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CHAETOTAXY OF CERCARIAE OF OPISTHIOGLYPHE RANAE (FRÖLICH, 1791) (TREMATODA: PLAGIIORCHIDAE)

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Abstract. A detailed observation of the sensory apparatus of *Opisthioglyphe ranae* (Frölich, 1791) cercariae was carried out. The species was determined on the basis of an experimental study of the life cycle and morphology of sporocysts, cercariae, metacercariae, and adults. In contrast to other species of the family Plagiorchidae, no papillae were present in the positions CId_1, CId_2, CII_0, CII_3 and CII_4. Papillae with markedly ventral situation on the postacetabular zone were found very rarely. Some of the cercariae possessed ventral papillae variously distributed in the preacetabular zone. A comparative analysis of the chaetotaxy revealed that the cercaria studied by us was very similar to *Cercaria* 4 of Richard, 1971, with the exception of the number of acetabular papillae and some groups of ventral papillae. Compared to the cercaria of *O. ranae* studied by Dobrovolskiy (1965). AID papillae and a part of ventral papillae were distributed differently. The chaetotaxy was identical with that of experimentally obtained cercariae of *O. ranae* from *L. stagnalis* and of cercariae of the same species obtained from Bulgaria.

During the last decade, several studies of cercaria chaetotaxy (Théron 1976, Bayssade-Dufour 1979, Samnaliev et al. 1982, 1983, Bušta 1987, Bock and Jansen 1987) have provided taxonomical criteria which can be used for the differential diagnosis of species of the genus *Plagiorchis*. Other authors (Richard 1971, Vaucher 1972, Bock 1983) studied the sensory apparatus of cercariae of trematodes belonging to *Opisthioglyphe*.

The results of these observations are interesting particularly for practical solution of complicated taxonomical problems in helminths with uncertain systematic position or of problems of species validity. An example is their application in the problem of validity of two trematode species parasitizing frogs — *O. ranae* and *Opisthioglyphe endoloba* (Dujardin, 1845). Some authors, as Dollfus (1958, 1968) and Combes and Knopffler (1965), consider *O. endoloba* to be an independent species, while others (Odening 1960, 1961 and Grabda-Kazubska 1967, 1968—1969) regard it as a synonym of *O. ranae*.

The topography of tegumentary receptors in *O. ranae* is known from the paper by Dobrovolskiy (1965) devoted to the life cycle of this species. In spite of the fact that the author could not use the nomenclature of papilla classification, which was later worked out by Richard (1971) and which is commonly used at the present time, the illustrations enable to range most of the sensillae according to this classification. The author presents only one variant of papilla distribution; in the head part he does not give papillae or groups of papillae which according to some authors (Richard 1971, Bayssade-Dufour 1979) are taken as characteristic for the cercariae of trematode species belonging to Plagiorchiidae. In Czechoslovakia, the papillae distribution in this species was studied by Nezvalová (1970) and her results are identical with that by Dobrovolskiy (1965).

In relation with the above data the subject of our study was the chaetotaxy of *O. ranae* cercariae originating from Czechoslovakia and a comparative analysis of the papillae distribution in *O. ranae* cercaria published by Dobrovolskiy (1965) and *Cercaria 4* of Richard, 1971.

MATERIALS AND METHODS

The chaetotaxy was studied in 50 cercariae obtained from naturally infected snails, *Lymnaea stagnalis* (L., 1758). The snails were collected in biotopes in the vicinity of the village Klec (30 km east of České Budějovice, Czechoslovakia). In addition, 20 cercariae from experimentally infected snails were used for a comparison (eggs from adults determined as *O. ranae* from *Rana ridibunda* were fed to snails *L. stagnalis* after incubation in a thermostat; cercariae for the study were obtained after 70 days) and 20 cercariae spontaneously passed by infected *L. stagnalis* collected in biotopes around the town Belene, Pleven region, Bulgaria.

The cercariae were impregnated by the method after Combes et al. (1976) and the papillae were described according to Richard's (1971) nomenclature.

