FOLIA PARASITOLOGICA 35: 97–103, 1988.

SCANNING ELECTRON MICROSCOPY OF THE EGG, SPOROCYST, CERCARIA, METACERCARIA, AND ADULT OF OPISTHIOGLYPHE RANAE (FRÖLICH, 1791) (TREMATODA: PLAGIORCHIIDAE)

J. BUŠTA and V. NAŠINCOVÁ

Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. Surface structures of developmental stages of *Opisthioglyphe ranae* (Frölich, 1791) were studied for the first time by scanning electron microscopy. The egg surface is smooth, with a distinct low ridge at the periphery or operculum. The sporocysts are cylindrical or club-shaped, with birth pore situated terminally at one end. Their surface is smooth or folded, in relation to their filling with the cercariae. The body surface of cercaria, including the tegument of suckers, is covered with spines. Tegumentary spines are situated also in the caudal pockets. Papillae without cilium, with a short cilium and with a long cilium were found on the cercariae. The tail of cercaria has a dorsal and ventral narrow fin fold. The surface of the cysts with metacercariae is finely granular. The bodies of the metacercariae released from the cysts are covered with tegumentary spines, except for the vicinity of the excretory pore. There are two types of papillae: large, dome-shaped papillae without cilium and small, conical papillae with a cilium. Tegumentary spines, dome-shaped papillae without cilium and conical papillae with a cilium were found also on the body surface of adult trematodes.

The life cycle of *Opisthioglyphe ranae* (Frölich, 1791), morphology of its larval stages and adults, as well as the variability and validity of the species have been studied by various authors, as Sinitsyn (1905), Komiya (1938), Brumpt (1944—1945), Joyeux and Baer (1953, 1958), Dollfus (1958), Odening (1961), Žďárská (1964), Dobrovolskiy (1965), and Grabda-Kazubska (1967, 1969). The first intermediate hosts of this obligatory parasite of frogs are water snails of the family Lymnaeidae, inside which the mother sporocysts develop from the miracidium. Further stage are daughter sporocysts in which the cercariae develop. Second intermediate hosts, into which the cercariae actively penetrate and encyst inside them into the metacercariae, are various species of water snails, tadpoles, small frogs, spotted salamanders and fishes.

O. ranae has often been found in Czechoslovakia, as reported in the papers by Kopřiva (1957). Prokopič (1957), Vojtková (1961, 1974, 1982), Moravec (1963), and Prokopič and Křivanec (1975). According to these authors, this species has been recorded in Czechoslovakia in 12 species of frogs, in *Triturus cristatus*, and *Natrix natrix*. Metacercariae of *O. ranae* were found in frogs, tadpoles and spotted salamanders, and adults were exceptionally recovered from tadpoles.

Oliver et al. (1984) studied the surface structures of adult *O. ranae* by means of scanning electron microscopy (SEM). In this paper, we present a new information about the surface structures and morphology of the larval stages obtained by SEM studies and extend the previous data obtained by light microscopy. At the same time, we described in detail some structures observed in the adult specimens and discuss the possibility of using the new obtained data for taxonomic purposes.

MATERIALS AND METHODS

Cercariae of *Opisthioglyphe ranae* (Frölich, 1791) were found in water snails *Lymnaea stagnalis* and *Galba corvus* from ponds in the vicinity of České Budějovice. Some of the cercariae and daughter sporocysts recovered from spontaneously infected snails of these two species were prepared for SEM studies. Other cercariae were used for the infection of snails, *Lymnaea stagnalis*, *Radix peregra peregra*, *Planorbatius corneus*, *Planorbis planorbis*, and *Biomphalaria glabrata* from laboratory breeding. The snails were kept at the temperatures of 20—24 °C during the experiment. They were dissected on day 23 p.i. Cysts with metacercariae were localized in the kidneys, heart, genital organs, hepatopancreas and mantle. Experimentally obtained metacercariae, both inside the cysts and excysted by a slight pressure, were fixed for SEM. Some of the cysts were fed to young frogs (*Rana ridibunda*) hatched from eggs under laboratory conditions. On day 40 p.i., the frogs were dissected and adult trematodes were found in their intestines. Some of the trematodes were fixed in 70% alcohol, stained with borax carmine and determined as adults of *O. ranae*. The remaining specimens and eggs spontaneously released by them in the saline were prepared for SEM. Daughter sporocysts, cercariae, metacercariae, adults, and eggs were prepared for the SEM studies using the previously described method (Bušta and Našincová 1986a). The measurements of spines and papillae given in the paper were taken from the published and other micrographs. In most cases 10—20 specimens were measured. The preparations were examined in Tesla BS-300 scanning electron microscope at 15 kV.

RESULTS

Egg (Pl. I, Fig. 1). The egg measures 42 × 25 μm and its surface is smooth. Distinctly separated large operculum is situated at one pole and forms a small ridge on the egg shell along its whole periphery.

Sporocysts (Pl. I, Figs. 2—4). Daughter sporocysts of different sizes and shapes are shown in Pl. I, Fig. 2. The majority of the sporocysts are cylindrical, with narrowed ends, others are club-shaped. The widened parts of sporocysts are filled with cercariae. The birth pore, through which the cercariae leave the sporocysts, was observed terminally in the widened part of club-shaped sporocysts (Pl. I, Fig. 3). There were no papillae or thickened tegument around the pore. The tegument on the widened parts of the sporocyst is stretched, on the narrowed parts it is folded and forms longitudinally orientated elevations separated from one another by deep grooves (Pl. I, Fig. 4).

