FOLIA PARASITOLOGICA (PRAHA) 30: 341–344, 1983.

SCANNING ELECTRON MICROSCOPY OF THE TREMATODE HASSTILESIA OVIS

Z. ŽĎÁRSKÁ, T. N. SOBOLEVA* and T. A. BAKKE**

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague, *Institute of Zoology, Academ y of Sciences of the Kazakh SSR, Alma-Ata, and **Zoological Museum of Oslo University, Oslo

Abstract. The trematode *Hasstilesia ovis* parasitic in sheep was studied by scanning electron microscopy. A single type of sensory papillae was found on the surface of oral and ventral suckers and around the genital pore. The whole surface of body, except the oral and ventral suckers and vicinity of genital pore, is covered with spines. The mostly multipointed and rarely singlepointed spines are of variable size and distribution on different parts of body. They irritate mechanically the intestinal mucosa of the definitive host and thus increase the pathogenic effect of the trematode.

This study is a part of complex investigations of *H. ovis*, which is a parasite of economic importance. Soboleva and Žďárská (1983) investigated the histochemistry of the species. The present study deals with the surface structures of the trematode as revealed by scanning electron microscopy (SEM) and aims to provide further information concerning the structures enabling the fixation of these minute (1 mm on an average) trematodes among the villi of small intestine, where they occur in large numbers (10–20 thousands).

MATERIAL AND METHODS

The trematodes were recovered from small intestine of sheep in the vicinity of Alma-Ata (USSR). They were washed in saline and fixed in 3 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 °C for minimally 2 hours or in Baker's fluid (Pearse 1968). Then they were washed in 0.1 M cacodylate buffer or distilled water and dehydrated through an alcohol series. The dehydrated trematodes were then critical-point dried (Anderson 1951), mounted on metal specimen stubs and coated with carbon and gold and examined in a Joel JSM-35 c scanning electron microscope operating at 15 kV.

RESULTS

The spines cover the entire ventral and dorsal sides of body. They are absent only in the region of oral (Plate I, Figs, 1, 2) and ventral suckers and around the genital pore (Plate III, Figs. 1, 2) situated halfway between the ventral sucker and excretory pore. The spines on both ventral and dorsal sides are in general irregularly distributed, however, at some places a chess-board arrangement occurs. The shape of spines varies from smooth, singlepointed (Plate II, Fig. 2, Plate IV, Figs. 1, 2) to wide, ribbed, multipointed ones, the distal part of which forms numerous pointed tips (Plate I, Fig. 3, Plate II, Figs. 1, 2). The width of some multipointed spines is twice the single ones and they resemble doubled spines (see Plate I, Fig. 3; Plate II, Fig. 1). In the close vicinity of the spineless regions around the suckers and genital pore, the spines are predominantly singlepointed and small. With increasing distance from these

regions the spines gradually become larger and turn to multipointed (Plate I, Fig. 1, Plate III, Fig. 2). Anterodorsally (Plate II, Fig. 1) the spines are more densely and more regularly distributed than posterodorsally (Plate II, Fig. 2), where they are not only irregularly distributed, but also of irregular shape. Anteroventrally (Plate IV, Fig. 1) the spines are larger than posteroventrally (Plate IV, Fig. 2). Generally the singlepointed spines are scarcely distributed on the whole armed body surface (Plate II, Fig. 2, Plate IV, Figs. 1, 2).

The sensory papillae inside the oral sucker are arranged in 3 circles (Plate I, Figs. 1, 2), the first from the inside consists of 12 large papillae, the second of 8 large papillae and the third of 36 smaller papillae (arrangement is demonstrated in Plate I, Figs. 1, 2). Also the ventral sucker is provided with large sensory papillae. All papillae are smooth, without cilia and openings.

A larger number of irregularly arranged sensory papillae (Plate III, Figs. 1, 2) are situated around the genital pore. The surface of evaginated cirrus is folded and long sperms often protrude from its duct (Plate III, Figs. 1, 2).

