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DEVELOPMENTAL CYCLE OF RUBENSTREMA EXASPERATUM (RUDOLPHI, 1819) (TREMATODA: OMPHALOMETRIDAE)

J. BUŠTA and V. NAŠINCOVÁ

Institute of Parasitology, Czechoslovak Academy of Sciences, Branišovská 31, 370 05 České Budějovice, Czechoslovakia

Abstract. The developmental cycle of the trematode *Rubenstrema exasperatum* (Rudolphi, 1819), a common parasite of insectivores of the family Soricidae, is described here for the first time. The first intermediate host in nature is the snail *Planorbarius corneus*. Metacercariae encysted experimentally in larvae of the mosquito *Culex molestus*; exceptionally, these were found encysted in daughter sporocysts. Adult specimens of *R. exasperatum* were found after experimental infections in the hamster *Mesocricetus auratus*. Daughter sporocysts, cercariae, metacercariae and adults are described in detail.

During studies on larval trematodes from water snails, we often found a xiphidiocercaria in *Planorbarius corneus*. We were not able to identify it using both Czechoslovak and foreign literature. The cercaria penetrated into the larvae of mosquitoes and encysted in them. Following several unsuccessful feeding experiments, we succeeded in infecting hamsters with experimentally obtained metacercariae. We have identified the adults as *Rubenstrema exasperatum* (Rudolphi, 1819).

The trematode *R. exasperatum* was found in Czechoslovakia in several localities of Slovakia and South Bohemia by Prokopič (1956, 1958, 1959), Mituch (1964, 1970), Prokopič and Genov (1974) and Matskási (1984) in *Sorex araneus*, *S. minutus*, *Neomys fodiens* and *N. anomalus*. Numerous data are available on its distribution in other European countries. Sołtys (1952, 1954), Pojmańska (1957, 1961) and Żarnowski (1960) found it in Poland, Shaldybin (1953), Merkusheva (1966), Arzamasov et al. (1969) and Andreyko (1973) in USSR, Genov and Dimitrova (1966) and Prokopič and Genov (1974) in Bulgaria, Chiriac and Hamar (1966) in Romania and Matskási (1970) in Hungary.

As far as we know, the life cycle and morphology of larval stages of this species have not yet been described. This contribution presents data on the development, under both natural and laboratory conditions, and a detailed description of larval stages of *R. exasperatum*.

MATERIALS AND METHODS

Cercariae spontaneously emerging from naturally infected snails (*P. corneus*) were used to study the life cycle of *R. exasperatum*. Snails were collected in ponds and their connecting canals near České Budějovice (150 km south of Prague, Czechoslovakia).

Water snails (*Lymnaea stagnalis*, *Radix auricularia*, *Planorbarius corneus*, *Biomphalaria glabrata*, *B. pfeifferi*, *Physa acuta*) and larvae of lower instars of mosquitoes (*Culex molestus*) originating from laboratory breeding were used to detect the range of second intermediate hosts. Twenty individuals of each snail species were used in the experiment. The snails were maintained in water containing large numbers of cercariae. The mosquito larvae were separately placed in 1 ml of water and infected with a dose of 5–10 cercariae.

The following animals were tested as the definitive hosts: hamsters (*Mesocricetus auratus* 22),

laboratory mice (*Mus musculus* 4), frogs (*Rana ridibunda* 2), duck (*Anas platyrhynchos* f. *dom.* 1), chicken (*Gallus gallus* f. *dom.* 1) and canary (*Serinus canaria* 1). These were infected with a dose of 50—100 metacercariae which were 7—10 days old.

Morphology of larval stages was studied in living specimens. Neutral red was used for vital staining. Sporocysts and cysts with metacercariae were measured alive under a slight pressure of the cover glass. Cercariae and excysted metacercariae were fixed in 4 % hot (70—80 °C) formaldehyde. The stylet was measured in dying cercariae. Provided that measurements of at least 20 specimens were available, the arithmetic means of the obtained sizes are given in parentheses in the following discussion. Metacercariae were released from cysts mechanically.

Adults were fixed in 70 % alcohol by a slight pressure of the cover glass and stained with borax carmine using a standard method. The description and measurements of adults were obtained from permanent mounts.