RESULTS

Distribution of papillae

1. Head part (Fig. 1A, B, C, D)

a) papillae around moth opening
CI = 1CIV, 6CIL
CII = $1CII_1$, $1CII_2$
CIII = $1CIII_1$, $2CIII_2$, $9CIII_3$

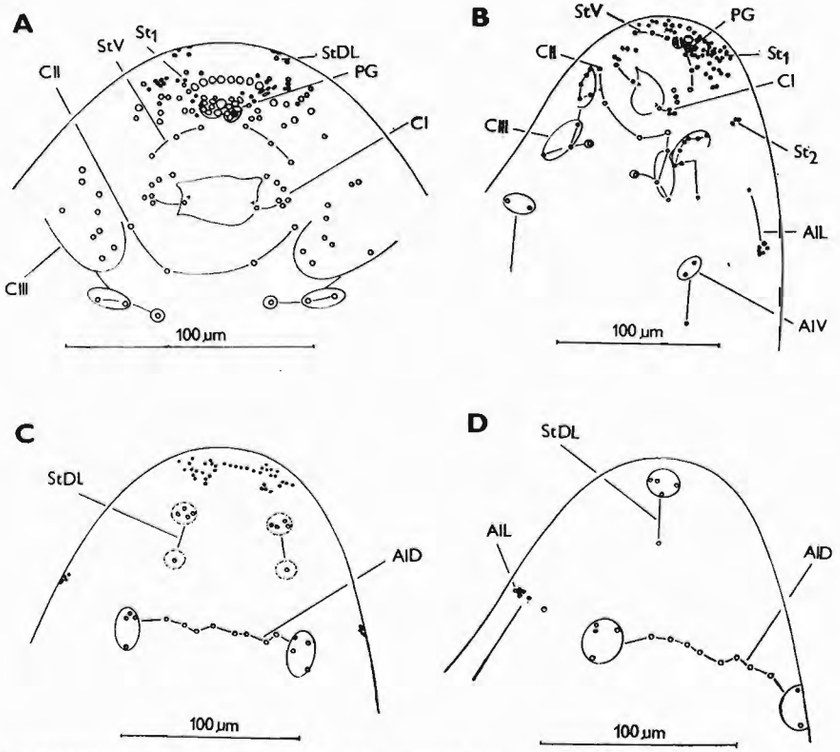

Fig. 1. *Opisthioglyphe ranae* cercaria. A—B — distribution of papillae around the mouth cavity; C—D — papillae of groups StDL and AID.

b) papillae on stylet
3StV
probably $35St_1$, $3St_2$
4 + 1STDL (another arrangement is also possible)