DISCUSSION

The distribution of spines in *H. ovis* differs from that in *H. tricolor* parasitizing rabbits, the surface structures of which were studied by SEM by Crites and Jilek (1981). In *H. ovis*, the whole ventral and dorsal sides up to the excretory pore are covered with spines, whereas in *H. tricolor*, the spines on posterior part of body, on both ventral and dorsal sides, are absent. The shape of spines of both trematodes is almost even. The basic types are pyramidal, singlepointed spines, and flat, multipointed spines. A comparison with the spines of other members of the superfamily Brachylaimoidea — *Leucochloridium* species and *Urogonimus macrostomus*, studied by SEM by Bakke (1976a, b, 1977, 1978, 1980, 1981, 1982), revealed that the morphology of multipointed spines of *H. ovis* is somewhat different. In the above species the rays of multipointed spines are more distinct and the points are longer. The singlepointed spines in *H. ovis* can be either spines where the development has ceased, or spines retarded in development. Their shape conforms to spines of a 10-day-old *Fasciola hepatica* (Bennett 1975 a). In this species the multipointed spines develop in older (2 and more weeks old) specimens (Bennett 1975b).

According to Crites and Jilek (1981) the trematodes *H. tricolor* are fixed between the microvilli of intestinal cells by spines projecting posteriorly and penetrating deep into the mucosa. The suckers are probably used by the parasite only for moving to its definitive location, but the fixation is performed by the spines. The anterior end of body of the trematode directed into the crypts is entirely buried in the intestinal mucosa, whereas the posterior part protrudes freely into the intestinal lumen. The morphology of spines suggests that the mechanical irritation of intestinal mucosa of the definitive host by the large number of sharp spines may be one of the factors increasing the pathogenic effect of the trematode as described by Vsevolodov and Soboleva (1981).

The differences between *H. ovis* and *H. tricolor* in the region of genital pore can be hardly assessed on the basis of the paper by Crites and Jilek (1981), since the genital pore is contracted in the photographs published by these authors and neither papillae nor cirrus are visible. The surface structure of the cirrus of *H. ovis* seems to be identical with that of members of Brachylaimoidea (Bakke 1976a, 1982) and of other trematodes (Page et al. 1980).

The shape and basic arrangement of sensory papillae on the oral sucker of *H. ovis* resembles that of *H. tricolor*. The papillae are arranged in 3 circles corresponding to the basic pattern described by Bayssade-Dufour (1974) in the cercaria of Brachylaimoidea. While comparing the shape of sensory papillae in the oral sucker of *H. ovis* with other species of Brachylaimoidea — *Brachylaimus aequans* (Žďárská and Soboleva 1980) it was found that, in contrast to *B. aequans*, only one type of papillae developed in the adults of *H. ovis*, namely papillae without processes. The papillae with finger-like process and papillae with opening, occurring in the metacercariae of *B. aequans*, were not found in *H. ovis*. Also the absence of somatic dorsal and ventral papillae in *H. ovis* corresponds to the basic type of argyrophilic surface structures of Brachylaimoidea established by Bayssade-Dufour (1974). The shape of papillae in the oral and ventral suckers in *H. ovis* is identical with that observed in *Leucochloridium* species and *Urogonimus macrostomus* (Bakke 1976a, b, 1978, 1980, 1982), *Phyllodistomum conostomum* (Bakke and Lien 1978) and *Ribeiroia marini* (Page et al. 1980). The papillae in *H. ovis* marita seem to correspond to the unciliated sensory receptors revealed by the transmission electron microscope by Žďárská and Soboleva (1982) in the metacercaria of this trematode, to the contact receptors without cilia observed by Bennett (1957a) in *F. hepatica*, or to the non-ciliated papillae described by Fujino et al. (1979) in the trematode *Clonorchis sinensis*. The three above-mentioned types of papillae contain inside sensory receptors not communicating with the surface of tegument.

Acknowledgement. We would like to thank Dr. T. Barnard of the Electron Microscope Unit for Biological Sciences, University of Oslo for the SEM facilities and we also greatly appreciate the technical assistance given by Mrs E. Braaten at the electron microscopical unit.

СКАНИРУЮЩАЯ ЭЛЕКТРОННАЯ МИКРОСКОПИЯ ТРЕМАТОДЫ *HASSTILESIA OVIS*

З. Ждярска, Т. Н. Соболева и Т. А. Бакке

Резюме. При помощи сканирующей электронной микроскопии изучали трематоду *Hasstilesia ovis*, паразитирующую у овец. На поверхности ротовой и брюшной присосок и в окрестности полового отверстия обнаружен только один тип сенсорных папилл. Целая поверхность тела, кроме ротовой и брюшной присосок и окрестности полового отверстия, покрыта шипиками. Размер, форма и распространение шипиков разные на разных частях тела. Большей частью встречаются многокончиковые и редко одноконичковые шипики. Эти шипики механически раздражают слизистую кишки окончательного хозяина и таким образом увеличают патогенное действие трематоды.