Cercaria (Pl. II, Figs. 1—3, Pl. III, Figs. 1—4). The cercaria has a convex dorsal side and strongly concave ventral side. The stylet is situated terminally, oral sucker subterminally. The openings of penetration glands are visible ventrally, immediately in front of the stylet (P. II, Fig. 3, white arrows). The tegument of almost the whole body is covered with fine tegumentary spines, orientated backwardly. They are arranged in regular horizontal and transversal rows. In the apical part of body, the spines in the first rows behind the stylet are very small, in the immediate vicinity of the stylet opening and on the rim of the caudal pocket the spines are lacking or they are enclosed in the tegument and do not prominate from the openings above its surface. The length of tegumentary spines (except those in the apical part, acetabulum vicinity and caudal pocket) is 0.9—1.2 μm at different sites of the first two thirds of the body. They reach the length of about 1.1 μm, e.g. on the dorsal side of body (Pl. III, Fig. 3), immediately behind the stylet, at the level of the first group of stylet dorsolateral papillae (position StDL according to the nomenclature after Richard, 1971). The size of spines decreases towards the posterior part of body. The longest tegumentary spines (1.9 μm) are on the stretched margin of acetabulum slightly protruding above the body surface (Pl. III, Fig. 2). Spines up to the length of 1 μm are present also on the tegument covering the acetabulum (Pl. III, Fig. 2). They are less densely distributed than on the remaining parts of body. On the dorsal side near the tail base (Pl. III, Fig. 1) on both sides inside

the caudal pockets, there are approximately 50 spines measuring up to 1.5 μm, which are wider near their base than those on the body surface. The tail of the cercaria bears no spines. Except for the terminal part, there is a conspicuous fin fold on its ventral (Pl. II, Fig. 1) and dorsal (Pl. III, Fig. 4) sides.

Sensory papillae between the tegumentary spines on the body surface are most frequent in the vicinity of stylet and both suckers. On the dorsal side of anterior part of body (Pl. II, Fig. 2), there are stylet papillae, stylet dorsolateral papillae (making groups of 4 + 1 papillae and marked by white arrows), and the majority of papillae of the first dorsal preacetabular row. The papillae of this part of body bear cilia measuring up to 10 μm in length. A detailed view of the apical part of body (Pl. II, Fig. 3) shows papillae without cilium and papillae with a short cilium (up to 1.5 μm long) in the vicinity of stylet and oral sucker. Like in the cercariae of other species belonging to Plagiorchiidae, the papillae on the body of *O. ranae* are arranged in two dorsal, two lateral and two ventral rows. The acetabulum (Pl. III, Fig. 2) bears 9 papillae in the inner circle (small white arrows) and 6 papillae in the outer circle (large white arrows). The papillae of both circles have a short cilium. Four papillae of the mentioned group of stylet dorsolateral papillae are shown in detail in Pl. III, Fig. 3. In the majority of the observed cercariae this group consists of a papilla with long cilium (about 9 μm) situated medially and three papillae with short cilium. Two papillae with cilia measuring about 2.5 μm are present close to one another on the dorsal side of the middle part of tail (Pl. III, Fig. 4). Their distance in the other observed cercariae is up to 5× greater than in the above figure.

Metacercaria (Pl. IV, Figs. 1—5). An oval cyst measuring 152 × 127 μm is illustrated in Pl. IV, Fig. 1. Its surface is granular. The encysted metacercaria (Pl. IV, Fig. 2) measures 195 μm in length and 118 μm in width. The anterior end of metacercaria body is widely rounded. The oral sucker, due to the contraction of the first third of body, is situated ventrally, and the ventral sucker lies in the middle of body. The tegument of the metacercaria (except for a small part around the excretory pore) is covered with tegumentary spines, orientated posteriorly. The tegumentary spines in the immediate vicinity of oral sucker (laterally and ventrally) are well developed. In the caudal direction, the shape of tegumentary spines changes and their size and density decrease. The shape of tegumentary spines arranged in horizontal and transversal rows in the first third of body on the dorsal side is shown in Pl. IV, Fig. 3. They measure 1.2 μm in length and 0.5 μm in width. Another figure (Pl. IV, Fig. 4) illustrates the spines immediately in front of the body end, laterodorsally, where they reach the length of about 0.9 μm and width of 0.3 μm. They resemble in their shape the spines of cercaria. Like in the cercaria, the tegumentary spines are present also on the tegument covering the acetabulum.

Sensory papillae are situated between the spines on the tegument of metacercaria (Pl. IV, Figs. 2 and 5). They are most frequent in the vicinity of both suckers. A circle of 10 large dome-shaped papillae without cilia, measuring about 2 μm at base, is visible around the oral sucker. Dorsally from this circle there are 3 conical papillae of approximately half the size and with short cilia. They are arranged symmetrically. Groups of small papillae with cilia are located lateroventrally. Two rows of papillae begin on the ventral side at the level of these groups and terminate behind the posterior margin of acetabulum. Two circles of papillae with cilia are present on the ventral sucker, the inner circle consisting of 9 papillae and the outer one of 6 papillae. On the anterior part of body, dorsally (Pl. IV, Fig. 5), there are papillae arranged in longitudinal rows at the sites corresponding to the position StDL and AfD in cercariae after Richard's (1971) nomenclature.

Adult (Pl. V, Figs. 1—4, Pl. VI, Figs. 1—5). The ventral tegument surface of an adult

specimen is shown in Pl. V, Fig. 1. The oral sucker is situated subterminally, the acetabulum lies at the end of the first third of body. The body surface is almost entirely covered with tegumentary spines. On sides they are present almost up to the posterior end of body, whereas dorsally and ventrally they are lacking on the last third of body and around the excretory pore. The spines are lacking also on the tegument covering the oral sucker (Pl. VI, Fig. 1), but they are present on the tegument of acetabulum (Pl. VI, Fig. 2). The spines on the body surface are orientated caudally and their shape, density and arrangement vary in the direction to the posterior end of body. Well developed arrow-shaped tegumentary spines arranged in horizontal and transversal rows can be seen in the first third of body (Pl. V, Fig. 2). Their parts prominating above the tegument are approximately 2.3 μm long and 1.2 μm wide. The density and size of tegumentary spines gradually decreases starting from the middle of the body. In the last fourth of body, laterally, where they are arranged in irregular rows (Pl. V, Fig. 3), they are at most 1.6 μm long and 0.9 μm wide. Irregularly arranged spines, situated laterodorsally immediately in front of the body end (Pl. V, Fig. 4), measure 0.9—1.5 μm in length and 0.5 μm in maximum width.

