

SCANNING ELECTRON MICROSCOPY OF CERCARIA AND ADULT OF OPISTHIOGLYPHE LOCELLUS KOSSACK, 1910 (TREMATODA: PLAGIORCHIIDAE)

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Dedicated to Dr. J. Prokopič, Corresponding Member of the CAS, on the occasion of his 60th birthday

Abstract. Surface structures of cercaria and adult of *Opisthioglyphe locellus* Kossack, 1910 parasitic in insectivores were studied by scanning electron microscopy. The surface of cercaria was covered with tegumentary spines, which were found also in the caudal pocket and on the tegument covering both suckers. Three types of papillae were distinguished in the cercaria: papillae without cilium, with short cilium, and with long cilium. A fold resembling the fin fold was visible on the tail of cercaria. The surface of the adult was also covered with tegumentary spines, the size and number of which gradually decreased from anterior to posterior part of body. Four types of papillae were observed: small and large dome-shaped papillae without cilium, papillae with empty middle depression, and conical papillae with cilium.

Cercariae fully conforming in their measurements, morphology and chaetotaxy to the description of *Opisthioglyphe locellus* Kossack, 1910 published by Bock (1982, 1983) were found in *Planorbarius corneus* in the vicinity of České Budějovice (150 km south of Prague, Czechoslovakia). The metacercariae were used for experimental infection of hamsters and the obtained adult trematodes were determined as the above species.

O. locellus was recorded in Czechoslovakia by Prokopič (1957, 1959) in *Neomys anomalus* and by Prokopič and Genov (1974) in *Sorex araneus* and *S. minutus*. However, this species has been reported more frequently from the neighbouring countries: the USSR (Shaldybin 1953, Morozov 1958, Merkusheva 1969), Poland (Żarnowski 1960), Hungary (Edelényi 1966), Germany (Stammer 1955, Bock 1982), and Bulgaria (Genov and Dimitrova 1966, Prokopič and Genov 1974, Genov 1979). Genov (1979) found it repeatedly in humid biotopes at a high intensity (up to 1 480 specimens). On the basis of comparative studies of a large number of specimens the author concluded that only *O. locellus* Kossack, 1910 parasitizes *Crocidura*, *Sorex* and *Neomys* in the Holarctic region. This species is very variable and has been synonymized with other six species recorded from insectivores by different authors.

The life cycle of *O. locellus* was studied by Macy and Moore (1958) and Bock (1982). Bock (1983) studied also its chaetotaxy.

This paper contributes to the knowledge of *O. locellus* by the scanning electron microscopic (SEM) study of the surface structures of cercaria and adult.

MATERIAL AND METHODS

The source of cercariae were spontaneously infected molluscs, *Planorbarius corneus*, collected at the periphery of České Budějovice in a canal uniting the ponds. In the laboratory, the molluscs were separated and put into flasks. The spontaneously released cercariae were collected and processed for SEM studies as described in a previous paper (Bušta 1985). They were used for experimental infection of 3rd instar larvae of *Culex pipiens molestus*, 4th instar larvae of *Eusimulium securiforme*, *Simulium noelleri* and *Odagmia ornata*, and molluscs *Lymnaea stagnalis*, *Radix peregra peregra*

and *Planorbarius corneus* (all originating from laboratory breeding). The larvae of insects and molluscs were then kept at laboratory temperature. An undefined number of 20-day-old metacercariae from snails were fed to 2 hamsters. On day 21 p.i. the hamsters were killed and 92 adult trematodes were recovered from them. Some of the trematodes were fixed for SEM studies using the same method as that used for the cercariae, and the remaining ones were stained by borax carmine for the determination.

The mounts were examined in a TESLA BS-300 scanning electron microscope operating at 15 kW.

