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THE LIFE CYCLE OF RUBENSTREMA OPISTHOVITELLINUM SOŁTYS, 1954 (TREMATODA: OMPHALOMETRIDAE)

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Abstract. The life cycle of *Rubenstrema opisthovitellinum* Soltys, 1954, a parasite of shrews, is elucidated. The following developmental stages are described in detail: daughter sporocyst, cercaria, metacercaria, and adult. The freshwater snail *Planorbarius corneus* was found to be the first intermediate host in nature. The metacercariae were obtained from the larvae of mosquitoes *Culex molestus* and only exceptionally from snails *Physa acuta*. The adults were obtained from the small intestine of hamsters *Mesocricetus auratus*. The life cycle and the larval stages of *R. opisthovitellinum* are described for the first time.

Adult specimens of *Rubenstrema opisthovitellinum* are known to parasitize the stomach and intestine of insectivores of the family Soricidae. The species was first described by Sołtys (1954). It has been reported from various countries of Europe, e.g., by Żarnowski (1960), Pojmańska (1961), Genov and Dimitrova (1966), Matskási (1970), Prokopič and Genov (1974), and Genov (1984). Prokopič (1958, 1959) and Mituch (1970) found adults of *R. opisthovitellinum* in Czechoslovakia.

During our studies of larval trematodes in water snails in Czechoslovakia, unknown xiphidiocercariae of "*armata*" type were found in *Planorbarius corneus*. We managed to demonstrate experimentally that these were the developmental stages of *R. opisthovitellinum* and we studied the life cycle of this species under laboratory conditions.

MATERIALS AND METHODS

Cercariae released from naturally infected snails *P. corneus* collected in ponds near České Budějovice (Czechoslovakia, 150 km south of Prague) were used for the study of the life cycle of *R. opisthovitellinum*.

Water snails, *Lymnaea stagnalis*, *Planorbarius corneus*, *Biomphalaria glabrata*, and *Physa acuta*, as well as larvae of lower instars of *Culex molestus* mosquitoes, all from laboratory breedings, were tested as second intermediate hosts. The mosquito larvae were infected with 5—10 cercariae each. The snails were kept in water containing a large number of *R. opisthovitellinum* cercariae. Adult parasites were obtained from the intestine of hamsters, *Mesocricetus auratus*, which had been experimentally infected with approximately 100 metacercariae, 10—15 days old.

The trematodes were fixed in 80 % alcohol under slight pressure and stained with borax carmine. The description of adults (including measurements) was made on the basis of permanent mounts. The morphology of larval stages was studied in living specimens, using vital staining by neutral red. The metacercariae were released from the cysts mechanically. The cercariae and excysted metacercariae used for measuring were fixed in hot (70—80 °C) 4 % formaldehyde. The sporocysts and cysts with metacercariae were measured alive under a slight pressure of the cover glass. The stylet was measured in dying cercariae. If more than 20 specimens were measured, their limit values were recorded and an arithmetical mean was calculated (in parentheses).

RESULTS

The snail *P. corneus* was found to serve as the first intermediate host of *R. opisthovitellinum* under natural conditions. Numerous daughter sporocysts were localized in the hepatopancreas of spontaneously infected snail hosts.

The cercariae readily penetrated into the *C. molestus* larvae and encysted in them. The cysts with metacercariae were found in all parts of body, but most frequently in the thorax. The water snails, *L. stagnalis*, *P. corneus*, *B. glabrata*, and *P. acuta*, were also tested as second intermediate hosts, using 30 specimens of each species. Although the experimental snails were kept in water containing a large number of cercariae, encysted metacercariae were found only in 7 specimens of *P. acuta*, the incidence being 1—3 cysts per snail. These results show that mosquito larvae are suitable second intermediate hosts for *R. opisthovitellinum*, whereas some snail species can only play the role of occasional intermediate hosts. This is also supported by the fact that *R. opisthovitellinum* metacercariae have never been found in snails from nature examined for the presence of larval trematodes. An exception was the case of encysted metacercariae found inside daughter sporocysts in naturally infected *P. corneus*.