REFERENCES

ANDERSON T. F.. Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope. Trans. N. Y. Acad. Sci. 13: 130—134, 1951.

BAKKE T. A., Shape, size and surface topography of genital organs of *Leucochloridium* sp. (Digenea) revealed by light and scanning electron microscopy. Z. Parasitenkd. 51: 99—113, 1976a.

—, Functional morphology and surface topography of *Leucochloridium* sp. (Digenea), revealed by scanning electron microscopy. Z. Parasitenkd. 51: 115—128, 1976b.

—, Shape, size and surface topography of genital organs of *Urogonimus macrostomus* (Rudolphi, 1803) Monticelli, 1888 (Digenea), revealed by light and scanning electron microscopy. Z. Parasitenkd. 54: 299—307, 1977.

—, *Urogonimus macrostomus* (Rudolphi, 1803) (Digenea): its taxonomy and morphology as revealed by light and scanning electron microscopy. Can. J. Zool 56: 2280—2291, 1978.

—, A revision of the family Leucochloridiidae Poche (Digenea) and studies on the morphology of *Leucochloridium paradoxum* Carus, 1835. Syst. Parasitol. 1: 189—202, 1980.

—, Systematics and biology of Leucochloridiidae Poche (Platyhelminthes; Digenea). Dissertation paper, University of Oslo, 188 pp., 1981.

—, The morphology and taxonomy of *Leuco-*

chloridium (*L.*) *variae* McIntosh (Digenea, Leucochloridiidae) from the Neartic as revealed by light and scanning electron microscopy. Zool. Scripta 11: 87—100, 1982.
—, LIEN L., The tegumental surface of *Phyllodistomum conostomum* (Olson 1876) (Digenea) revealed by scanning electron microscopy. Int. J. Parasitol. 8: 155—161, 1978.
BAYSSADE-DUFOUR C., Chétotaxie d'une cercaire de Brachylaimoidea (Trematoda). Bull. Mus. nat. Hist. nat. Paris, 3e sér., no 204, Zoologie 134: 67—71, 1974.
BENNETT C. E., Surface features, sensory structures, and movement of the newly excysted juvenile *Fasciola hepatica*. J. Parasitol. 61: 886—891, 1975a.
—, Scanning electron microscopy of *Fasciola hepatica* L. during growth and maturation in the mouse. J. Parasitol. 61: 892—898, 1975b.
CRITES J. L., JILEK R., Surface topography of *Hasstilesia tricolor* (Trematoda: Brachylaemidae) as demonstrated by scanning electron microscopy. Ohio J. Sci. 81: 120 to 124, 1981.
FUJINO T., ISHII Y., CHOI D. W., Surface ultrastructure of the tegument of *Clonorchis sinensis* newly excysted juveniles and adult worms. J. Parasitol. 65: 579—590. 1979.
PAGE M. R., NADAKAVUKAREN M. J., HUIZINGA H. W., *Ribeiroia marini* surface ultrastructure of redia, cercaria and adult. Int. J. Parasitol. 10: 5—12, 1980.
PEARSE A. G. E., Histochemistry theoretical and applied. Vol. 1, 2. Third edition. Churchill Ltd., London, 1518 pp., 1968.
SOBOLEVA T. N., ŽĎÁRSKÁ Z., Histochemistry of *Hasstilesia ovis* (Trematoda: Hasstilesiidae). Folia parasit. (Praha) 30: 141—145, 1983.
VSEVOLODOV B. P., SOBOLEVA T. N., Morpho-biological peculiarities of *Hasstilesia ovis* (Trematoda: Brachylaimoidea) and patho-morphological changes caused thereby in the guts of sheep. Parazitologiya 15: 407—415, 1981. (In Russian.)
ŽĎÁRSKÁ Z., SOBOLEVA T. N., Scanning electron microscopy of the cercaria and metacercaria of *Brachylaimus aequans* (Looss 1899). Folia parasit. (Praha) 27: 127—130, 1980.
—, —, Ultrastructure of the tegument of *Hasstilesia ovis* larval stages. Folia parasit. (Praha) 29: 367—370, 1982.