RESULTS

Cercaria. The shape of body, situation of suckers and stylet, and arrangement of tegumentary spines and papillae are shown in Plates I-II. The majority of studied cercariae have the ventral side concave and the dorsal one convex. The stylet is situated terminally, the oral sucker subterminally. The tegument of the whole body is covered with fine tegumentary spines (Plate I, Figs. 1-4). They are orientated backwardly, only at the periphery of the ventral sucker they are orientated obliquely towards the end of body, and exceptionally some of the spines are horizontal (Plate I, Fig. 4). The spines are arranged in regular horizontal and transverse rows. Around the stylet opening (up to 15 μm) the spines are lacking or they are completely sunken in the tegument and do not protrude above it. The length of cuticular spines (except those around acetabulum and caudal pocket) is 1.2-1.7 μm . On the dorsal side of body, immediately behind the stylet (Plate I, Fig. 2), they are 1.2-1.3 μm long. Cuticular spines of the same length are also on the lateral and ventral sides, at the same distance from the stylet. On the dorsal side of body (Plate II, Fig. 3) at the level of first preacetabular zone of papillae (position A₁D after Richard's (1971) nomenclature) they are 1.5-1.7 μm long, and near the caudal pocket (Plate II, Fig. 1) they measure 1.4-1.5 μm . The spines on the margin of acetabulum which slightly protrude above the body surface, are 2.3-2.8 μm long (Plate I, Fig. 4). The figure shows well the situation of large spines on the tegument covering the acetabulum. The spines inside the caudal pocket (Plate II, Figs. 1,2) are 2.2-2.5 μm long, i.e., they are approximately twice as long as the cuticular spines on the surface of cercaria. They are also much thicker at the base (by 0.2-0.3 μm).

The sensory papillae are arranged in two ventral (Plate I, Fig. 1), two dorsal and two lateral rows on the body surface. However, they are most numerous on the head part in the vicinity of stylet and oral sucker. It is apparent from the detail of the apical part (Plate I, Fig. 3) that the papillae without cilium (small white arrow), with short cilium (black arrow) and with long cilium (large white arrow) may occur close to one another in a small area. The cilia of lateral papillae (Plate I, Fig. 1) are 7.5-9.3 μm long, at some sites of the head part they measure up to 8 μm (Plate I, Fig. 3). The cilia of dorsolateral papillae (Plate I, Fig. 2-black arrow) measure 1.5-3.5 μm . The cilia of four of the nine acetabular papillae shown in Plate I, Fig. 4 (white arrow) measure only 1.5 μm ; consequently, the papillae belong to the type with short cilium. The papillae in the preacetabular zone (Plate II, Fig. 3) possess cilia measuring 11 μm in maximum length. The longest cilia (13 μm) were found on the dorsal side immediately behind acetabulum (Plate II, Fig. 5). The papillae with short cilium (up to 2 μm) are situated mediodorsally, in the middle of the tail length (Plate II, Fig. 4). The longitudinal folds of the cuticle on the dorsal and ventral side of tail resemble the fin fold observed in other groups of cercariae.

Adult. The shape and size of body and situation of suckers, cuticular spines and papillae are shown in Plates III and IV. The anterior end of body is widely rounded. The oral sucker is situated subterminally and the ventral one approximately at the end of the first third of body (Plate III, Fig. 1). The cirrus opening is in front of the ventral sucker,

but it is closed by a cuticular fold in this figure. Almost the whole surface of the adult trematode is covered with tegumentary spines orientated backwardly. Their arrangement, size and shape are shown in Plate III, Figs. 1-4 and Plate IV, Figs. 1 and 5. The first row of spines on the ventral side begins immediately behind the margin of oral sucker (Plate IV, Fig. 1). On the dorsal side, it is more distant from the sucker margin, behind a space filled with numerous papillae. The cuticular spines situated at the end of the first third of body are 4.2-4.7 μm long (Plate III, Fig. 2) and 2.8-3.3 μm wide. They are arranged in horizontal and transverse rows. The spines in posterior fifth of body (Plate III, Fig. 4) are arranged in the same way, but they are smaller, 3.5-4.0 μm long and 3.0-3.5 μm wide. By contrast with the spines in the first third of body, they are more bluntly ended and the distance between individual spines is greater, particularly in the transverse rows. On the ventral side, only a small middle part of body and the immediate vicinity of the excretory system opening is without spines (Plate III, Fig. 3). The lateral part of body end and dorsal surface, however, are covered with small spines.