The tegument also bears some papillae, which are most dense in the vicinity of both suckers. A symmetrical and circular arrangement of papillae around the oral sucker is shown in Pl. VI, Fig. 1. Two papillae are situated outside this circle, laterally, in the oral sucker (Pl. VI, Fig. 1 — white arrows). There are two types of papillae there: large, dome-shaped papillae without central cilium (size of papillae about 4 μm at the base) and smaller conical papillae with central cilium (size up to 2.8 μm at the base). Both types of papillae are illustrated in detail in Pl. VI, Fig. 3. The acetabulum has two rings of papillae on its surface. The inner ring consists of 9 papillae with cilium (about 3.5 μm wide at the base) and the outer ring consists of 6 papillae without cilium (about 5 μm wide at the base). The detail of a papilla of the inner ring is shown in Pl. VI, Fig. 5. Like in the metacercaria, longitudinal rows of papillae with cilium are present between the spines on the dorsal and ventral surface of the anterior part of body. Papillae of the dorsal side of body, which measure about 2.6 μm, are shown in detail in Pl. VI, Fig. 4.

DISCUSSION

The trematode *O. ranae*, which belongs to the most common parasites of amphibians, has often been studied by the helminthologists. In Czechoslovakia, it has been the subject of faunistical studies, whereas the foreign authors have solved the problems of its existence from many other aspects. The morphology of developmental stages has already been studied (Ginetsinskaya 1959, Palm 1966), but no author has dealt with the surface structures of this species using SEM, except for adult specimens (Oliver et al. 1984).

The smooth egg shell observed by us in *O. ranae* is characteristic also for other trematode species, e.g. *Fasciola hepatica* (Wilson 1967, Koie et al. 1976). The data on the sporocyst morphology are very scanty, only Brumpt (1944—1945) mentioned a warty surface of the narrowed parts of sporocysts, which he observed in the light microscope. Our findings in SEM confirm the presence of a similar surface in these parts. A pore through which the cercariae leave the sporocyst was visible in a small number of sporocysts. The authors who studied the morphology of *O. ranae* sporocysts by means of a light microscope did not mention this pore at all. Only Brumpt (1944—1945) observed that the cercariae leave the sporocyst at one of its ends.

The tegumentary spines on the body surface of cercaria, diminishing in caudal direction, have been commonly observed by the light microscope. However, the authors did

not give their length (Sinitsyn 1905, Komiya 1938, Brumpt 1944—1945 and others). Like in *O. locellus*, the longest spines were found on the margin of the ventral sucker and thick and long spines were present in caudal pockets. Also the distribution of sensory papillae on the cercariae was of the same basic type as that observed by us in other species of the family Plagiorchiidae. However, the distribution of some groups of papillae on *O. ranae*, as observed by us, does not fully correspond with the data published by Dobrovolskiy (1965), who studied the chaetotaxy of cercariae in preparations treated with silver nitrate.

The presence of spines (similar to the spines of the cercaria) on the tegument of metacercaria has been recorded by Grabda-Kazubska (1969). The spines around the oral sucker are well developed and resemble in their shape the spines found in adult specimens. This also concerns some of the spines in the first half of body. If the light microscopy was used, no papillae were recorded in the metacercariae. Although no special attention was paid to the morphogenesis of papillae in the metacercariae, an identical number of papillae was observed in some parts of the cercaria and metacercaria body (position StDL, both rings of suckers), other papillae were reduced in number (dorsally from the stylet), or their distribution changed (middle papillae in the $A_I D$ row in the cercaria are lacking in the metacercaria).

The tegumentary spines of adults can also be observed by the light microscope. Oliver et al. (1984) studied these spines in detail by means of SEM. They described the shape and size of spines in the anterior and middle parts of body and observed smaller and irregularly distributed spines in the posterior half of body. We have therefore studied the size, shape and arrangement of tegumentary spines in different parts of body of the adults, including the posterior half, and supplemented thus the data published by the above authors. The spines observed by us in the anterior part of body were slightly smaller than those described by the French authors. In our opinion, the differences may be caused by many factors, as different definitive hosts, different age of the trematodes, different processing of the preparations for SEM, individual variability and others. It is also possible that we did not examine exactly the same body part as Oliver et al. (1984) did. This is not specified in their paper.

In contrast to the tegumentary spines, the sensory papillae of adults have not been observed in the light microscope. Oliver et al. (1984) found ciliary sensory receptors in SEM. Our studies evidently confirm the presence of large, dome-shaped papillae without cilium, in addition to ciliary papillae. This type of papillae was observed by us also in adult specimens of relative species, *Opisthioglyphe locellus* and *Plagiorchis elegans*.

Our previous papers on *Plagiorchis elegans* (Bušta 1985), *Plagiorchis neomidis* (Bušta and Našincová 1986a), *Opisthioglyphe locellus* (Bušta and Našincová 1986b) and this study enable to evaluate the taxonomic significance of the surface structures of cercariae and adults.

