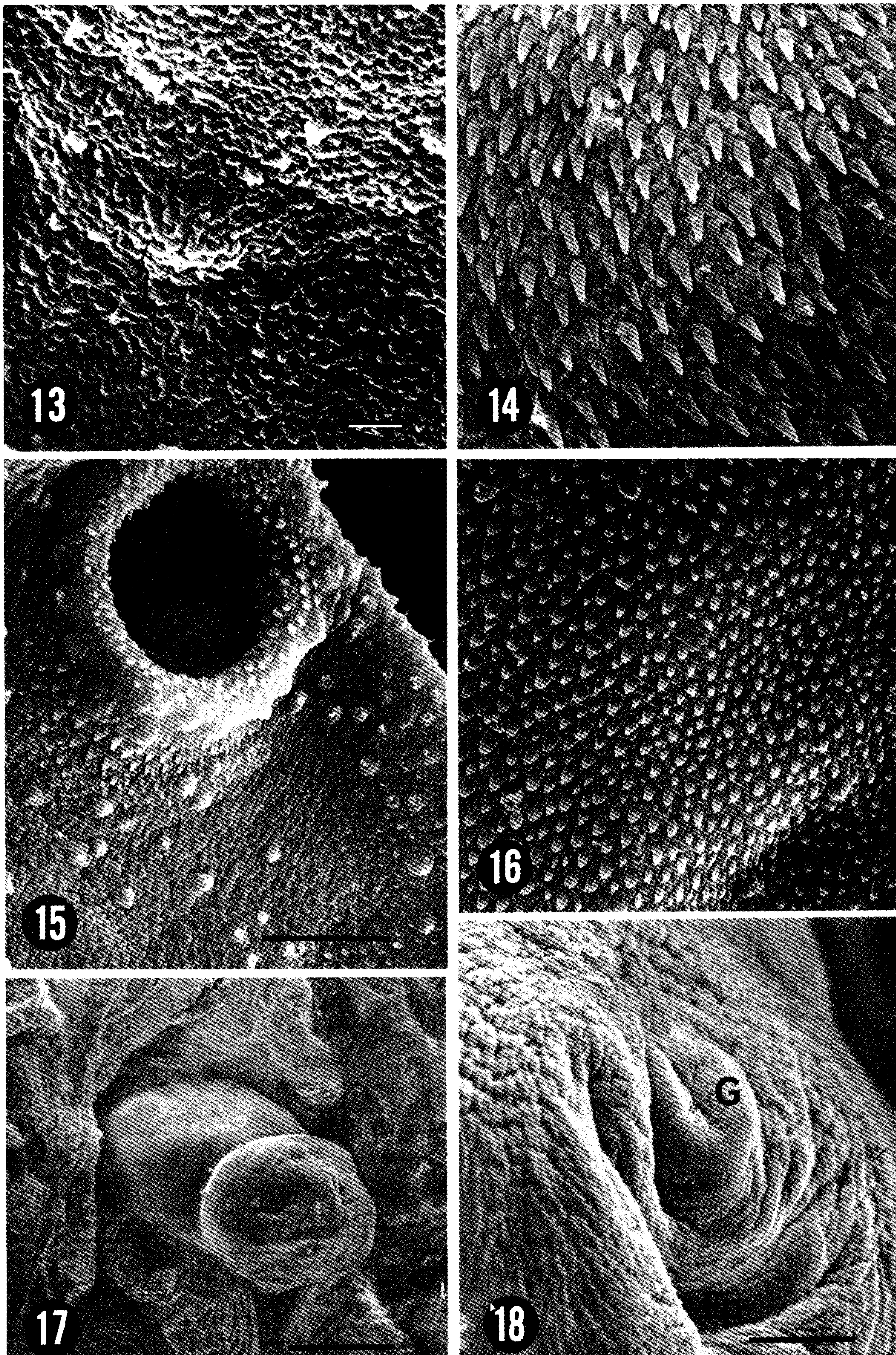
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