Received 26. January 1983.
Translated by: M. Dašková

Z. Ž., Parasitologický ústav ČSAV,
Flemingovo n. 2, 166 32 Praha 6,
ČSSR

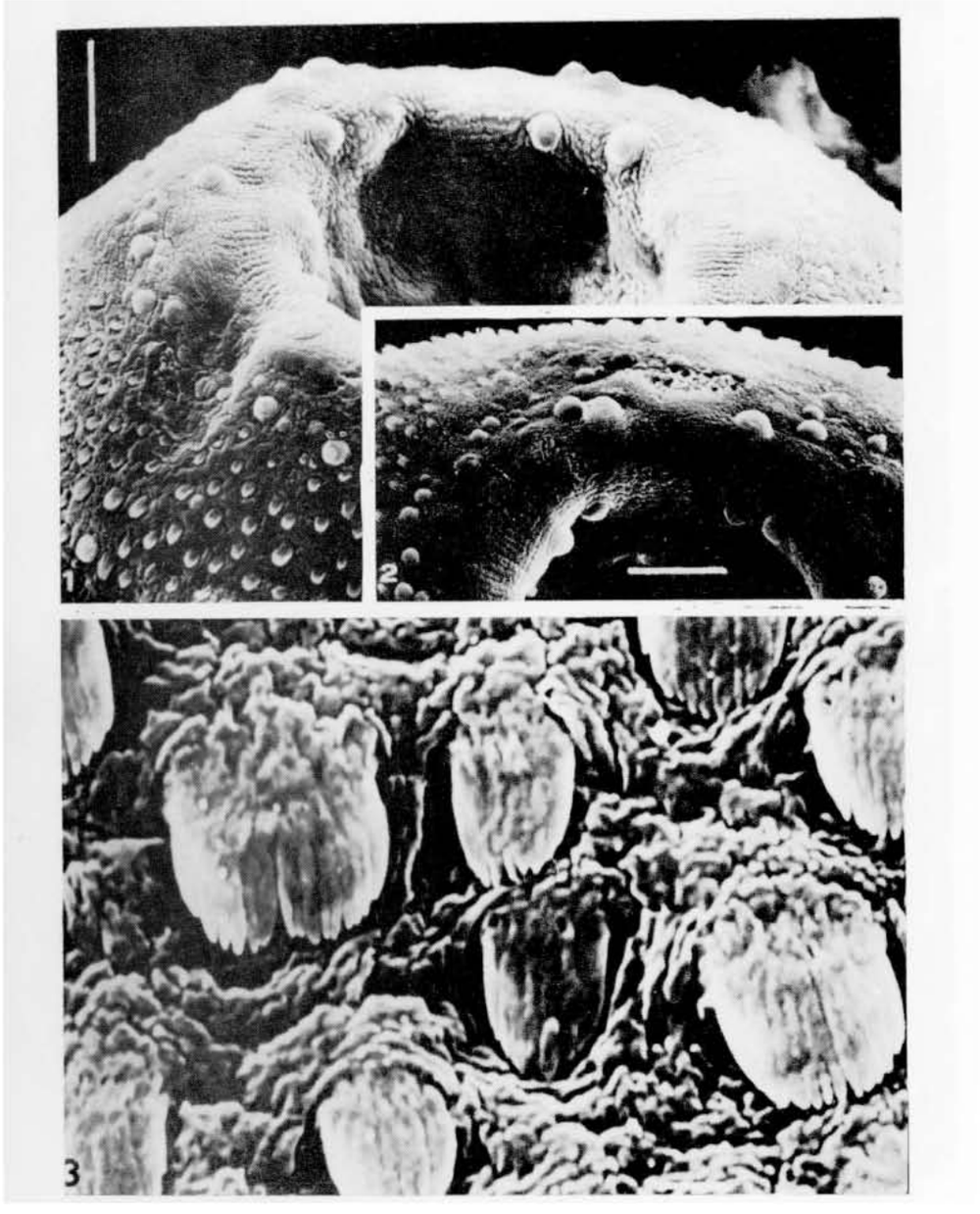

Fig. 1. Oral sucker of *H. ovis* with typically distributed sensory papillae and spines (vetral view). (Scale bar 0.01 mm.) **Fig. 2.** The same as in Fig. 1 in frontal view. (Scale bar 0.01 mm.) **Fig. 3.** Detail of multipointed spines from anterior part of dorsal side of body. Note the difference in the width of individual spines. (Scale bar 0.001 mm.)