Sensory papillae are situated in the vicinity of both suckers and between the spines of the tegument (Plate IV, Figs. 1, 2, 4, 5). Like in the cercaria, they are most frequent in the vicinity of the oral sucker. The first outer circle of papillae consists usually of 12-14 large (base width 2.8-3.4 μm) and 5-7 small (base width about 1.5 μm) dome-shaped papillae without cilium. The papillae are sometimes arranged asymmetrically (Plate IV, Fig. 1).

Six papillae symmetrically arranged in one inner circle are situated inside the oral sucker. Only one of them (lateral) is visible in Plate IV, Fig. 1 (small white arrow). Due to their situation inside the sucker, it is impossible to see them all at once in the frontal view. A larger number of papillae of the first type (dome-shaped, base width 2.5-4.0 μm) are symmetrically distributed dorsally to the first outer circle of papillae. Between them, there are smaller papillae with empty middle depression (base width up to 2 μm -black arrow) and laterally also conical papillae with short central cilium (large white arrow). The acetabulum (Plate IV, Fig. 2) bears an inner circle of 9 and outer circle of 6 dome-shaped papillae.

From the oral sucker up to the level of midlength of body the papillae are arranged in two lateroventral bands (Plate III, Fig. 1); in the second half of body they are distributed irregularly. A dome-shaped papilla with short cilium (Plate III, Fig. 2). 2.6 μm high and 3.3 μm wide at base, is situated between the spines, ventrolaterally in the first third of body. The last papilla of the lateroventral complex is shown in Plate IV, Fig. 5. It has a short cilium and its base is about 3 μm wide. Longitudinal rows of papillae were not observed on the dorsal surface of body. The papillae are distributed irregularly and both conical papillae with cilium and dome-shaped papillae without cilium were found on the dorsal and ventral surface. Some of the trematodes possessed an evaginated cirrus (Plate IV, Fig. 3) expelling spermiae from its opening.

DISCUSSION

The surface structures of trematode developmental stages have been studied by many authors (Lo et al. 1975, Edwards et al. 1977, K  ie 1977,   d  rsk   1980, Hoole and Mitchell 1981,   d  rsk   et al. 1983, Fried and Fujino 1984 and others).

This study is the fourth of the papers devoted to surface structures of developmental stages of Plagiorchiidae and published within a short period (Oliver et al. 1984, Bu  ta 1985, Bu  ta and Na  incov  , in press).

The general metrical and morphological characterization (including chaetotaxy) of the cercariae studied by us fully corresponds to the data published by Bock (1982,

1983) for this species. The majority of cercariae fixed for SEM studies by the mentioned method have a characteristic position which is visible in Plate I, Fig. 1. This position makes it possible to observe the head and ventral part; the dorsal part adheres to the stub.

The whole body tegument of cercaria (including the parts covering both suckers) is covered with spines arranged in horizontal and transverse rows. According to Bock (1982), they are visible also in the light microscope. The mean length of spines (1.2 to 1.7 μm) markedly differs from the length of the spines on the ventral sucker margin (2.3—2.8 μm). This fact can be partly explained by stretching of the tegument at the observed site. The possible effect of other factors (functional?) might be elucidated by further observations of more species.

As it was published in our previous papers (Bušta 1985; Bušta and Našincová, in press), the cuticular spines measure 1.1 μm in *Plagiorchis elegans* and 1.0—1.2 μm in *Plagiorchis neomidis*. Consequently, the cercariae of *O. locellus* can be differentiated from those of *P. elegans* and *P. neomidis* by the length of their cuticular spines. However, this character cannot be used for the differentiation of *P. elegans* from *P. neomidis*. Longer and thicker spines were observed also in the caudal pockets. This also corresponds with the data published by Bock (1982), who found extremely large spines in the caudal pockets on body end. However, it should be stressed that these pockets can be observed in less than one fifth of cercariae, which seems to be associated with the position and shape of body during fixation.

This cercaria does not substantially differ from those of *P. elegans* and *P. neomidis* in the general characteristics of papillae distribution and presence of their basic types (papillae without cilium, papillae with short cilium and papillae with long cilium). However, it differs markedly in the length of cilia at some parts of body (e.g., on the head and laterodorsal papillae). The large papilla observed by Bock (1983) near the stylet was not found by us.