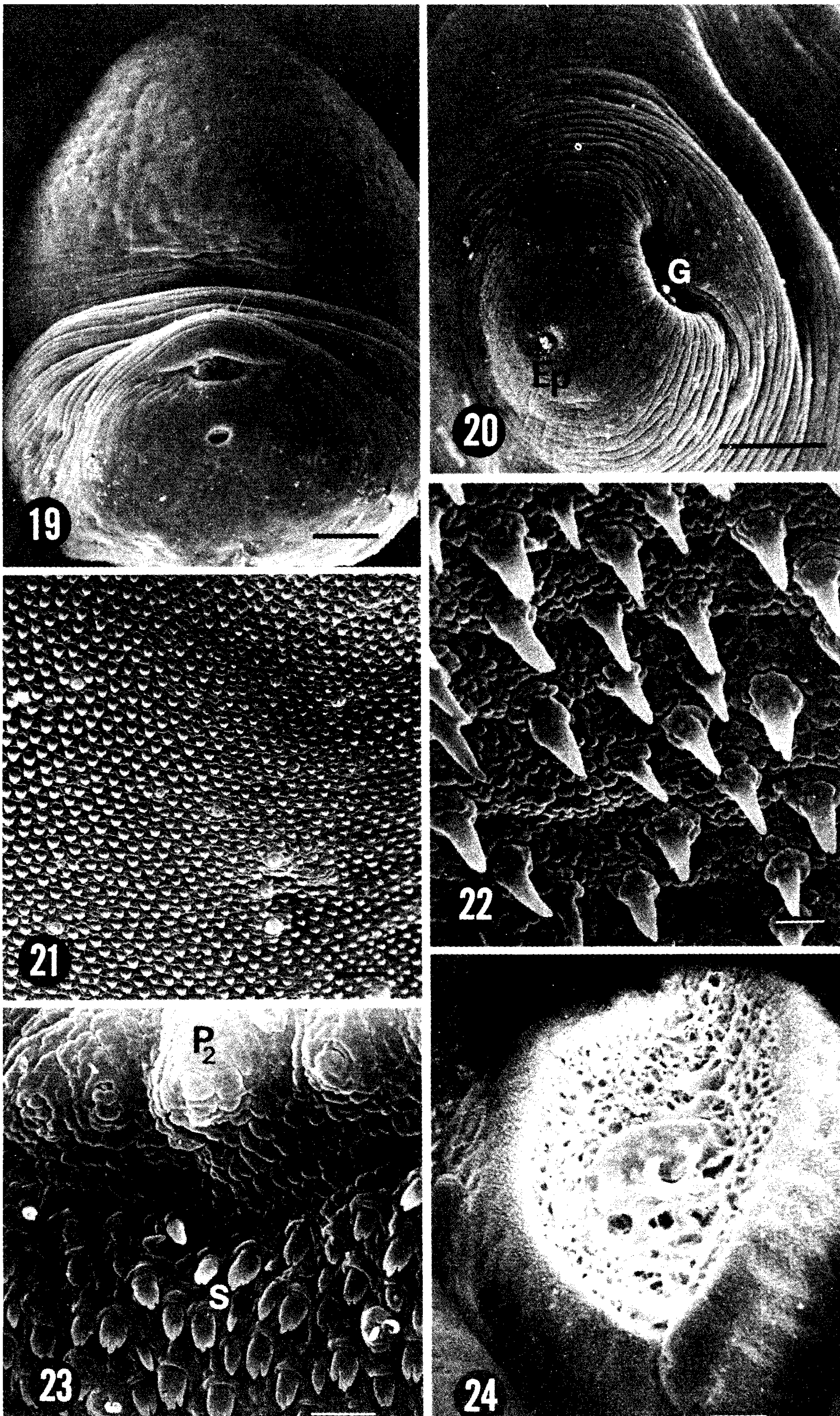
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