The arrangement of tegumentary spines on the body surface of cercaria is identical in all of the four species. The differences in their size are negligible. Only the cercaria of *O. locellus* can be partly differentiated from the others on the basis of the spine length (1.2—1.7 μm). The species *P. elegans*, *P. neomidis* and *O. ranae* cannot be differentiated from one another on the basis of this character, since the length of their spines is 0.9 to 1.2 μm. Three types of papillae were found in all of these cercariae: without cilium, with a short cilium, and with a long cilium. However, some differences were recorded in the length of cilia in the corresponding groups of papillae in individual species. It will be necessary, however, to verify the stability of this character. We have not studied the general distribution of sensory papillae (chaetotaxy) on the cercariae of these species, because it has already been studied by various authors in $AgNO_3$-treated preparations

using the light microscopy (Dobrovolskiy 1965, Theron 1976, Samnaliev et al. 1982, Bock 1983). However, some of the papillae in the apical part are hardly visible if this method is used. Therefore the observations of the surface structures of cercaria in the SEM suitably supplement the results obtained by the classical method.

The distribution, arrangement and shape of tegumentary spines on the body surface of adults are similar. The body is covered by tegumentary spines with the exception of a larger or smaller part of the last third of body. There were differences in the size of spines in the anterior third of body (1.5—1.9 μm in *P. elegans*, 2.3 μm in *O. ranae*, 4.2—4.7 μm in *O. locellus*). On the other hand, no differences enabling the specific differentiation of individual species have been observed between the types of papillae in adult specimens (smaller conical papillae with cilium and larger dome-shaped papillae without cilium were present in all species).

Due to the close relationship, the studied species are very similar in the shape, size and arrangement of tegumentary spines, as well as in the type and basic distribution of sensory papillae in the cercariae and adults. Although some of the surface structures of cercariae and adults observed by SEM can be used for the differentiation of related species, at the present stage of knowledge of the problem it is necessary to be precautious in making practical decisions and conclusions. A prerequisite for the mutual comparative analysis of surface structures of several species is to obtain a suitable material providing comparable data.

Acknowledgements. The authors thank Ing. M. Tůma and Mr. J. Horák of the Laboratory of Electron Microscopy, South-Bohemian Biological Centre, České Budějovice for their technical assistance in microscoping and Mrs. J. Růžičková, L. Nováková and A. Prokopičová for making the preparations.

СКАНИРУЮЩАЯ ЭЛЕКТРОННАЯ МИКРОСКОПИЯ ЯИЦ, СПОРОЦИСТ, ЦЕРКАРИЙ, МЕТАЦЕРКАРИЙ И ПОЛОВОЗРЕЛЫХ ЭКЗЕМПЛЯРОВ ТРЕМАТОДЫ *OPISTHIOGLYPHE RANAE* (FRÖLICH, 1971) (TREMATODA: PLAGIORCHIIDAE)

Я. Бушта и В. Нашинцова

Резюме. Впервые были изучены поверхностные структуры стадий развития *Opisthioglyphe ranae* (Frölich, 1791) с помощью сканирующей электронной микроскопии. Поверхность яйца гладкая, с четким, низким валом на окружности крышечки. Спороцисты цилиндрические или палковидные, с родильной порой на одном конце. Их поверхность гладкая или складчатая, в зависимости от их наполнения церкариями. Поверхность тела церкарий, включая тегумент присосок, покрыта шипами. Тегументарные шипы находятся также в каудальных карманах. На церкариях обнаружены сосочки без реснички, с короткой ресничкой и с долгой ресничкой. На хвосте церкарии находится дорзальная и вентральная, узкая, плавниковая складка. Поверхность цисты с метацеркариями мелко зернистая. Тела метацеркарий, освободившихся из цисты, покрыты тегументарными шипами, кроме части, окружающей экскреторное отверстие. Встречаются два типа сосочков: большие, куполовидные сосочки без реснички и небольшие, конусовидные сосочки с ресничкой. Тегументарные шипы, куполовидные сосочки без реснички и конусовидные сосочки с ресничкой найдены также на поверхности тела половозрелых трематод.