RESULTS

The snail *P. corneus* was found to be the first intermediate host of the trematode *R. exasperatum* under natural conditions. Daughter sporocysts are localized in the hepatopancreas of the host snails.

Larval stages of *R. exasperatum* belong to the most frequent species occurring in water snails in Czechoslovakia. Until now, snails collected in 48 localities of Bohemia have been examined for the presence of larval trematodes. The snail *P. corneus* was found in 15 of these localities and larval stages of *R. exasperatum* parasitizing *P. corneus* were found in 8 localities. The total prevalence ranged between 2.5—33.3 %. Cercariae were released from naturally infected snails from early spring to late autumn.

The second intermediate hosts are mosquito larvae. These are attacked by cercariae immediately. The encysted metacercariae can be found in all body parts within a few minutes after infection. All attempts were unsuccessful to infect the following snails: *L. stagnalis*, *R. auricularia*, *P. corneus*, *B. glabrata*, *B. pfeifferi* and *P. acuta*. The same situation was observed under natural conditions: metacercariae of *R. exasperatum* were not found in snails. The rare exceptions were naturally infected *P. corneus* snails in which cercariae encysted inside the daughter sporocysts.

During our feeding experiments only 3 golden hamsters were infected successfully. Two hamsters dissected 14 days p.i. contained one and three subadult flukes in the small intestine. In the third animal, one adult with eggs was observed 28 days p.i.

Descriptions are of the following developmental forms given below; daughter sporocysts and cercariae from the naturally infected snail (*P. corneus*), 1—10 days old metacercariae from larvae of the mosquito (*Culex molestus*), the adult from the hamster (*M. auratus*).

Daughter sporocyst (Fig. 1A). White to orange coloured, body club-shaped, 0.96 to 1.92(1.31) × 0.16—0.34(0.25) mm, with birth pore at narrower end, containing cercariae at different stages of development and sometimes also encysted metacercariae.

Cercaria (Fig. 1B). Body 189—246(221) × 99—123(110) μm, tail 151—183 (164) μm long and 22—27(24) μm wide at base. Surface of body covered with regularly arranged small tegumental spines. Caudal pockets conspicuous, spines of caudal pockets larger than body spines. Oral sucker 48—53(50) × 48—62(54) μm, prepharynx short, muscular pharynx 17—24(20) × 17—22(20) μm, oesophagus bifurcating at level of first pair of penetration glands, thin caeca reaching to posterior part of excretory vesicle. Ventral sucker 39—43(41) × 38—52(43) μm, just postequatorial. Penetration glands anterolateral to acetabulum, arranged successively, five on one side and six on the other side, their ducts in two bundles on each side, opening

besides stylet; two anterior pairs containing somewhat coarse granulated plasma, in contrast to others are not stainable with neutral red. Stylet (Fig. 1C) 35—38(36) μm long, 6.5—7 μm wide at base. Flame cells visible readily, flame cell formula 2 [(3 + + 3 + 3) + (3 + 3 + 3)] = 36. Excretory vesicle Y-shaped, thick-walled, situated posteriorly. Genital primordia as a mass of cells at level of ventral sucker.