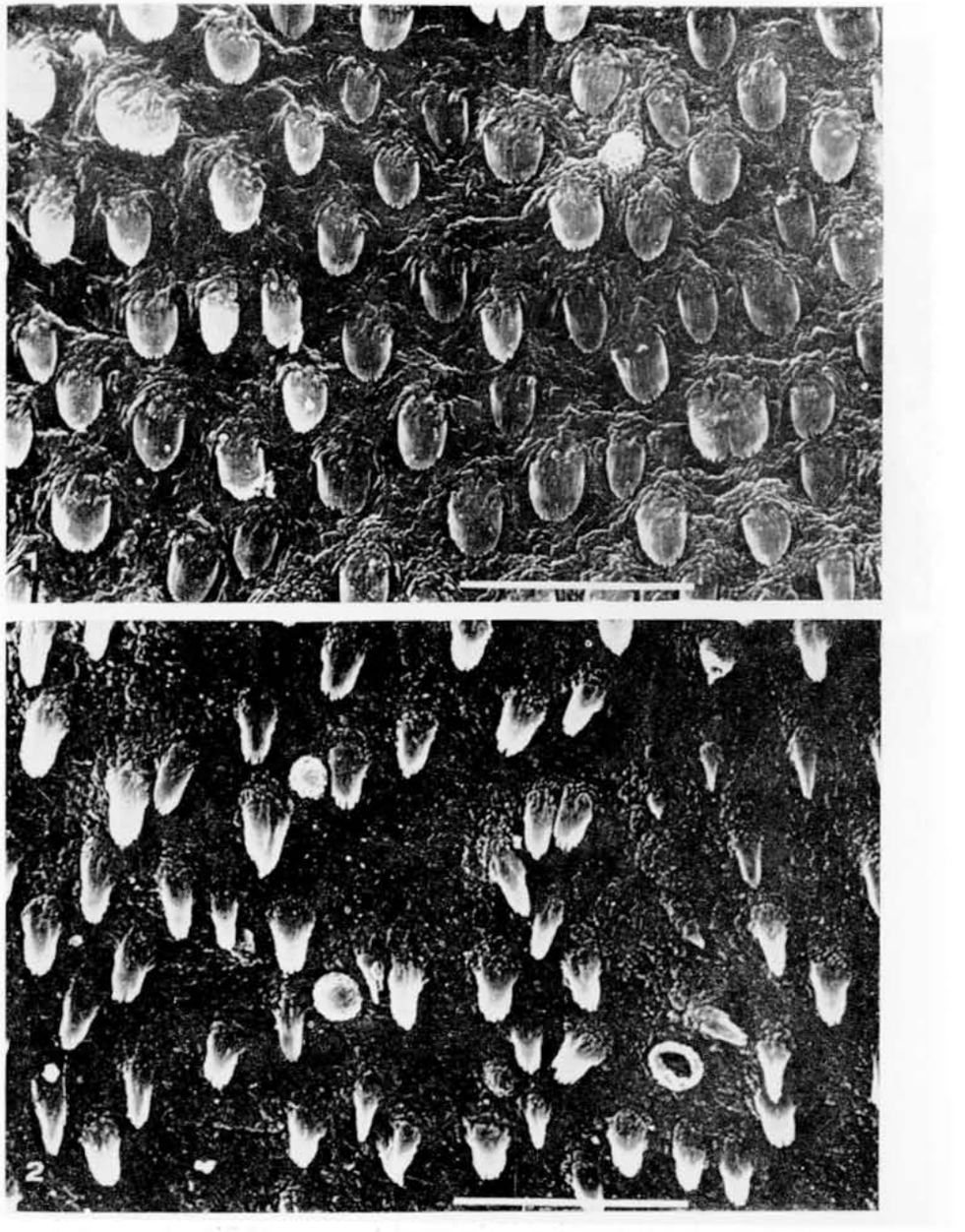

Fig. 1. Shape and distribution of spines on anterior part of dorsal side of body in *H. ovis* (see Plate I, Fig. 3 for details). (Scale bar 0.01 mm.) **Fig. 2.** The same on posterior part of dorsal side of body. (Scale bar 0.01 mm.)