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Received 7 May 1987

J. B., Parazitologický ústav ČSAV,
Branišovská 31,
370 05 České Budějovice, ČSSR

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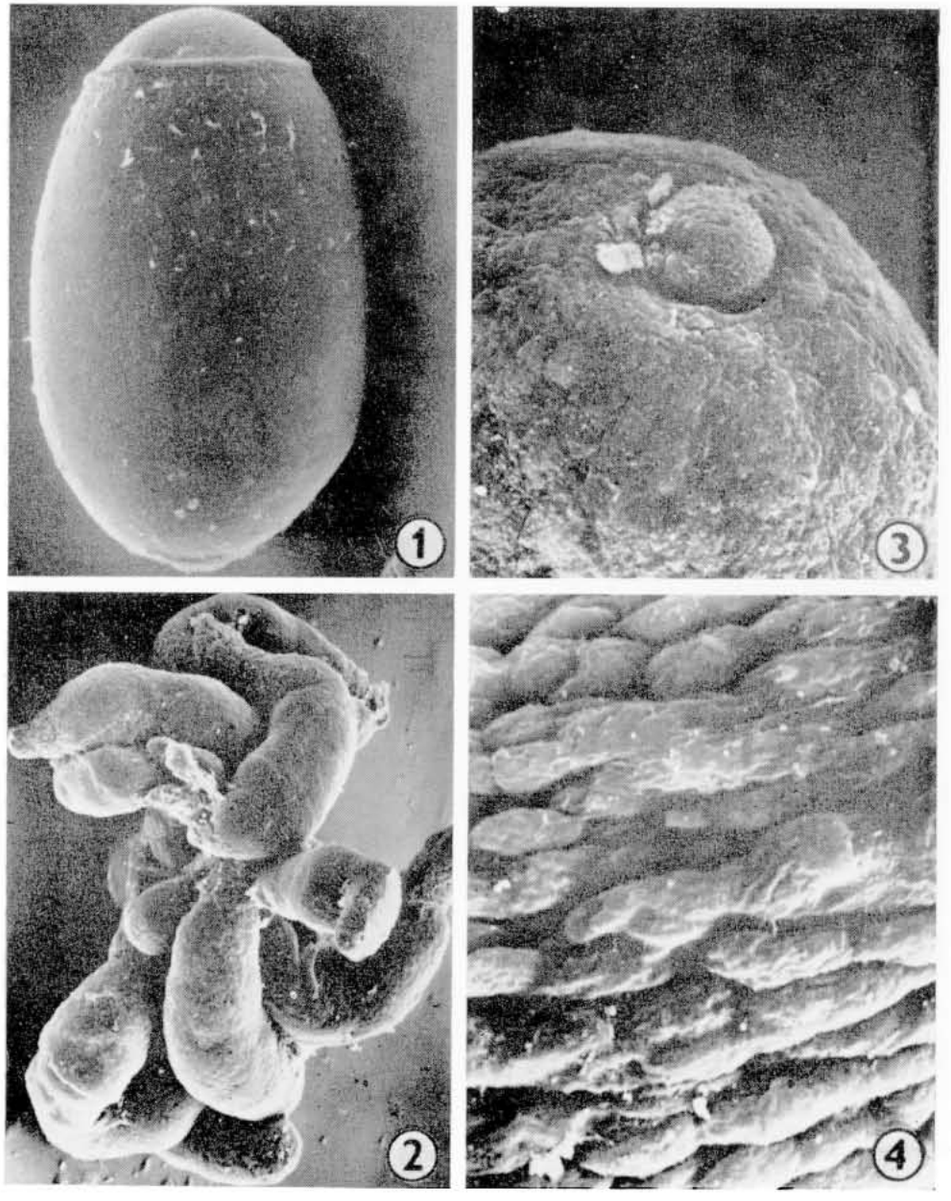
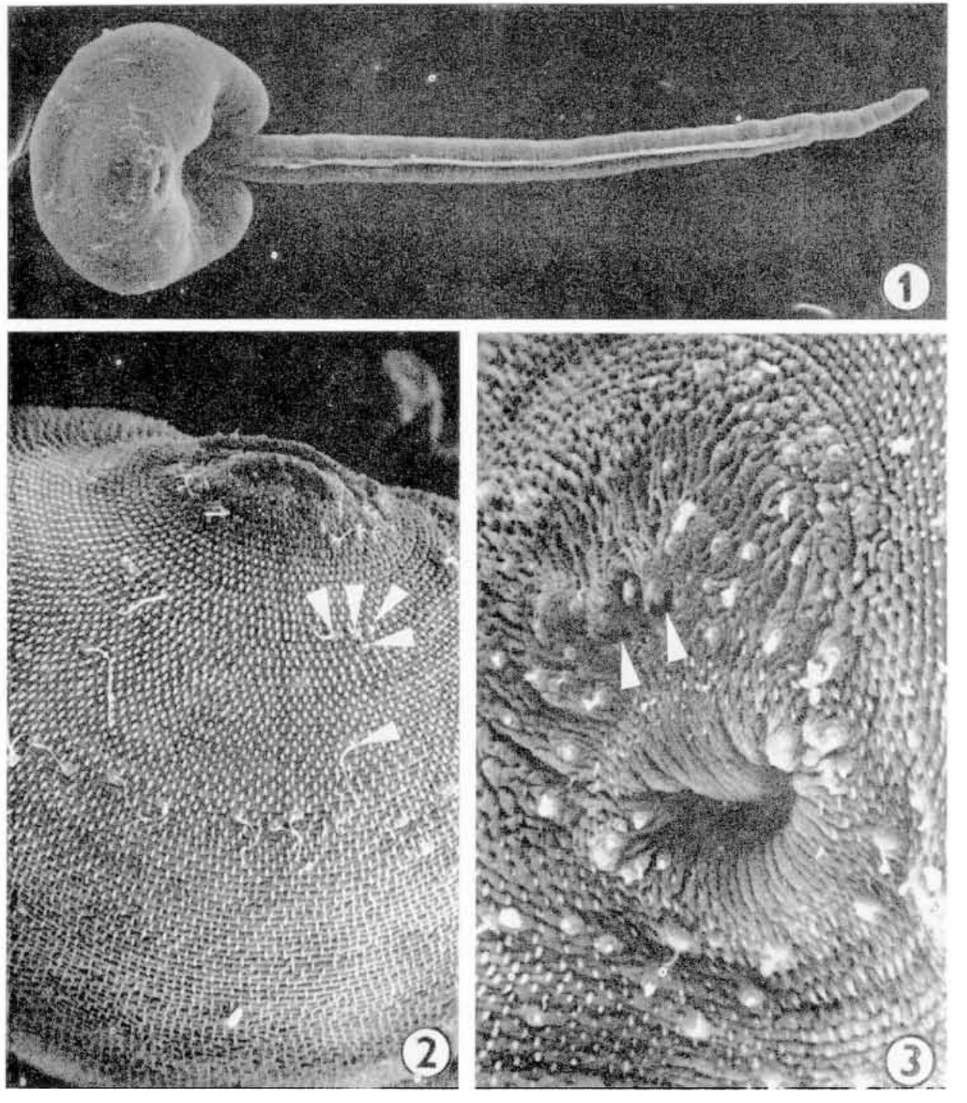
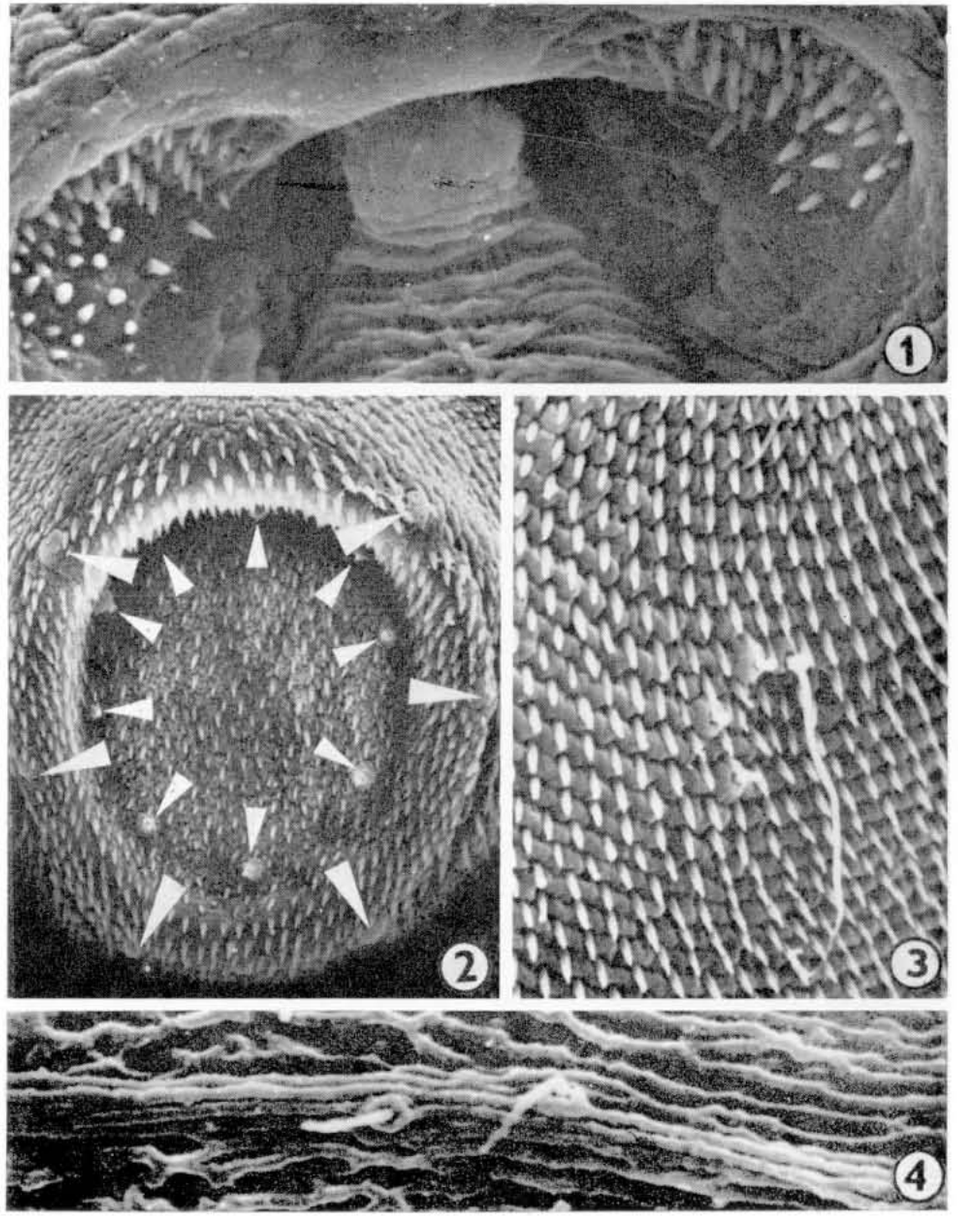