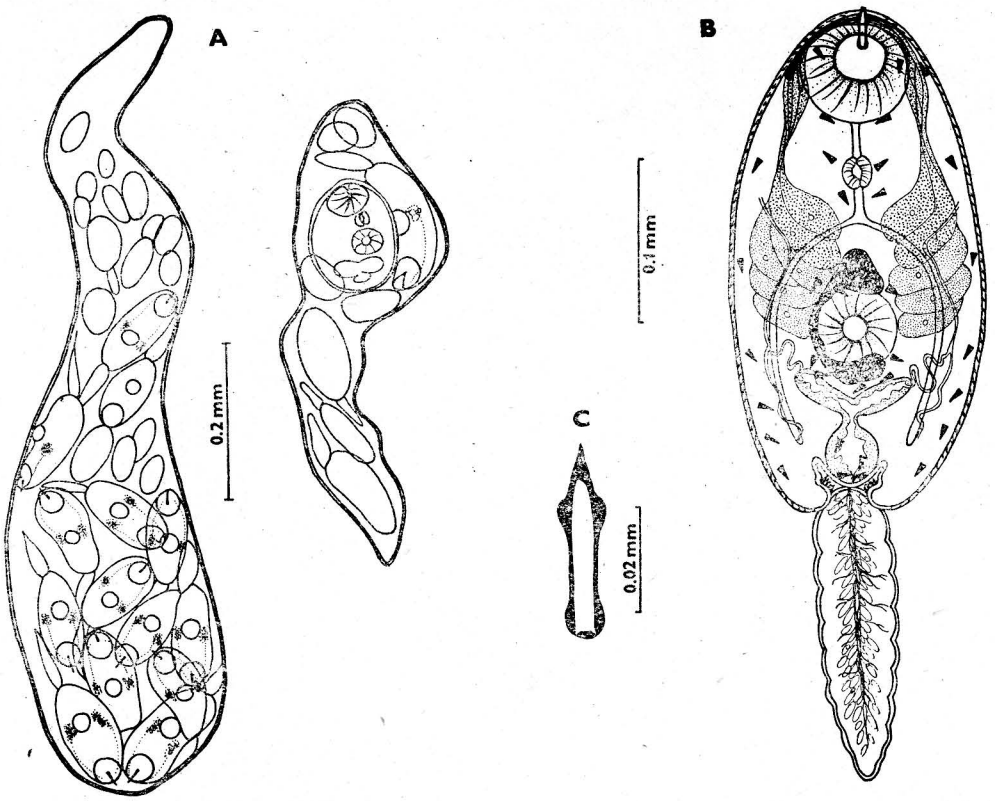

Fig. 1. *Rubenstrema exasperatum* (Rudolphi, 1819). A — sporocysts, B — cercaria, C — stylet.

Metacercaria (Figs. 2A—D). Metacercariae enclosed in oval thin-walled cysts size of which slightly increasing during development. Cysts with metacercariae measure 134—173(153) × 105—159(130) μm, 141—183(158) × 118—162(137) μm, 147 to 185(162) × 126—164(142) μm, 147—196(166) × 130—171(146) μm after 1, 3, 7, and 10 days, respectively.

Eight-day-old excysted metacercariae measure 378—473 × 136—167 μm. Body surface, including extruded caudal pockets, covered with tegumental spines. Oral sucker 90—100 μm in diameter, prepharynx short, pharynx measuring 31—35 by 29—31 μm, oesophagus about 20 μm long bifurcating just in front of acetabulum, wide caeca reaching nearly to the caudal end of the body. Ventral sucker 71—86 by 74—82 μm. Y-shaped excretory vesicle without granules situated in posterior quarter of body. Slightly differentiated genital primordia at level of ventral sucker and anterior part of excretory vesicle.

