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

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Abstract. Surface structures of developmental stages of Opisthiolyphe 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 foliated, in relation to their filling with the cercariae. The body surface of cercariae, including the tegument of suckers, is covered with spines. Tegumentary spines are situated also in the caudal pockets. Papillae without cillum, 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 cillum and small, conical papillae with a cilium. Tegumentary spines, dome-shaped papillae without cillum and conical papillae with a cilium were found also on the body surface of adult trematodes.

The life cycle of Opisthiolyphe 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 Sinitays (1905), Komiyama (1938), Brumpt (1944—1945), Joyeux and Baer (1953, 1958), Dollfus (1958), Odening (1961), Žďárská (1964), Dobrovolskij (1965), and Grabda Kazubaska (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 Kopečka (1967), Prokopčík (1957), Vojtková (1961, 1974, 1982), Moravec (1963), and Prokopčík and Krivanec (1975). According to these authors, this species has been recorded in Czechoslovakia in 12 species of frogs, in Triturus cristatus, and Natricis 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 *Opisthiophryne ranus* (Frolich, 1791) were found in water snails *Lymnea stagnalis* and *Helix aspersa* from ponds in the vicinity of Český Budeč. Specimens of the cercariae were recovered from spontaneously infected snails of these two species prepared for SEM studies. Other cercariae were used for the infection of snails, *Lymnea stagnalis*, *Radix pepera* peregra, *Planorbea cornea*, *Planorbis planorbis*, and *Biomphalaria glabris* from laboratory bred snails. 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 pericardial cavity. Externally operated metacecariae, both inside the cysts and extracystically, were fixed 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 the cercariae were found in their tracheae. Some of them were preserved in 70% ethanol, stained with benzyl carmine and determined as adults of *O. ranus*. The remaining specimens and eggs spontaneously released by the salmon were prepared for SEM. Daughter sporocysts, cercariae, metacercariae, adults, and eggs were prepared for the SEM studies using the previously described method (Bušta and Našínová 1986a). The measurements of spines and papillae given in the paper were taken from the same 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. There are spherical sporocysts. 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 longitudinal eleonae 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 (Pl. 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 the 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 protramine from the openings above its surface. The length of tegumentary spines (except those in the apical part, acutum ventrally and caudal pocket) is 0.1–1.2 μm at the 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 dorssal papillae (position StDl according to the nomenclature after Richard, 1971). The size of spines decreases towards the posterior part of body. The longests tegumentary spines (1.9 μm) are on the stretched margin of acetabulum slightly protruding above the body surface (Pl. III, Fig. 3). 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 the body. On the dorsal side near the tail base (Pl. II, Fig. 1) on both sides inside the caudal pockets, there are approximately 50 spines measuring up to 1.5 μm, which are shorter 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, Figs. 2), there are styli papillae, stila dorssal papillae (making groups of 4 + 4) papillae and marked by white arrowheads and the majority of papillae of the first dorsal proaetabular 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 cilia and papillae with a short cilia (up to 1.5 μm long) in the vicinity of stylet and oral sucker. Like in the cercariae of other species belonging to Plagiorchidae, the papillae on the body of *O. ranus* are arranged in two dorsal, lateral and two ventral rows. The acetabulum (Pl. III, Figs. 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 cilia. Four papillae of the mentioned group of styli dorssal 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 cilia (about 9 μm) situated in the circle and three papillae with short cilia. Some of the cercariae and daughter cercariae, 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. 1–8). An oval cyst measuring 182 × 127 μm is illustrated in Pl. IV, Fig. 1. Its surface is granular. The encysted metacercaria (Pl. IV, Fig. 2) measures 100 × 60 μm in length and 118 × 65 μm in width. The anterior end of metacercaria is rounded, the posterior end slightly pointed. The 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 0.4 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 3). They are most frequent in the vicinity of both suckers. A circle of 4 + 4 dorssal papillae without cilia, measuring up to 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 consists of 4 + 4 papillae and the outer circle of 6 papillae. On the lateral part dorssally (Pl. IV, Fig. 5), there are papillae arranged in longitudinal rows at the sites corresponding to the position StDl and ADD 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
not give their length (Sinitsyn 1905, Komja 1938, Brumpt 1944–1945 and others).
Like in O. ocellata, 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 Plocorchidae. However, the distribution of some groups of papillae
on O. ranae, as observed by us, does not fully correspond with the data published by
Dobrovolskij (1960), 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 meta-
cercaria 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 spec-
imens. The cercariae also have a certain number of spines in the first half of body. If the light micro-
scopy was not used, no papillae were recorded in the metacercaria. 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 A2D row in the
cercaria, its 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
stomach and posterior part of the arrangement and distribution of tegumentary spines in different parts of body
of the adults, including the posterior half of body, have been 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 differ-
ences 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 ciliation, in addition to ciliary papillae. This type of papillae was observed by us
also in adult specimens of relative species, Opisthorchys liscus and Plagiorchis
delena.

Our previous papers on Plagiorchis elegans (Buža 1988), Plagiorchis neomidia
(Buža and Našínová 1986a), Opisthorchys liscus (Buža and Našínová 1988b) and this study enable to evaluate the taxonomic significance of the surface structure of cercaria 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. ocellata can be partly differentiated from the others on the basis of the spine length
(1.2–1.7 μm). The species P. elegans, P. neomidia and O. ranae cannot be differentiated
from one another on the basis of this character, since the length of their spines is 0.8 to
1.2 μm. Three types of papillae were found in all of these cercariae: without cillum, with
a short cillum, and with a long cillum. 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 AgNO3-treated preparations.


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. Ceraria of *Opisthioglyphe ranae*. Fig. 1. General view (×380). Fig. 2. Anterior part of body with stylet dorsolateral papillae (white arrow) 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 (×1,000).
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 (×3,800).

Figs. 1-5. Metacercaria of *Opisthioglyphe ranae*. Fig. 1. Cyst (×400). Fig. 2. Excreted metacecercaria. 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. Lateral dorsal view (×4,000). Fig. 5. Anterior part of body with tegumentary spines and papillae. Dorsal view (×2,150).
Figs. 1—4. Adult *Opisthobdyla ranae*. Fig. 1. General view, ventral side (x 200). Fig. 2. Tegumentary spines in the first third of body. Dorsal view (x 4,000). Fig. 3. Tegumentary spines in the last fourth of body. Lateral view (x 2,000). Fig. 4. Tegumentary spines immediately in front of body end. Lateral-dorsal view (x 4,000).

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