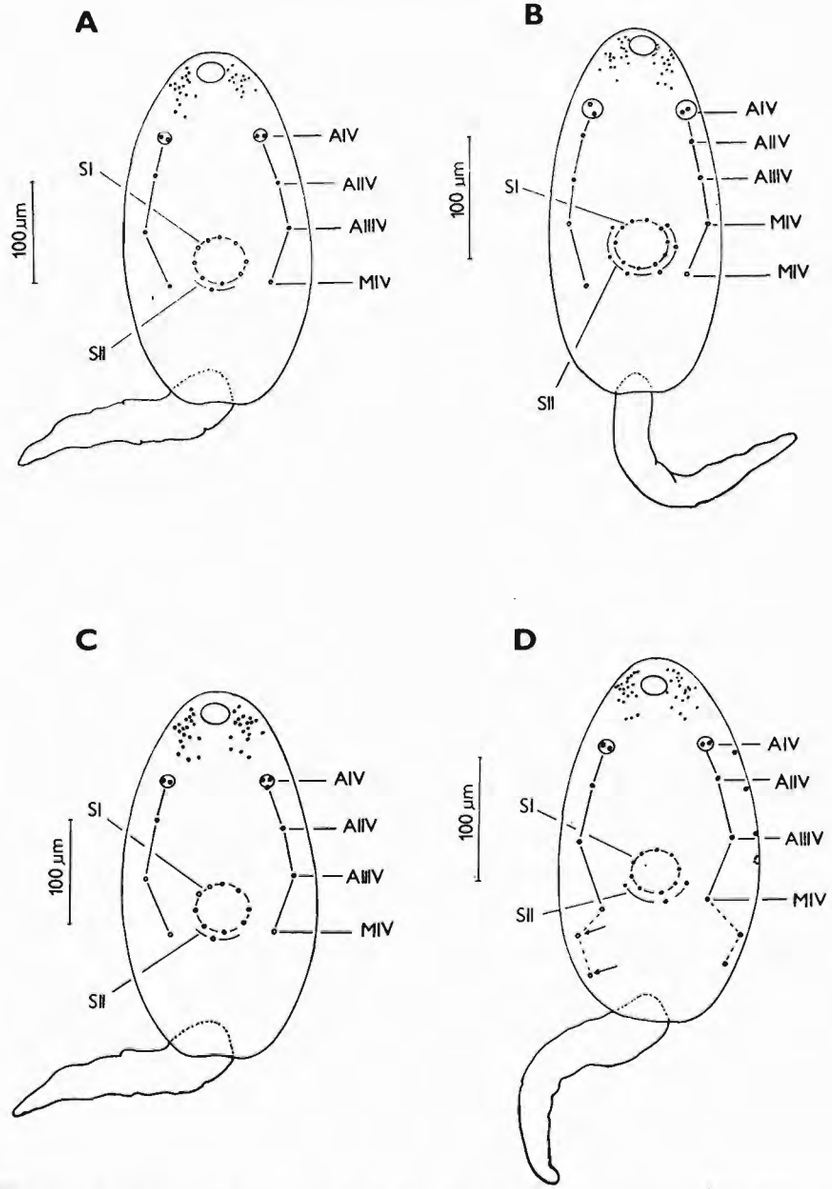

Fig. 2. *Opisthioglyphe ranae* cercaria. A, B, C, D — distribution of ventral and acetabular papillae.

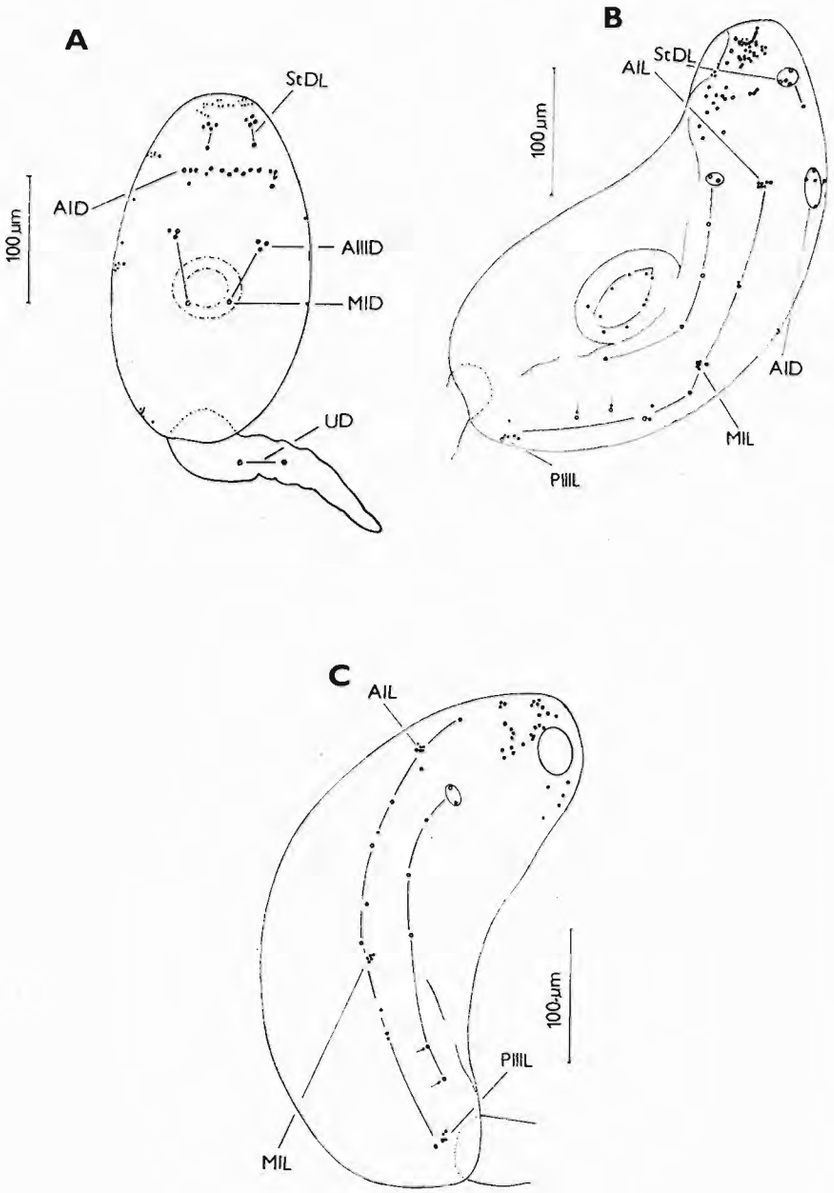

Fig. 3. *Opisthioglyphe ranae* cercaria. A — dorsal body papillae and tail papillae, B—C — distribution of lateral papillae.