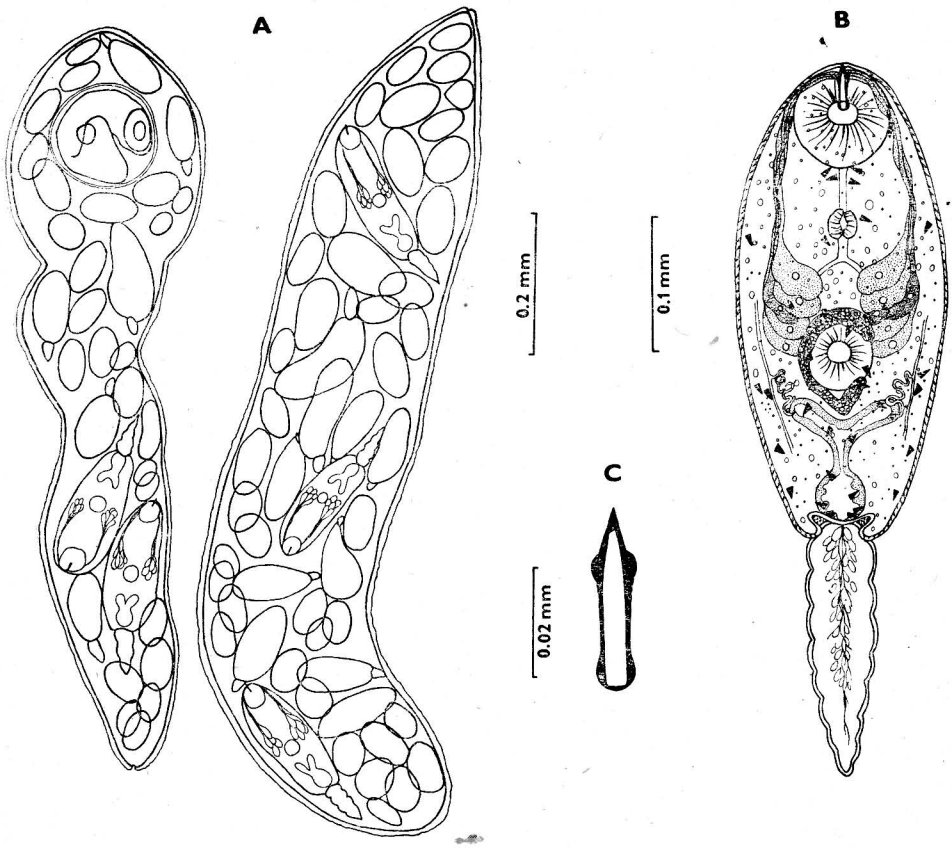

Fig. 1. *Rubenstrema opisthovitellinum* Soltys, 1954. A — sporocysts, B — cercaria, C — stylet.

Golden hamsters (*M. auratus*) were used as experimental definitive hosts. Of the eleven animals tested, only one could be successfully infected. Three mature parasites with eggs were isolated from its small intestine on day 14 p.i.

The descriptions given below are based on daughter sporocysts and cercariae from spontaneously infected *P. corneus*, 1—10 days old metacercariae from experimentally infected larvae of mosquitoes *C. molestus*, and adults from experimentally infected hamsters.