The surface of adult trematodes, except for a small middle part on the ventral side, is also covered with cuticular spines. By contrast with our observations, Bock (1982) did not find spines on the whole posterior part of body in trematodes. This fact could be affected by the differentiating ability of the light microscope. Approximately the same shape of cuticular spines, but of smaller size, and the change of the shape, size and density towards the posterior part of body were reported by Oliver et al. (1984) in *Opisthioglyphe ranae*.

Sensory papillae of adult trematodes were not observed in the light microscope. Oliver et al. (1984) found papillae with one cilium in *O. ranae*. However, in our studies of *O. locellus*, the dome-shaped papillae without cilium, of two different sizes, were found particularly in the vicinity of oral sucker and only near acetabulum. The papillae with cilium, approximately of the same shape as in *P. elegans* (Bušta 1985), were encountered less frequently. Both types of papillae, however, did not reach the size of those found in *P. elegans*. Whether the third type of papillae, papillae with empty middle depression, can be regarded as an independent type of papillae in this species, or whether a papilla with cilium not penetrating above the tegument is involved, will be decided by further histological studies.

The number of spermiae leaving the opening of evaginated cirrus was different in almost all of the evaginated cirruses. A similar phenomenon was observed also by Žďárská et al. (1983) in *Hasstilesia ovis*.

Some data (length of cuticular spines, length of cilia in sensory papillae of cercariae, occurrence of individual types of papillae in adults) obtained during the studies of surface structures of cercariae and adults of *P. elegans* and *O. locellus* and cercariae of *P. neomidis* make possible their mutual differentiation, but a wider use of the results

of scanning electron microscopy in the solution of taxonomic problems is not possible for the time being due to the incomplete and limited amount of obtained information.

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СКАНИРУЮЩАЯ ЭЛЕКТРОННАЯ МИКРОСКОПИЯ ЦЕРКАРИИ И ВЗРОСЛОГО ЭКЗЕМПЛЯРА ТРЕМАТОДЫ *OPISTHIOGLYPHE LOCELLUS* KOSSACK, 1910 (TREMATODA, PLAGIORCHIIDAE)

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Резюме. При помощи сканирующей электронной микроскопии изучали поверхностные структуры церкарии и взрослого экземпляра трематоды *Opisthioglyphe locellus* Kossack, 1910, паразитирующей у насекомых. Поверхность церкарии покрыта тегументальными шипами, которые находятся также в каудальном кармане и на тегументе присосок. Обнаружены три типа сосочков: без реснички, с короткой ресничкой и с длинной ресничкой. На хвосте церкарии встречается складка похожая на плавательную мембрану. Поверхность взрослого экземпляра также покрыта тегументальными шипами, размер и количество которых постепенно уменьшаются в направлении к задней части тела. Обнаружены четыре типа сосочков: малые и большие куполовидные сосочки без реснички, сосочки с пустой средней ямкой и конусовидные сосочки с ресничкой.