2. Body (Fig. 2-A, B, C, D; 3-A, B, C)

a) ventral papillae
2AIV, 1AIIV, 1AIIIV
1 + 1MIV (also another arrangement occurs)
b) dorsal papillae
16 AID, 3AIIID
1MID (other arrangement occurs exceptionally)
c) acetabular papillae
9SI, 0-6SII
d) lateral papillae
26—29 papillae on each half of body

3. Tail papillae (Fig. 3-A)

2UD

The circle CI is incomplete, the papillae in positions CId_1 and CId_2 are absent. 1CIV papillae are invaginated. The papillae of groups CII_0, CII_3 and CII_4 are lacking in the circle CII. The papillae of the group St_1 are of different size, as it is shown in Fig. 1A. This was detected at high magnification (over 1000×) and during observations under immersions. On each half of the body immediately around the stylet there are 8 papilla-like structures of irregular form. These are the openings of penetration glands. Individual papillae sometimes occur among them. In 39 of 50 studied cercariae the StDL groups consisted of 4 + 1 papillae (Fig. 1C, D). The following combinations of the situation and number were observed in other cercariae: 3—4 + 1 papillae in 7 cercariae; 3 + 1 in 2 cercariae; 2—4 + 1 in one cercaria, and 2—3 + 1 in on cercaria.

A great variability in distribution was observed in ventral papillae, as it is evident from Table 1. Some variations are shown in Fig. 2A, B, C. The classification of some ventral papillae is problematical with regard to their position on the boundary between two neighbouring circles. These papillae can be therefore described in any of them.

The majority of dorsal papillae belonged to the groups 16AID, 3AIIID and 1MID. In 44 of the studied cercariae, AID consisted of 16 papillae. Their formula was usually 4 + 8 + 4, but when the tegument surface is very stretched in the embedding medium, the exact distribution in groups may be damaged. The following changes in the number and distribution of papillae were observed: 4 + 7 + 4 in 3 cercariae; 4 + 6 + 4 in one cercaria; 4 + 9 + 4 in one cercaria; 5 + 6 + 4 in one cercaria. Only in one cercaria occurred 3—4 papillae in AIIID position.

The acetabular papillae were distributed as follows: 9SI, 1SII in 16 cercariae; 9SI, 2SII in 5 cercariae; 9SI, OSII in 11 cercariae; 9SI, 3SII in 5 cercariae; 9SI, 4SII in 2 cercariae; 9SI, 5SII in one cercaria, and 9SI, 6SII in 2 cercariae.

Altogether 26—29 lateral papillae were present on each half of body. Characteristic is the separation of groups of tegumentary receptors AIL, MIL and PIIIL. Four to five papillae of these groups are smaller than the others. Two of the lateral papillae of the postacetabular zone (marked by arrows in Figs. 2D and 3C) are usually atypically situated outside the lateral axis of papillae and tend to approximate to the logintudinal ventral row of papillae. However, they reach the line of this row only rarely in an extreme position and in such case they can be described as 1PIIV and 1PIIIV papillae and placed to the ventral papillae (Fig. 3C). In all studied *O. ranae* cercariae there were two papillae (2UD) situated medially on the dorsal surface of tail.

No differences were observed in the chaetotaxy of *O. ranae* cercariae originating from spontaneously and experimentally infected *L. stagnalis* from Czechoslovakia. The same topography of papillae was observed also in the cercariae from Bulgaria.