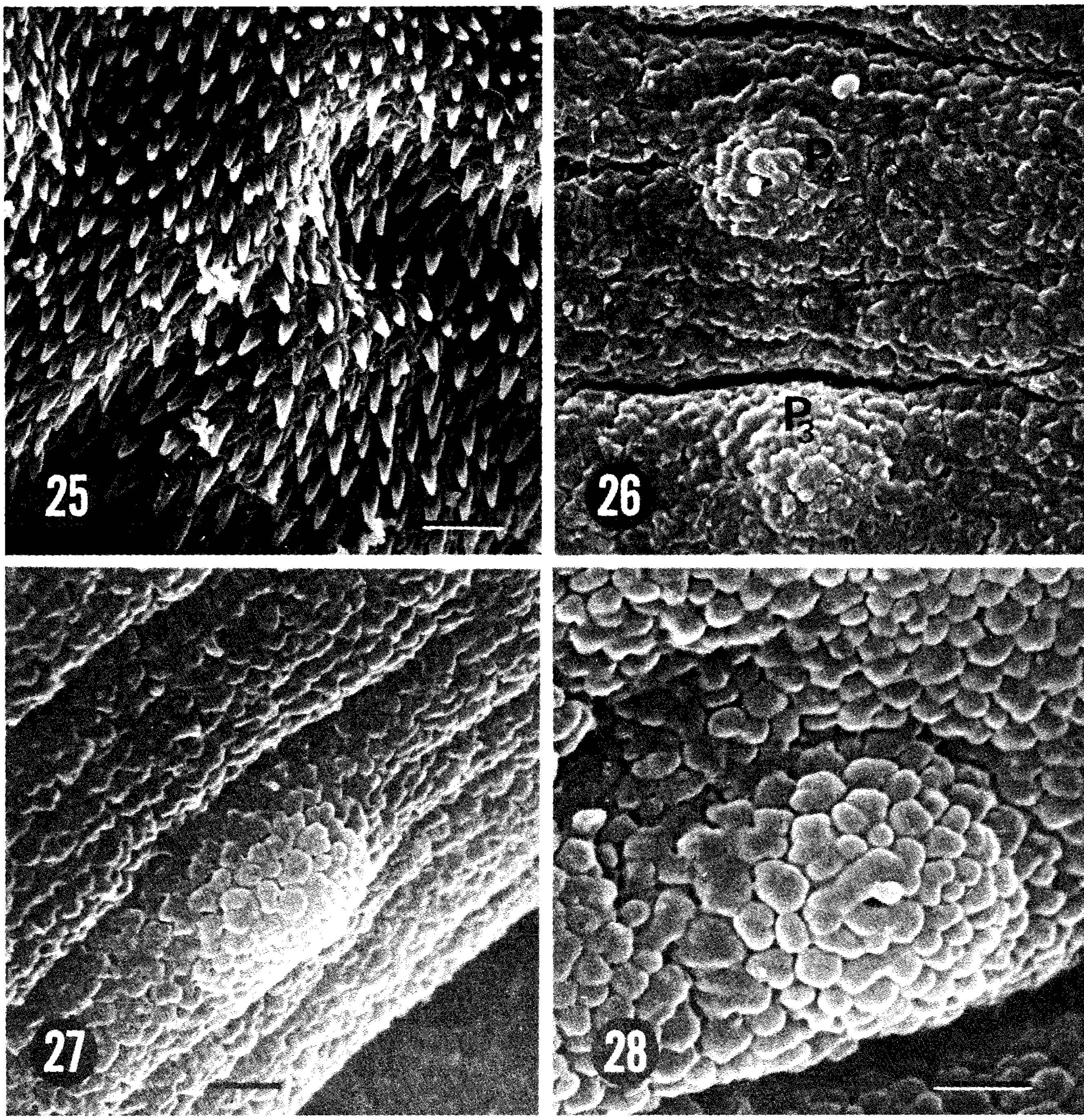
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EXPLANATIONS FOR FIGURES