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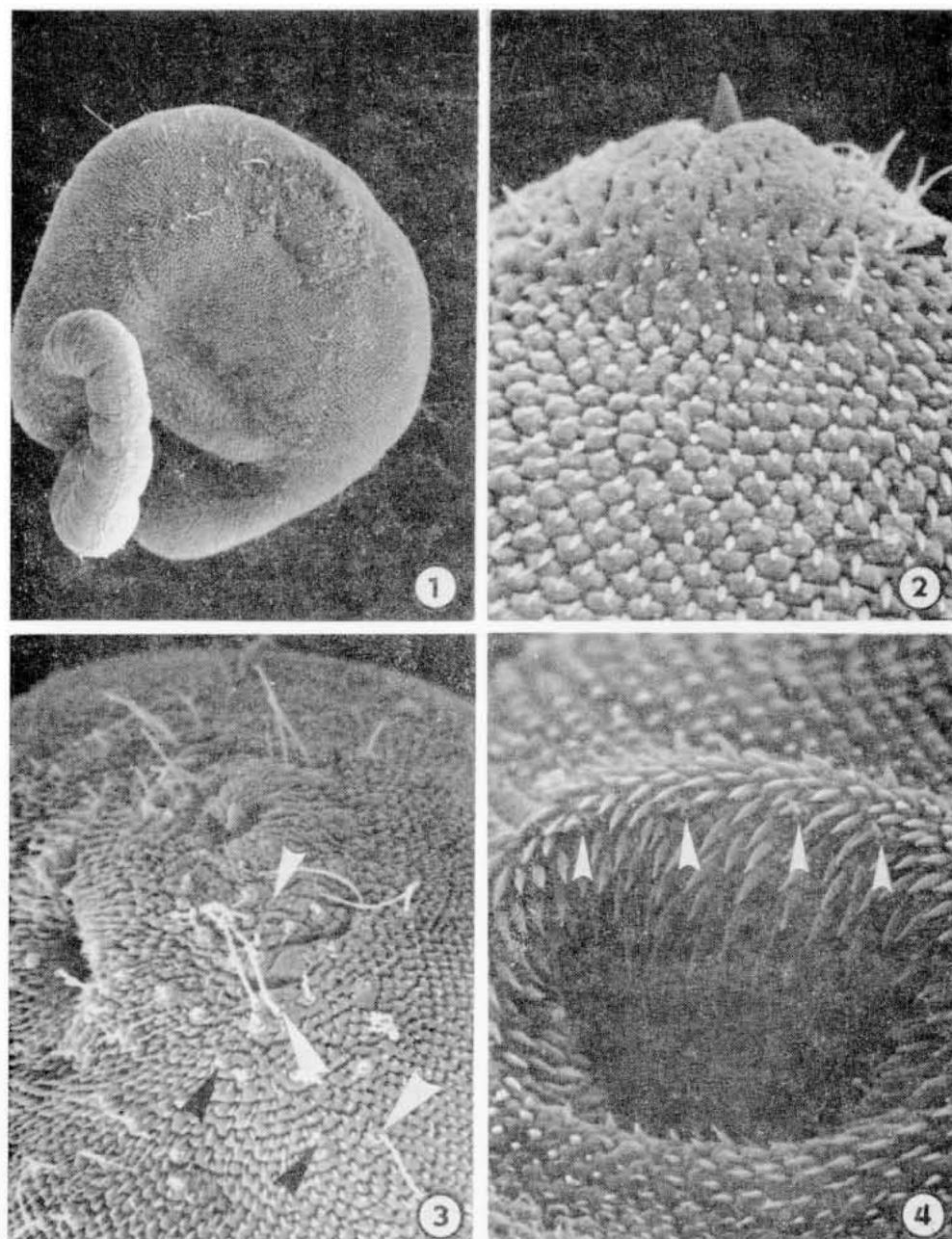
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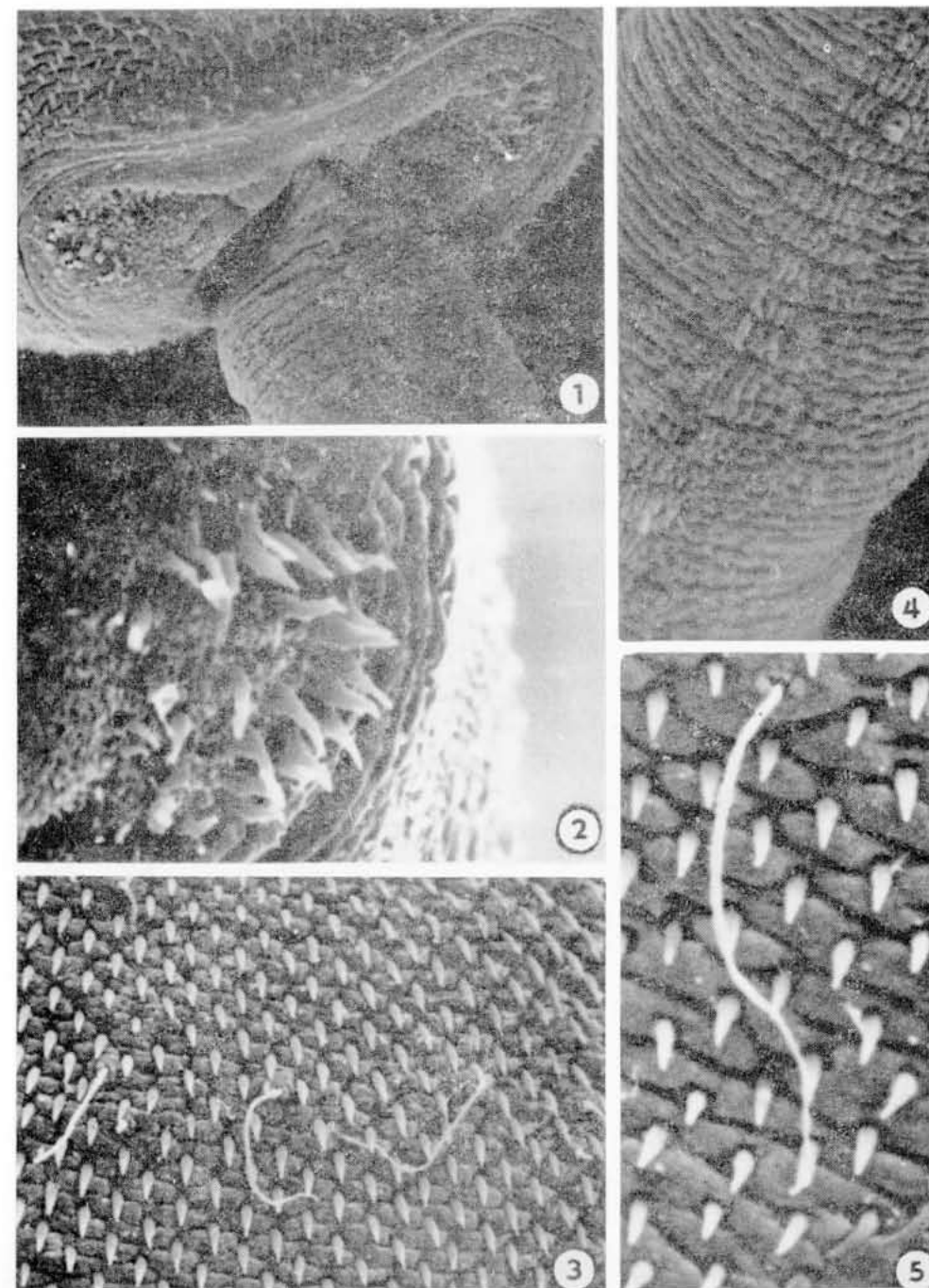
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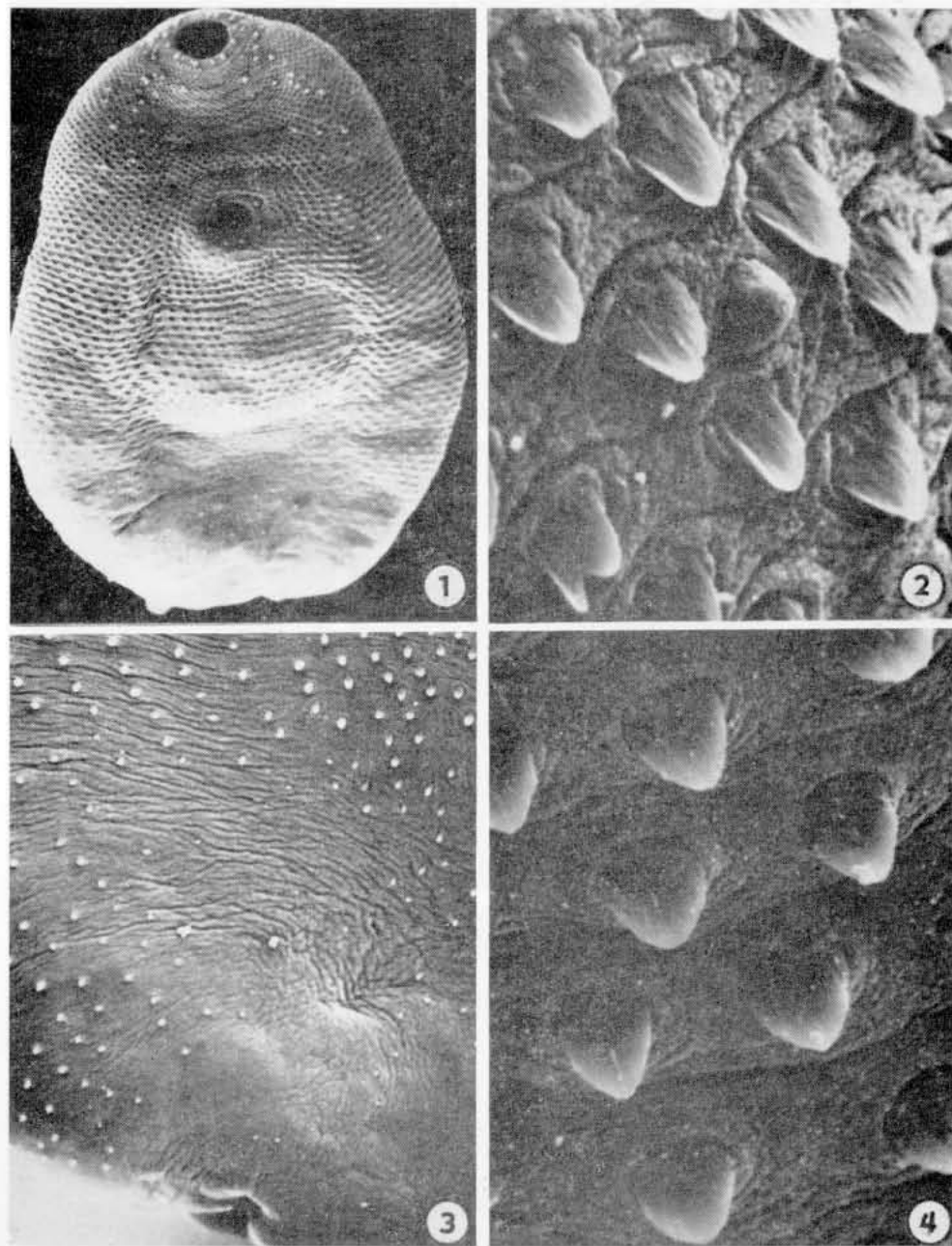