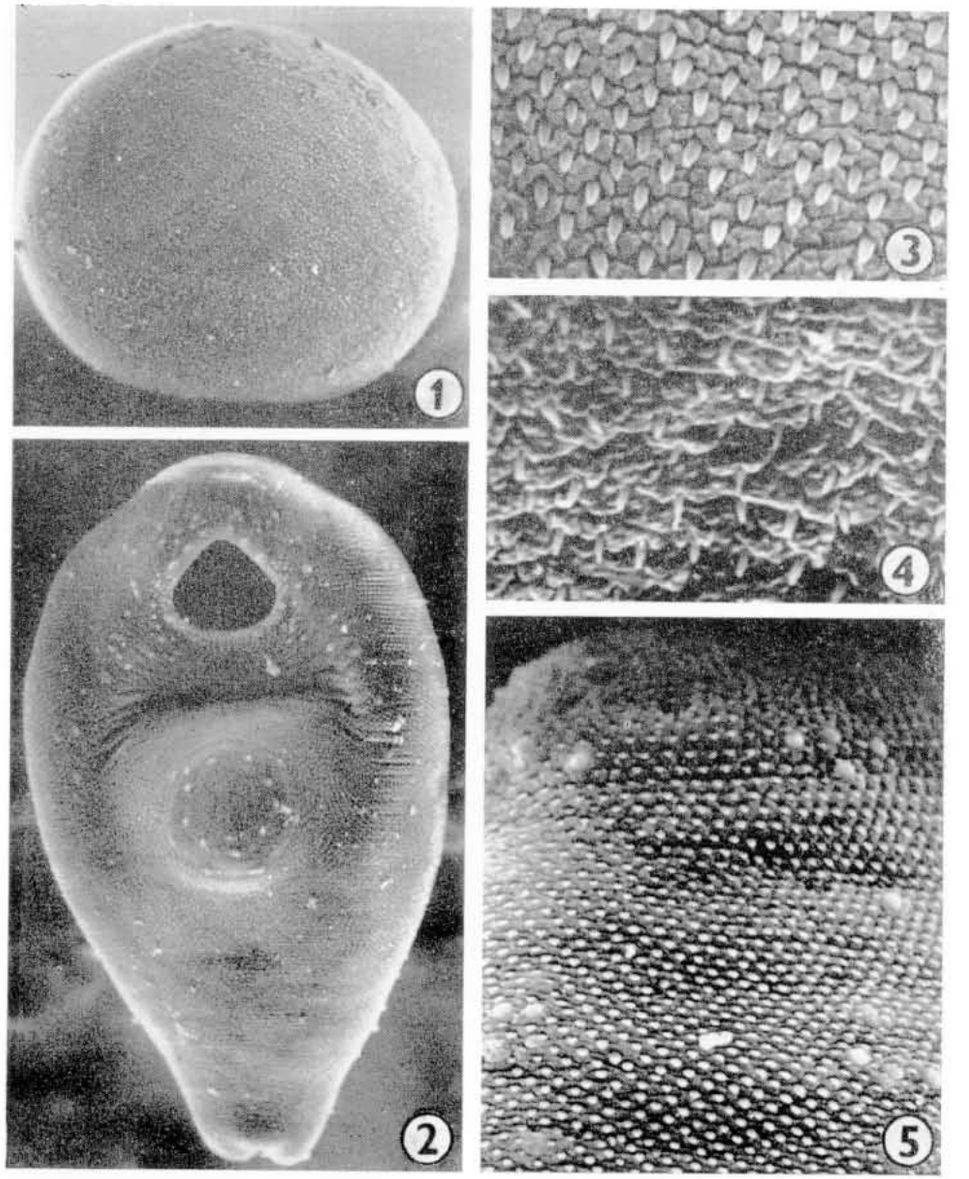
Fig. 1. Egg of *Opisthioglyphe ranae*. General view (×1,800). **Figs. 2—4.** Sporocysts of *O. ranae*. **Fig. 2.** Accumulation of daughter sporocysts (×100). **Fig. 3.** Birth pore of the sporocyst (×1,300). **Fig. 4.** Detail of the wrinkled part of sporocyst (×2,250).