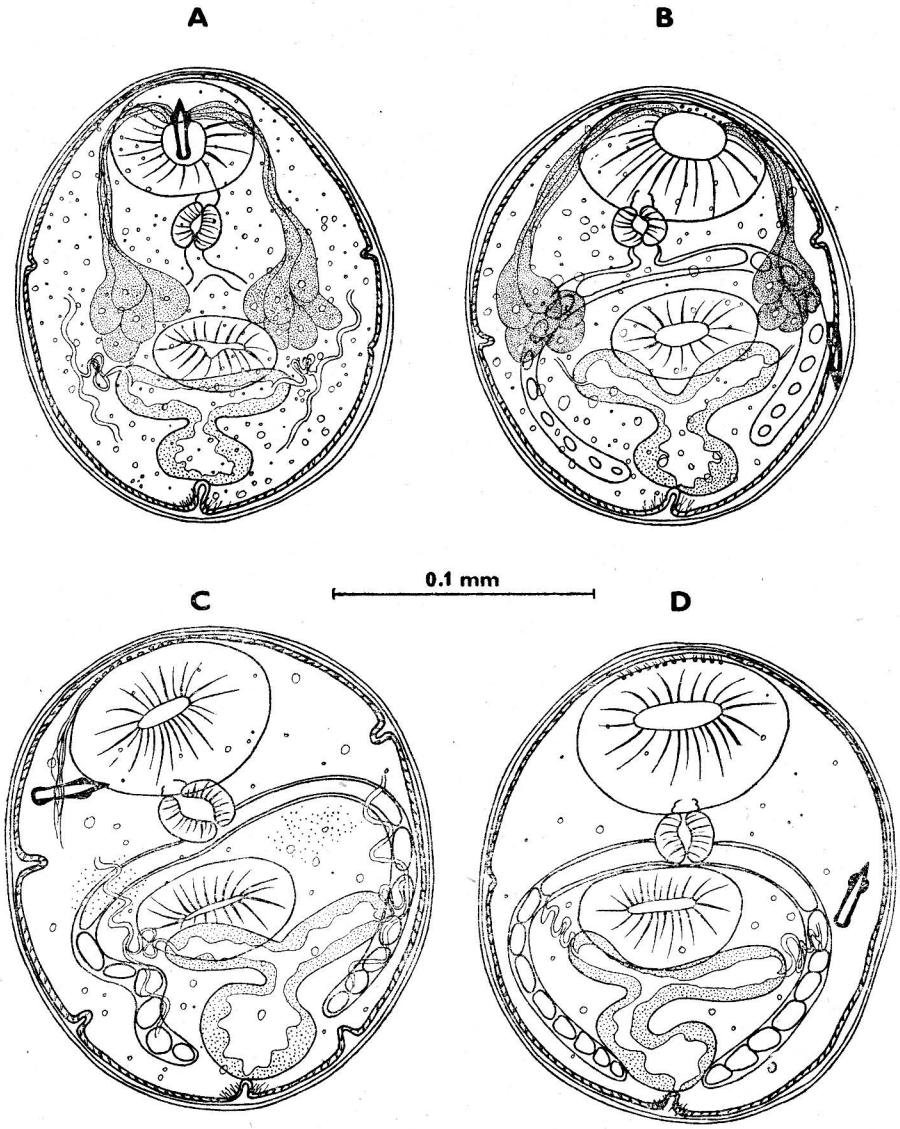

Fig. 2. *Rubenstrema opisthovitellinum* Sołtys, 1954 — metacercariae in cysts. A — on day 1, B — on day 3, C — on day 7, D — on day 10.

Description of developmental stages

Daughter sporocyst (Fig. 1A). Orange, club-shaped, 0.85—3.20 (1.33) mm long and 0.13—0.32 (0.20) mm wide, with birth pore on narrower end, containing a large number of cercariae at various stages of development (usually only 1—4 of them were mature) and, sporadically, also encysted metacercariae.

Cercaria (Fig. 1B). Body 208—240 (222) μm long and 81—93 (86) μm wide. Tail 155—195 (176) μm long and 21—25 (23) μm wide at base. Small tegumental spines regularly arranged on whole body surface. Caudal pockets bearing larger spines. Oral sucker measuring 40—45 (43) × 41—49 (44) μm, followed by conspicuous prepharynx, pharynx measuring 11—15 (12) × 13—15 (14) μm. Oesophagus bifurcating at level of first pair of penetration glands. Caeca not visible. Ventral sucker 30—34 (31) μm in diameter, situated at the beginning of posterior half of body. Penetration gland cells usually asymetrically developed, 5 on one side and 6 on the other, anterior two pairs not staining with neutral red. Other penetration glands well stainable with neutral red. Ducts of penetration gland cells opening beside stylet (Fig. 1C). Stylet 30—32 (31) μm long and 6—6.5 μm wide at base. Flame cells 36 in number. Excretory bladder Y-shaped, situated in posterior part of body. Genital primordia as a mass of cells at level of ventral sucker. Numerous refractive granules scattered in parenchyma. Cercariae exhibiting negative geotaxis.

Metacercaria. The development of metacercariae in experimentally infected mosquito larvae was followed for 10 days. The metacercariae were situated in thin-walled cysts, the size of which slightly increased during their development (Table 1). Figure 2 A—D shows 1, 3, 7, and 10 days old metacercariae in cysts. The metacercariae of the same age but released from the cysts are shown in Fig. 3 A—D.