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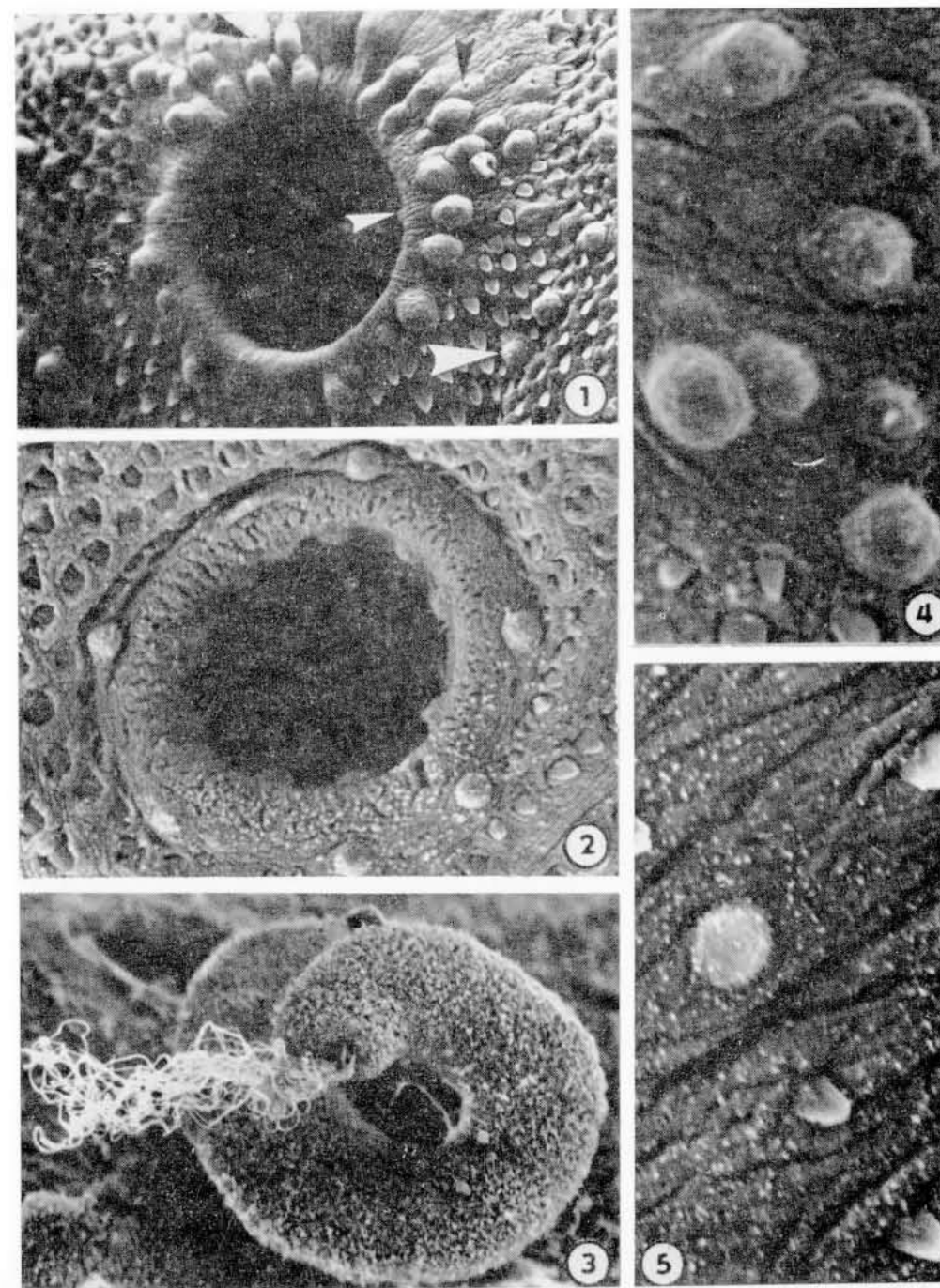
Figs. 1—4. Scanning electron microscopy of the cercaria of *Opisthioglyphe locellus* Kossack, 1910. **Fig. 1.** General ventral view ($\times 640$). **Fig. 2.** Head end with stylet and dorsolateral papillae (black arrows), dorsal view ($\times 3\,000$). **Fig. 3.** Apical part of anterior end with sensory papillae (papillae without cilium—white arrow; papillae with short cilium—black arrows; papillae with long cilium—large white arrows) ($\times 2\,050$). **Fig. 4.** Acetabulum, sensory papillae (white arrows), frontal view ($\times 3\,000$).