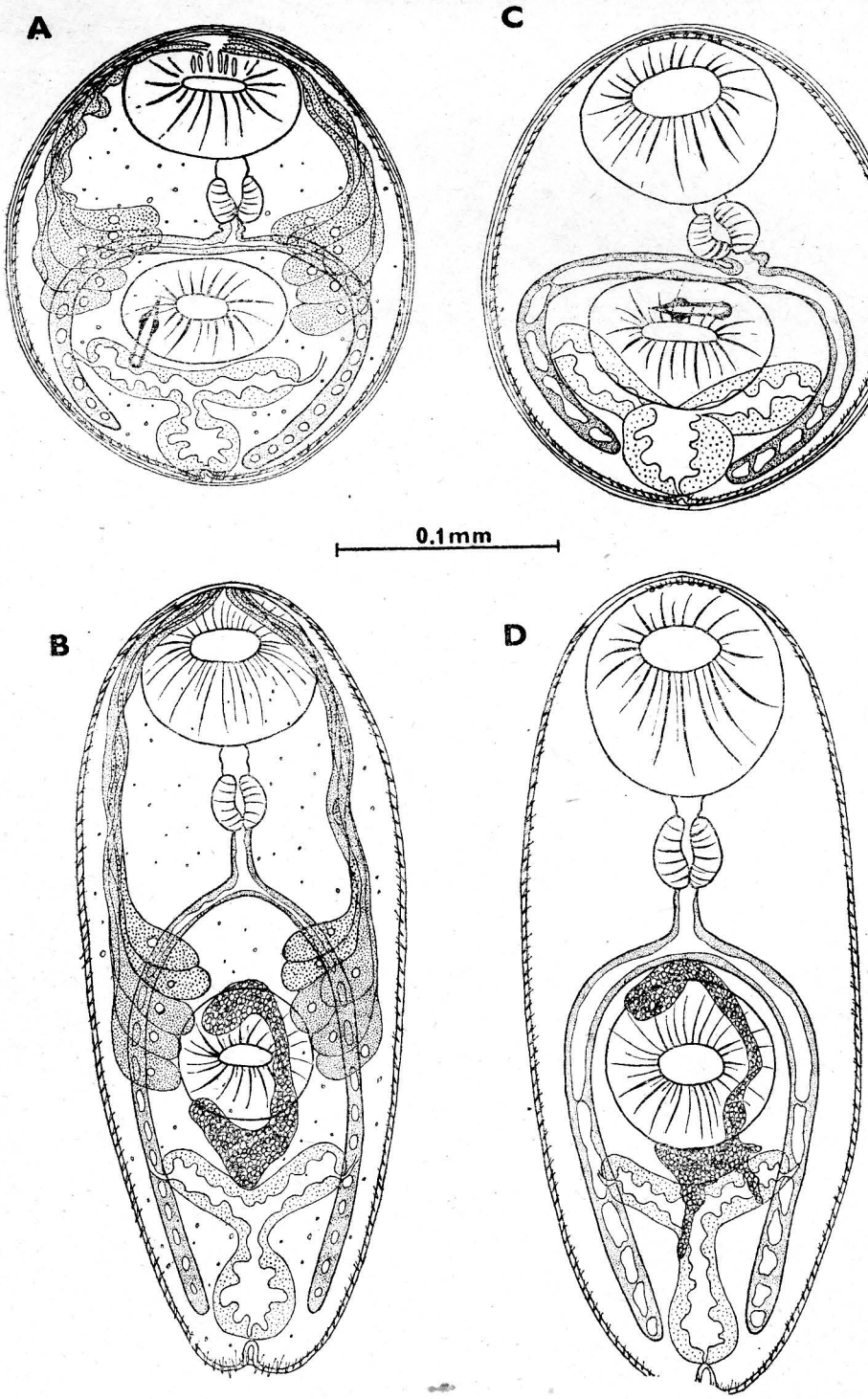

Fig. 2. *Rubenstrema exasperatum* (Rudolphi, 1819) — encysted and released metacercariae. A, B — on day 3, C, D — on day 8.

Usually during the first day after the cercaria encystation in the second intermediate host, the stylet releases from the oral sucker. It then freely moves in the cyst. Soon after encystation, the extrusion of caudal pockets can be observed. The size differences of body and caudal pocket spines remain apparent in the early stages of development. The intestinal lumen forms gradually. The caeca of the mature metacercaria are rather wide and visible readily in comparison with the cercaria. In three-day-old metacercaria, penetration glands and their ducts are still preserved (Figs. 2A,B). However, they are gradually destroyed in the next few days and they are not visible at all in eight-day-old metacercaria (Figs. 2C,D). After a few days of development, eight narrow ducts can be observed on the dorsal lip of the oral sucker. They are not perceptible, however, in the body of the metacercaria.

Adult (Fig. 3). Medium sized trematode. Body elongated, 3.07 × 0.91 mm, forebody rounded, hindbody lanceolate. Body surface covered with small tegumental spines. Oral sucker subterminal, 361—387 μm, prepharynx very short, about 20 μm; pharynx rounded, 175 × × 188 μm, oesophagus practically absent; caeca running from pharynx first laterally then turning caudally, terminating close to posterior extremity. Acetabulum preequatorial, measuring 533 × 455 μm. Testes of kidney-like shape, postequatorial, diagonal, separated one from the other by uterus. Anterior testis 283 × 146 μm, posterior testis 258 × 120 μm. Cirrus pouch situated diagonally anterior to acetabulum, entirely preacetabular (its posterior part reaching the level of the anterior margin of the acetabulum); enclosing convoluted cirrus, pars prostatica and vesicula seminalis; cirrus partly extruded in the described specimen. Genital pore submedian, situated beyond intestinal bifurcation.