After the first day of its development in the second intermediate host the me-

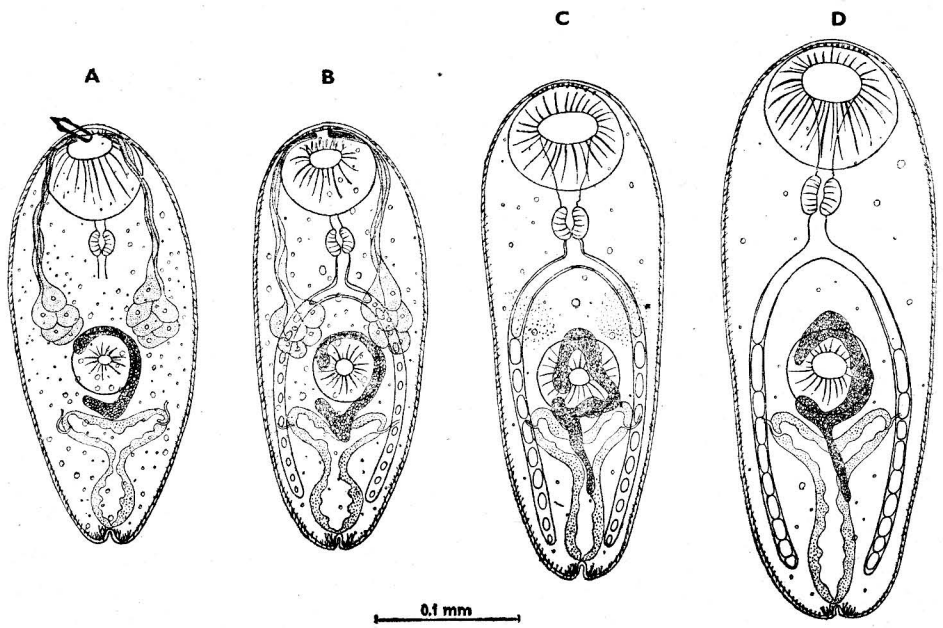

Fig 3. *Rubenstrema opisthovitellinum* Sołtys, 1954 — released metacercariae. A — on day 1, B — on day 3, C — on day 7, D — on day 10.

tacercaria preserves the shape and features of the cercaria body. The penetration glands are still distinct, the body contains numerous granules, and the stylet of most of the metacercariae remains anchored in the oral sucker. The caudal pockets extrude and in the first days of their development their relatively long spines markedly differ from the spination of the remaining body surface. The stylet is usually released during the second day of development and then freely moves in the cyst. Starting on days 2—3, the intestinal lumen gradually forms and the intestinal branches become distinct. In the first days, there is a conspicuous membranous prepharynx surface which gradually decreases during the growth in relation to other parts of the digestive system. Later on, the penetration glands and their ducts gradually disappear and the number of stored granules decreases. The dorsal lip of the oral sucker of 3—10 days old metacercariae bears 10—13 narrow canals, whose further parts in the metacercaria body are not visible.

The body of 10-day-old metacercaria is 309—334 μm long and 120—151 μm wide. The oral sucker measures 75—82 × 75—88 μm. It is followed by short prepharynx and pharynx measuring 25—35 × 25—30 μm. The oesophagus of approximately the same length as the pharynx. The intestinal bifurcation is about midway between the posterior margin of the oral sucker and anterior margin of the ventral sucker. The intestinal caeca reach close to the posterior end of body. The ventral sucker is smaller than the oral sucker, measuring 47—52 × 47—57 μm. The Y-shaped excretory bladder is situated in posterior third of body and does not contain excretory granules. The genital primordia are only little differentiated and are situated at the level of the ventral sucker and anterior part of the excretory bladder.

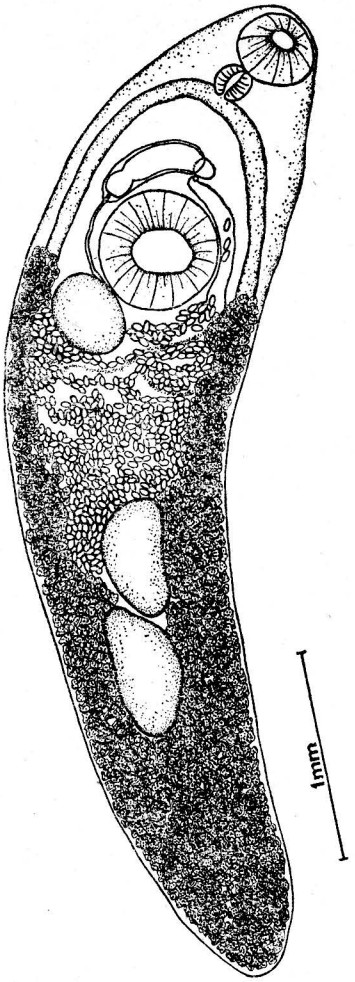

Fig 4. *Rubenstrema opisthovitellinum* Soltys, 1954. Adult trematode.