DISCUSSION

The general distribution of tegumentary papillae in the studied *O. ranae* cercariae is of a plagiorchid type (Bayssade-Dufour 1979). Some significant deviations were found separating this species from all other species of the family Plagiorchidae the chaetotaxy of which has been studied. The basic difference is the arrangement of papillae in the circles CI and CII. These circles are incomplete, non-closed, without papillae in positions CId_1, CId_2, CII_0, CII_3, and CII_4. The absence of papillae in these positions has not yet been observed in the cercariae of the family Plagiorchiidae. The papillae with distinctly ventral situation in the postacetabular zone can be found only rarely. The distribution of ventral papillae in the preacetabular zone was variable in some of the cercariae.

In the majority of studied cercariae, however, the sensory apparatus has a constant arrangement. The constant distribution of papillae in the high number of studied cercariae is important for an objective evaluation if our results are compared with those of other authors who worked with a small number of cercariae or did not give the number of cercariae under study.

For a detailed comparative analysis we have used besides our results also the data on all clearly visible groups of papillae of *O. ranae* cercariae published by Dobrovolskiy (1965) and of *Cercaria* 4 as published by Richard (1971). A detailed information on the distribution of papillae in these cercariae is given in Table 2.

Dobrovolskiy (1965) did not exactly define the circles and groups of papillae in the head part. Richard (1971) gave only one variant of the arrangement of ventral and acetabular papillae.

The cercariae from Czechoslovakia and Bulgaria studied by us, as well as *Cercaria 4* of Richard, 1971, have a very similar distribution of sensory papillae. Small deviations were recorded only in the number of acetabular papillae of the outer circle (0-6SII or 6SII) and in an exceptional presence of papillae in positions PIIV and PIIIV. However, we consider the papillae in these positions to be extremely situated papillae of the lateral row, with regard to other registered cases of transient position of these two papillae in the space from lateral longitudinal line to the ventral one.

The chaetotaxy of the cercariae studied by us markedly differs from that of *O. ranae* studied by Dobrovolskiy (1965) in the number of papillae of AID complex and in most cases also in the absence of papillae in positions PIV and PIIIV. *Cercaria 4* of Richard, 1971 differs from *O. ranae* cercaria studied by Dobrovolskiy (1965) also in the number of papillae of AID complex and in an absolute absence of papillae of the postacetabular zone.

Richard (1971) cited in his paper the opinion of Combes that *Cercaria 4* may be a larval stage of *O. endoloba*.

The determination of the cercariae studied by us as the larval stage of *O. ranae* was made on the basis of an experimental study of all its developmental stages by means of scanning electron microscopy (Bušta and Našincová 1988) and their confrontation with the Czechoslovak and foreign literature. According to the data published by Czechoslovak authors (Prokopič 1957, Zajíček 1963, Žďárská 1964, Prokopič and Křivanec 1975, Vojtková 1982), only the species *O. ranae* has been recorded in frogs in Czechoslovakia. Vojtková (1982) states that this is one

Table 1. Distribution of ventral papillae in *O. ranae*

No. of cercariae	No. of papillae in positions			
	A_IV	$A_{II}V$	$A_{III}V$	M_IV
25	2	1	1	1
9	2	1	1	1 + 1
3	2	1	1 — 2	1 — 2
2	1 — 2	1	1 — 2	1
1	2	1	0 — 1	1
1	2	0 — 1	1	1
1	2 — 3	1	1	1
1	2	0 — 1	0 — 1	1
1	1 — 2	1 — 2	1	1
1	2	1	0	1
1	1 — 2	1	1	1
1	2 — 3	1	1	0 — 1
	2	1	0 — 1	1 + 1
1	2	.	1	0 — 1 + 1
1	2	1	?	1 + 0 — 1

Table 2. Data for comparative analysis of cercaria chaetotaxy in *O. ranae* and *Cercaria 4* of Richard, 1971

Groups of papillae	*O. ranae* (our data)	*O. ranae* (after Dobrovolskiy, 1965)	*Cercaria 4* (after Richard, 1971)
CIV + CIL + CId_1 + + CId_2	1 + 6 + 0 + 0	cannot be exactly	1 + 6 + 0 + 0
CII_1 + CII_2 + + CII_3 + CII_4	1 + 1 + 0 + 0	identified	1 + 1 + 0 + 0
$CIII_1$ + $CIII_2$ + + $CIII_3$	1 + 2 + 9	1 + 2 + ?	1 + 2 + 9
StV	3	not described	3
StDL	4 + 1	4 + 1	4 + 1
AIV — PIIIV	2 + 1 + 1 + 1; 2 + 1 + 1 + 1 + 1; 1PIIV, 1PIIIV usually absent; exceptionally 1 papillae in each position shifted from the lateral row	2 + 1 + 1 + 1 + + 1 + 1; present se 1PIIV, 1PIIIV	2 + 1 + 1 + 1; 1PIV — 1PIIIV absent
AID	4 + 8 + 4	4 + 4 + 4	4 + 8 + 4
AIID — PID	3 + 1	3 + 1	3 + 1
SI + SII	9SI + 0-6SII	9SI + 3SII	9SI + 6SII
AIL — PIIIL	about 29 on each half of body	about 29 on each half of body	about 29 on each half of body