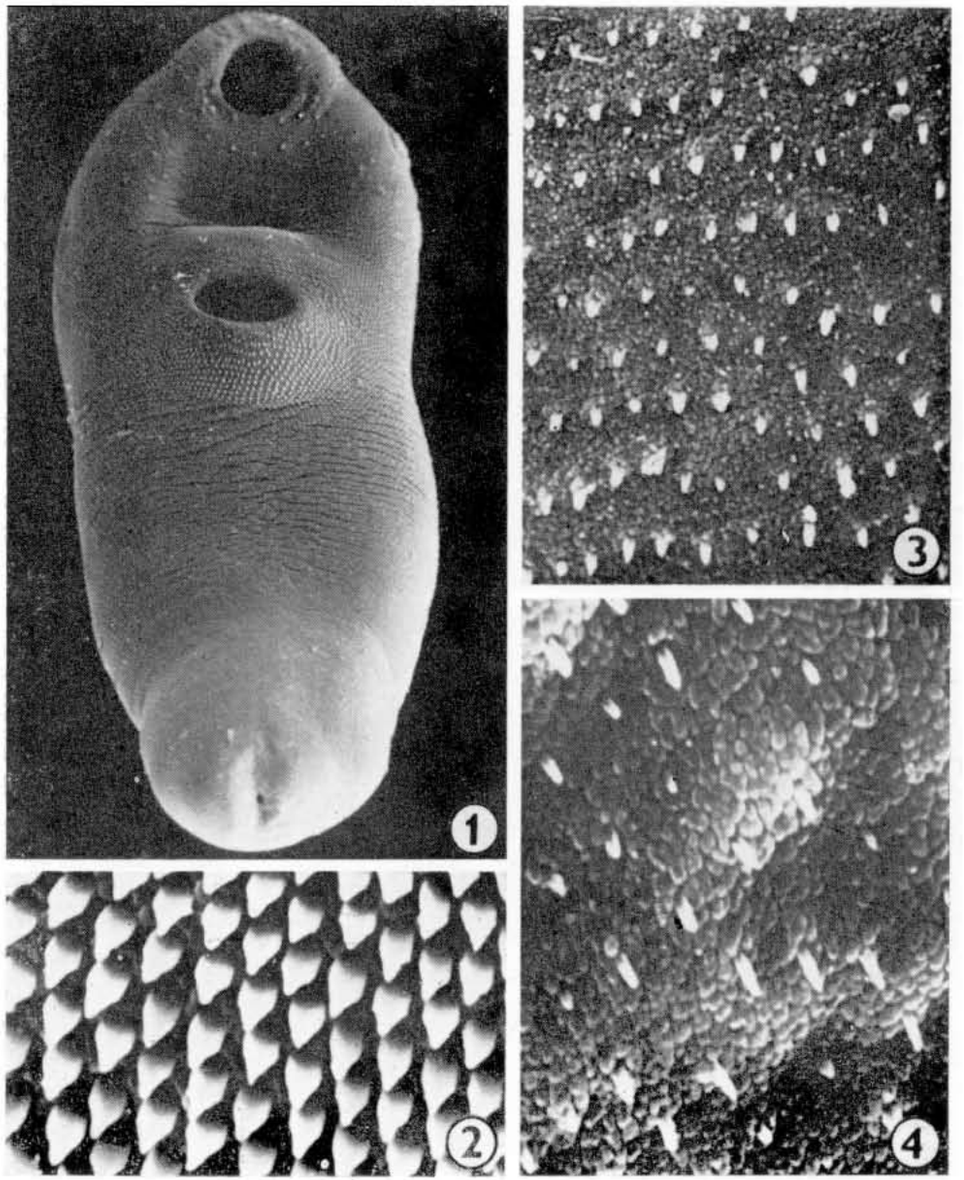
Figs. 1—3. Cercaria of *Opisthioglyphe ranae*. **Fig. 1.** General view (×380). **Fig. 2.** Anterior part of body with stylet dorsolateral papillae (white arrows) and dorsal row of papillae. Dorsal view (×1,400). **Fig. 3.** Apical part of anterior end with stylet, openings of penetration glands (white arrow), oral sucker, and papillae. Ventral view (×1,900).