Fig. 1-8. Tegument of metacercaria; SEM.

1. Ventral view. Bar=10 μm.
2. Dorsal view. Bar=10 μm.
3. Notched spines in the ventral tegument in the vicinity of the tribocytic organ. Bar=1 μm.
4. Single-pointed spines in the dorsal tegument. Bar=1 μm.
5. Oral sucker. Note type II papilla(P_2) on the lip and single-pointed spines(S) on the posterior surface and the funnel. Bar=2 μm.
6. Ventral sucker. Note type I papillae(arrow heads) on the margin of the funnel and type II papillae on the lip. Bar =5 μm.
7. Oral sucker. Note type I papillae(arrow heads) on the lip. Bar=2 μm.
8. The primordium of the hindbody with excretory pore(Ep), central. Bar=2 μm.

Fig. 9-12. Tegument of 1-day old diplostomulum; SEM.

9. Ventral view. Bar=10 μm.
10. Ventral tegument of the developing hindbody. Bar=1 μm.
11. Ventral sucker and tribocytic organ. Bar=2 μm.
12. Ventral tegument exhibits single-pointed spines and type I papilla(P_1). Bar=1 μm.

Fig. 13-16. Tegument of 2-day old diplostomulum; SEM.

13. The third type papilla(P) on the developing tegument of hindbody. Bar=1 μm.
14. Stout spines on the tegument of tribocytic organ. Bar=1 μm.
15. Oral sucker and anterior ventral tegument. Bar=10 μm4.
16. Anterior dorsal tegument with 2-3 pointed spines. Bar=2 μm.

Fig. 17-18. Scanning electron micrographs of 3-day old diplostomulum.

17. Diplostomulum attached to an intestinal villus of the albino rat. Bar=100 μm.
18. Genital atrium(G) and excretory pore(Ep). Bar=10 μm.

Fig. 19-28. Tegument of 8-day adult; SEM.

19. Dorsal view. Bar=50 μm.
20. Posterior end shows excretory pore(Ep), genital atrium(G) and papillae. Bar=50 μm.
21. Dorsoanterior tegument of the forebody. Bar=5 μm.
22. Dorsoposterior tegument of the forebody. Bar=1 μm.
23. Tegument of the ventral sucker. Note the serrated spines(S) and type II papilla(P_2). Bar=2 μm.
24. Tribocytic organ shows the honeycomb-like internal structure and spinous tegument. Bar=15 μm.
25. Stout spines on the outer surface of tribocytic organ. Bar=3 μm.
26. Type III(P_3) and type IV (P_4)papillae in the hindbody. Bar=2 μm.
27. Type III papilla on the tegument of the hindbody. Bar=1 μm.
28. Type IV papilla on the tegument of the hindbody. Bar=1 μm.

= 국문초록 =

한국의 장흡충에 관한 연구
XV. *Fibricola seoulensis* 표피 미세구조의 발육단계별 변화

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이순형 · 홍성종 · 채종일 · 서병설

이 연구는 *F. seoulensis*의 발육단계별 표피미세구조의 변화를 주사전자현미경으로 관찰하고자 실시하였다. 피낭유충은 유혈목이(*Natrix tigrina lateralis*)의 근육과 내장으로부터 분리수집하였다. 수집된 피낭유충의 일부분은 표피관찰용으로 고정하고, 나머지는 흰쥐에 약 200개씩 감염시킨 후 1, 2, 3 및 8일에 도살하여 십이지장으로부터 충체를 회수하였다. 수집된 충체는 PBS로 3번 씻고 2.5% glutaraldehyde에 고정한 후 ethanol로 탈수하고 냉동건조시킨 다음 순금으로 표면처리하여 한국 ISI社의 SS-60 및 DS-130 주사전자현미경으로 관찰하였다.

피낭유충은 복면이 함몰된 숫가락모양이었으며, tribocytic organ은 복흡반보다 작고 수축되었으며 그 후방에 접하고 있었다. 배설공은 충체후방에 돌출한 후반부원기의 중심에 개구하였다. 끝이 뾰족하고 납작한 가시가 충체전면을 덮고 있었다. 한개의 섬모를 가진 제 I 형 감각유두가 충체전면에 퍼져있었는데 특히 구 · 복흡반주위 및 충체외연에 밀집되어 있었다. 섬모가 없는 제 II 형 감각유두는 구 · 복흡반의 구순에 국한되어 있었다.

감염후 1일된 유약충의 표피구조는 피낭유충과 큰 차이를 보이지 않았으나 감염 2일후에는 tribocytic organ에 분포하는 가시를 제외한 전 체표에 분포하는 가시의 끝이 3~4개로 나뉘어졌다. 감염후 3일에는 후반부의 길이가 전반부의 1/3로 자랐으며 그 위에 제3의 감각유두원기가 나타났다.

감염후 8일된 성충은 전반부와 후반부의 크기가 거의 같아졌으며 표면은 융단모양이었다. Tribocytic organ의 내부구조는 봉소와 같았으며 외면의 송곳모양가시는 더욱 커졌다. 섬모가 없는 제 III 형과 섬모가 있는 제 IV 형 감각유두가 후반부에 나타났다.

끝이 분지된 가시는 숙주조직을 마멸하기에 효과적일 것으로 생각되며, 표피의 원형질막돌기는 흡수면적을 확대시키기 위해 융단모양으로 분화된 것으로 보인다. 섬모가 있는 I, IV형 감각유두는 촉-혹은 주류성수용기로, 섬모가 없는 II, III형 감각유두는 촉-혹은 화학수용기로 생각된다.