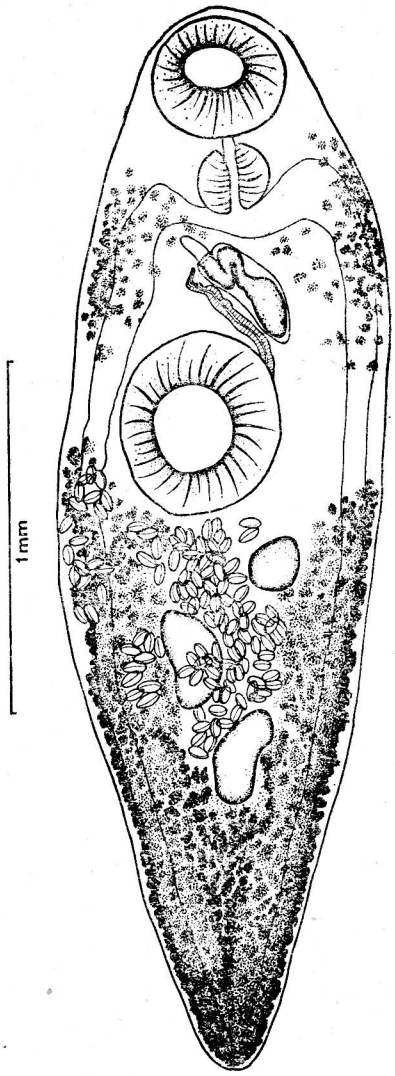

Fig. 3. *Rubenstrema exasperatum* (Rudolphi, 1819). Adult trematode.

Ovary posterolateral to acetabulum, measuring 161 × 136 μm. Uterus winding in intracaecal field between middle of posterior testis and genital pore, laterally overreaching caecum on one side; large eggs numerous, size of eggs on the permanent mount 59—67 × 29—32 μm. Vitellaria composed of large number of mutually overlapping follicles of different size; extending in lateral fields from pharynx to posterior body end but missing in acetabular zone, anterior groups of follicles not confluent in median line, posterior parts of vitellaria developed more profusely, united beyond posterior testis and fully filling posttesticular space.

DISCUSSION

The life cycle of the trematode *R. exasperatum* (Rudolphi, 1819) has not been known. This is in contrast with the fact that it has been 180 years since the description of this species. Dollfus (1949) reported that this parasite had been found only four times before that year. Since that time it has been reported more often in helminthfaunistic studies by numerous authors from various places in Europe, but without mention of its development. The only exception is the note of Pojmańska (1961) who presumed that the first intermediate hosts were water snails and the second ones could be larvae and imagoes of insects. Historical reports concerning this species and its systematic classification are given by Skryabin (1966). He separated within the family Omphalometridae an independent genus *Rubenstrema* on the basis of vitellaria interrupted at the acetabular zone and the entirely preacetabular arrangement of the cirrus pouch. He placed *R. exasperatum* in this genus. Yamaguti (1971) assigned the same status to this species.

The adult of *R. exasperatum* which we obtained does not differ essentially in its size and morphology from reports of various authors (Dollfus 1949, Shaldybin 1953, Pojmańska 1961). Only the very short prepharynx observed by us was not described earlier.

R. exasperatum cercariae were found only in *P. corneus*. In addition, other closely related xiphidiocercariae, e.g. *Neoglyphe locellus* and *Rubenstrema opisthovitellinum*, have been described in the same snail (Bock 1982, Bušta and Našincová 1986, Našincová and Bušta 1991). In Czechoslovakia, and according to the literature data also in neighbouring countries, these species represent an eudominant group of xiphidiocercariae parasitizing *P. corneus*. We suppose that, due to their great similarity, they were not distinguished and identified as separate species. The great variability in size of some organs (especially the stylet) supports our assumption that numerous earlier descriptions include two or more cercaria species under the same name. Our opinions concerning this possibility have already been mentioned elsewhere (Našincová and Bušta 1991). The differential diagnosis of developmental stages of *R. exasperatum*, *R. opisthovitellinum*, and *N. locellus* will be published separately.

Out of all known reports on larval stages of this type of xiphidiocercariae, only that one described as *Xiphidiocercaria* sp. 7 Odening, 1962 by Ginetsinskaya and Dobrovolskiy (1968) can be considered identical with *R. exasperatum*. According to Odening's original description (Odening 1962), the *Xiphidiocercaria* sp. 7 has a shorter stylet (30—34 μm) and, therefore, probably belongs to a different pecies.

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