of the most common species of frog trematodes in Czechoslovakia. None of the above authors has found *O. endoloba* in Czechoslovakia, not even its larval stage. On the contrary, during the studies of the morphology and chaetotaxy of cercariae from a large number of snails of many species, carried out in the last two years in two regions in Czechoslovakia, none of the cercariae had a chaetotaxy identical with that of

Scheme 1. Basic differences in the cercaria chaetotaxy of individual species of the genus *Opisthioglyphe*

O. ranae cercaria reported by Dobrovolskiy (1965), while the cercaria species studied by us was very frequent.

The view of some authors (Odening 1960, 1961, Grabda—Kazubska 1967, 1968—1969) that *O. endoloba* is a synonym of *O. ranae* was considered in the determination of species. Bychvarov (1986) arrived at the same conclusion in his dissertation paper where he compared morphological and metrical characteristics of a large number of maritae from Bulgaria, France and Poland.

The chaetotaxy of *O. ranae* cercariae studied by us differs from that of other species of the genus *Opisthioglyphe*, namely *O. rastellus* (Olsson, 1876), *O. megastomus* Baer, 1943, and *O. locellus* Kossack, 1910, which was studied by Richard (1971), Vaucher (1972), and Bock (1983). Comparative data on the distribution of papillae in these species enabling the species differentiation were given by Bock (1983). Richard (1971) paid a special attention to AID papillae and ventral papillae. The differences in the cercaria chaetotaxy in different species of the genus *Opisthioglyphe* are shown in Scheme 1.

The data given in this scheme indicate that there are considerable differences in the arrangement of AID complex and ventral papillae and that they have a special taxonomic importance for the differential diagnosis of cercariae of the genus *Opisthioglyphe*. We have used both these criteria for the differentiation of the cercariae studied by us and for those of *O. ranae* studied by Dobrovolskiy (1985).

In our opinion, the great similarity in the chaetotaxy of the cercariae studied by us with *Cercaria 4* of Richard, 1971, as well as its dissimilarity with *O. ranae* cercaria studied by Dobrovolskiy (1965), requires further information about the life cycle of *O. ranae* and other relative species from different areas of their distribution, particularly from Czechoslovakia, Bulgaria, the USSR, France, and Spain.

ХЕТОТАКСИЯ ЦЕРКАРИЙ *OPISTHIOGLYPHE RANAE* (FRÖLICH, 1971) TREMATODA; PLAGIORCHIIDAE)

В. Димитров, Я. Бушта и И. Канев

Резюме. Проводили детальные наблюдения сенсорного аппарата церкарий *Opisthioglyphe ranae* (Frölich, 1971). Видовая принадлежность была определена на основе экспериментального изучения цикла развития и морфологии спороцист, церкарий, метацеркарий и взрослых экземпляров. В отличие от других видов церкарий семейства Plagiorchidae у *O. ranae* не были обнаружены сосочки в положениях CId_1, CId_2, CII_0, CII_3 и CII_4. Очень редко встречались сосочки выразительно лежащие позади брюшной присоски. У некоторых сосочков в зоне перед брюшной присоской. На основе сделанного анализа хетотаксии изучаемая нами церкария сходна с *Cercaria 4* of Richard, 1971 с исключением количества сосочков на брюшной присоске и некоторых групп вентральных сосочков. По распределению сосочков AID и части вентральных сосочков изучаемая нами церкария отличается от церкарии *O. ranae*, изучаемой Добровольским (1965). Не были обнаружены отличия от хетотаксии экспериментально полученных церкарий *O. ranae* от L. *stagnalis* и церкарий того же вида, полученных из Болгарии.

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