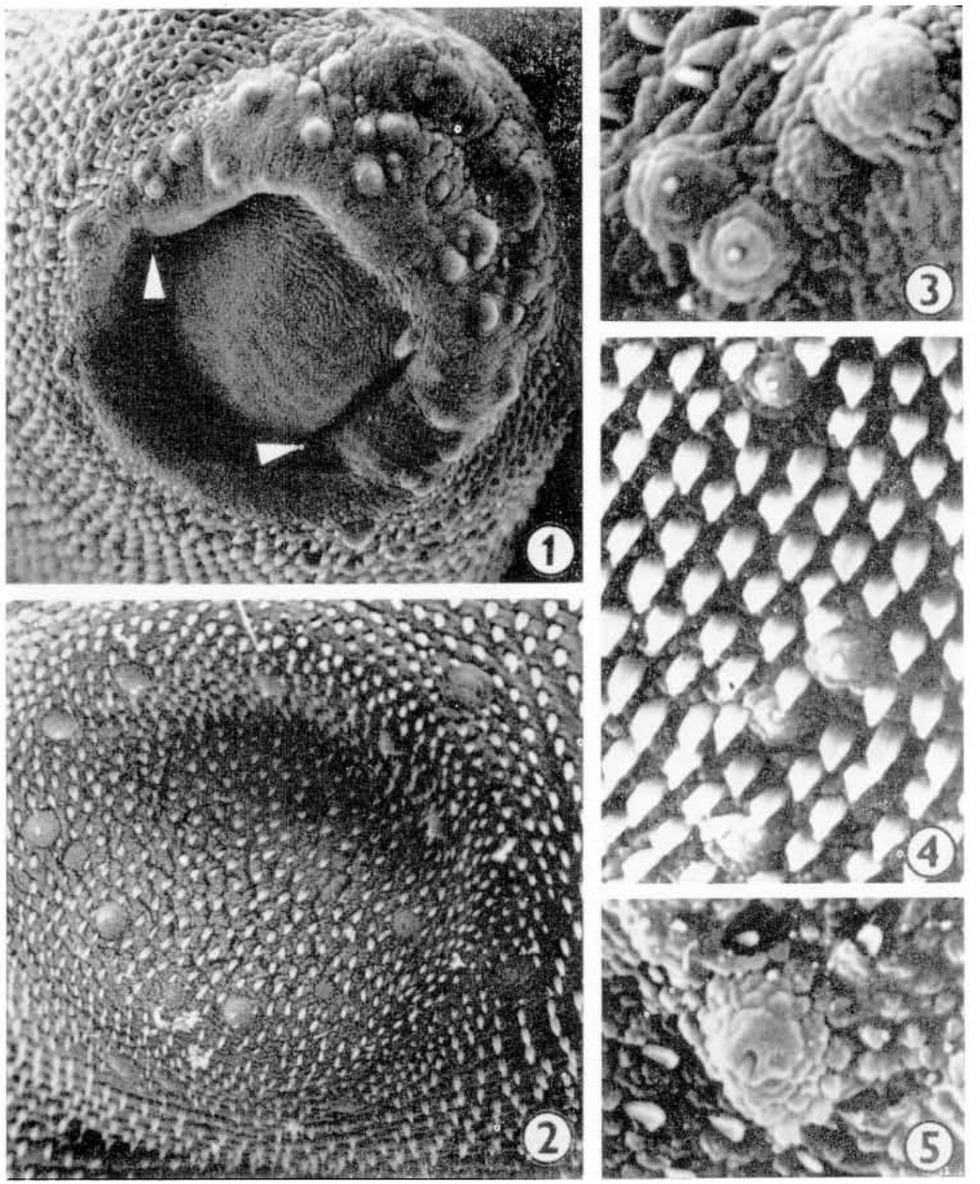
Figs. 1—4. Cercaria of *Opisthioglyphe ranae*. **Fig. 1.** Caudal pocket with spines. Dorsal view (×4,000). **Fig. 2.** Acetabulum with two rings of papillae; inner one designated by small arrows, outer one by large white arrows. Frontal view (×2,100). **Fig. 3.** Group of stylet dorsolateral papillae (×5,300). **Fig. 4.** Tail papillae. Dorsal view (×5,900).



Figs. 1—5. Metacercaria of *Opisthioglyphe ranae*. **Fig. 1.** Cyst (×400). **Fig. 2.** Excysted metacercaria. Ventral view (×500). **Fig. 3.** Tegumentary spines of metacercaria in the first fourth of body. Dorsal view (×4,050). **Fig. 4.** Tegumentary spines immediately in front of body end. Laterodorsal view (×4,800). **Fig. 5.** Anterior part of body with tegumentary spines and papillae. Dorsal view (×2,150)



Figs. 1—4. Adult *Opisthioglyphe ranae*. **Fig. 1.** General view, ventral side (×260). **Fig. 2.** Tegumentary spines in the first third of body. Dorsal view (×4,300). **Fig. 3.** Tegumentary spines in the last fourth of body. Lateral view (×2,200). **Fig. 4.** Tegumentary spines immediately in front of body end. Laterodorsal view (×4,600).



Figs. 1—5. Adult *Opisthioglyphe ranae*. **Fig. 1.** Oral sucker with papillae. Ventral view (×1,200). **Fig. 2.** Acetabulum with papillae (×1,700). **Fig. 3.** Detail of papillae of oral sucker. Lateral view (×5,200). **Fig. 4.** Papillae in the first third of body. Dorsal view (×3,800). **Fig. 5.** Papilla of ventral sucker (×7,000).