Figs. 1—5. Scanning electron microscopy of the cercaria of *O. locellus* Kossack, 1910. **Fig. 1.** Caudal pocket with spines, dorsal view ($\times 1\,950$). **Fig. 2.** Detail of caudal pocket spines ($\times 5\,000$). **Fig. 3.** Cuticular spines and papillae with long cilia in preacetabular zone, dorsal view ($\times 2\,600$). **Fig. 4.** Tail papillae, dorsal view ($\times 3\,500$). **Fig. 5.** Papilla with long cilium in postacetabular zone, ventral view ($\times 5\,800$).



Figs. 1—4. Scanning electron microscopy of *O. locellus* Kossack, 1910. **Fig. 1.** General ventral view ($\times 245$). **Fig. 2.** Cuticular spines and papilla in the first third of body, ventrolateral view ($\times 4\,200$). **Fig. 3.** Caudal part of body with opening of excretory system, ventral view ($\times 450$). **Fig. 4.** Spines in posterior fourth of body, ventrolateral view ($\times 3\,200$).



Figs. 1—5. Scanning electron microscopy of *O. locellus* Kossack, 1910. **Fig. 1.** Oral sucker with papillae (papilla of inner circle—small white arrow, papilla with empty middle depression—black arrows, papilla with central cilium—large white arrow) ($\times 1\,750$). **Fig. 2.** Ventral sucker, frontal view ($\times 1\,800$). **Fig. 3.** Cirrus with spermiae leaving its opening ($\times 1\,250$). **Fig. 4.** Papillae laterally to oral sucker ($\times 4\,500$). **Fig. 5.** Papillae in posterior fifth of body, ventral view ($\times 3\,000$).