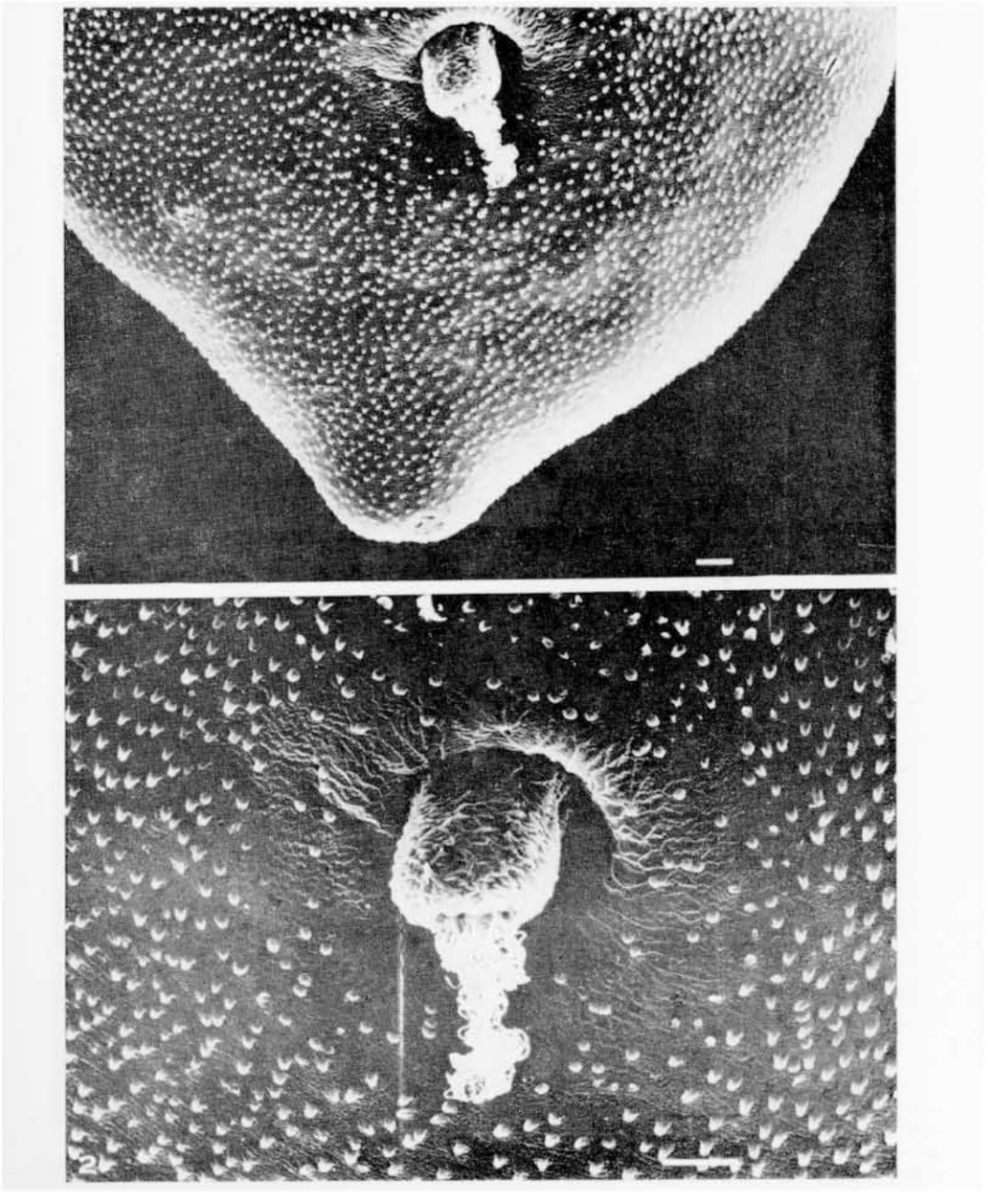

Fig. 1. Distribution of spines on posterior part of ventral side of body behind genital pore in *H. ovis*. (Scale bar 0.01 mm.) **Fig. 2.** Genital pore with cirrus (detail from Fig. 1). Note the distribution of papillae around genital pore, surface structure of cirrus and sperms protruding from it. (Scale bar 0.01 mm.)