Adult (Fig. 4). Trematode of medium size. Body elongate, 4.69—4.94 mm long and 1.00—1.18 mm wide. Body surface covered with fine tegumental spines, their density and size decreasing towards caudal end. Oral sucker subterminal, measuring 369—412 × 352—447 μm. Pharynx 146—183 × 126—142 μm. Intestinal caeca long, reaching close to posterior end of body. Acetabulum at end of anterior third of body and measuring 559—610 × 541—602 μm. Testes oval, elongate, mostly with entire margine, exceptionally lobed, situated in posterior half of body. Anterior

Table 1. Size of cysts with metacercariae *of R. opisthovitellinum* from experimentally infected larvae of *C. molestus*

Age of metacercariae (days)	Size of cysts (μm)
1	135 – 163 (150) × 113 – 149 (122)
3	145 – 170 (153) × 123 – 148 (136)
7	133 – 173 (158) × 125 – 157 (141)
10	153 – 187 (166) × 133 – 163 (148)

testis 498—636 × 275—344 μm, posterior testis 636—713 × 344—361 μm. Cirrus sac arched, oblique, measuring 464—602 × 172—197 μm, its basal part overlapping anterior margin of ventral sucker. Wide cirrus and distinctly separated seminal vesicle contained in cirrus sac. Genital pore submedian, preacetabular. Ovary oval, submedian, overlapping posterior margin of acetabulum and measuring 387—438 × × 292—361 μm. Uterus with numerous eggs filling the space limited anteriorly by posterior margin of acetabulum and ovary and by vitelline follicles on sides, posteriorly reaching at most to middle of anterior testis. Vitellaria consisting of numerous small follicles covering one another and reaching up to posterior margin of acetabulum. Posterior part of body behind posterior testis completely filled with vitellaria. Eggs rather large, measuring 62—65 × 23—26 μm.

DISCUSSION

The life cycle of *R. opisthovitellinum* has not been known previously. The first descriptions of its daughter sporocysts, cercariae and metacercariae are given in this paper.

The adult specimens of *R. opisthovitellinum*, which were obtained experimentally from the above-described xiphidiocercaria, completely conform in their morphology to the original description of this species (Sołtys 1954). The differences in measurements do not exceed the range of species variability. It should be noted, however, that the body length of 2.28—3.5 mm and maximum body width of 1.2 mm recorded by Sołtys (1954) do not correspond to the data evident from the accompanying figure. If compared with the given scale, the body length should be about 4.8 mm, which corresponds to the measurements of the trematodes obtained experimentally by us.

The cercariae of the type recovered from snails *P. corneus* have been reported by many authors, as Brumpt (1944), Zdun (1959), Odening (1962), Nouveau and Matricon-Gondran (1962), Palm (1966), Ginetsinskaya and Dobrovolskiy (1968), Frolova (1975), Bock (1982, 1985), and Chernogorenko (1983); in Czechoslovakia by Žďárská (1963), Nezvalová (1970), and Balůsek and Vojtek (1973). However, the above-described larval stages did not conform to any of these descriptions available due to differences in their morphology and biology or to incomplete or inexact descriptions. Moreover, we assume that the descriptions of this cercariae often concern several related species. For example, Brumpt (1944) recorded the length of stylet in *Cercaria polyxena* to be 28—38 μm. Odening (1962)

described his *Xiphidiocercaria* sp. 7 with a 30—34 μm long stylet. The stylet lengths recorded by other authors for the same species were as follows: 36 μm (Ginetsinskaya and Dobrovolskiy (1968), 30—36 μm Palm (1966), 28—36 μm Nezvalová (1970), and 40 μm Frolova (1975). According to our experience, such variability in the stylet length is improbable in one species. Bock (1985) is of the same opinion. He found 9 cercaria species with 5 + 6 penetration gland and about 30 μm long stylet in snails *P. corneus*.

The life cycles of three related species belonging, according to Skryabin (1966) and Yamaguti (1971), to the family Omphalometridae have been recently described: *Neoglyphe locellus* (Bock 1982), *Rubenstrema exasperatum* (Bušta and Našincová 1991), and *R. opisthovitellinum* (this paper). Whereas the adults of each of these species can be easily differentiated on the basis of morphology, it is difficult to distinguish their very similar larvae. The cercariae of these three closely related species have the same number of penetration glands (5 + 6). They differ in the arrangement of the penetration gland cells, length of stylet, size of suckers, presence of refractive granules in cercaria body, and chaetotaxy. The differential diagnosis of developmental stages of these species will be published elsewhere.

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