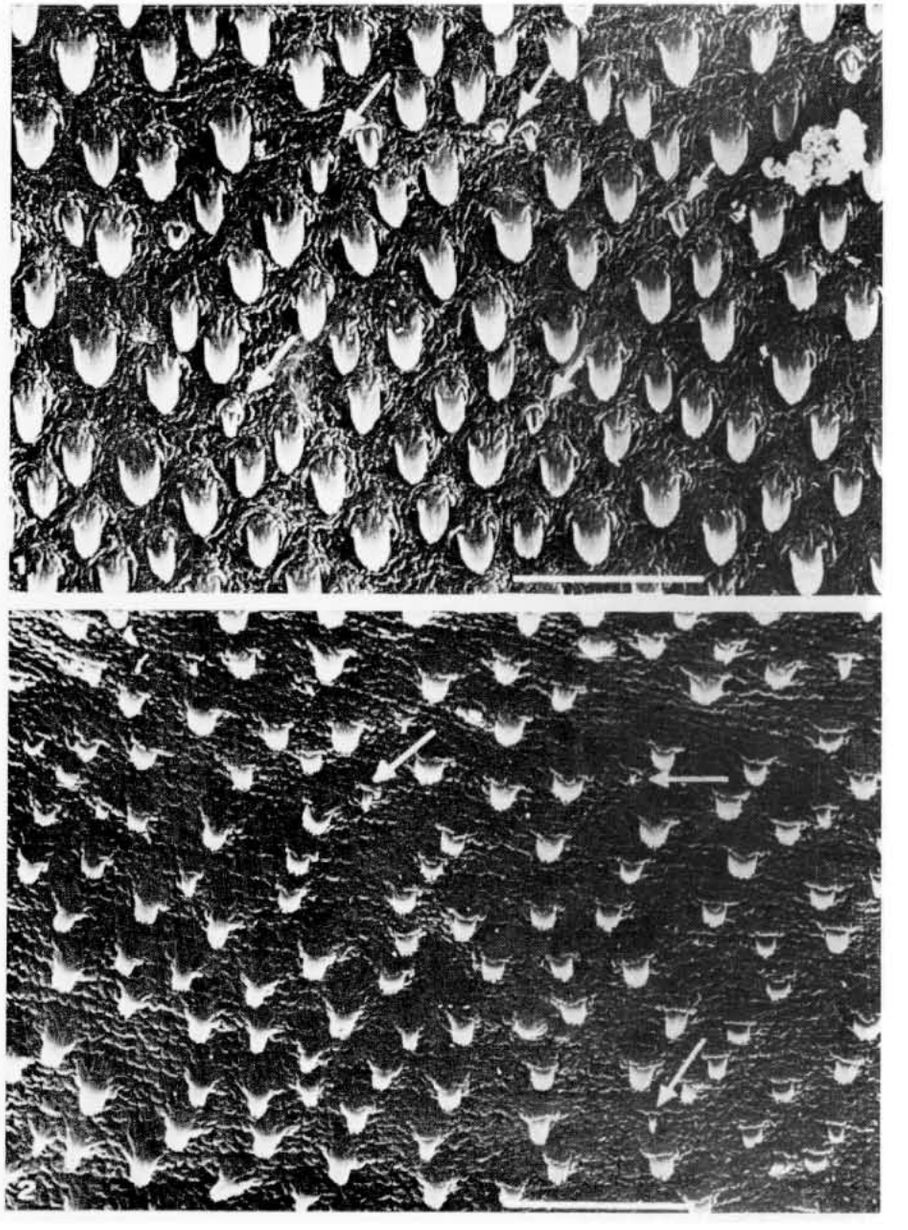

Fig. 1. Shape and distribution of spines on ventral side of body near ventral sucker in *H. ovis*. Note the singlepointed spines (arrows). (Scale bar 0.01 mm.) **Fig. 2.** The same behind genital pore. Note singlepointed spines (arrows) among the multipointed spines. (Scale bar 0.01 mm.)