NATURAL INFECTION OF THE SNAIL *BIOMPHALARIA OBSTRUCTA* IN LOUISIANA WITH *RIBEIROIA ONDATRAE* AND *ECHINOPARYPHIUM FLEXUM*, WITH NOTES ON THE GENUS *PSILOSTOMUM* [1]

EMILE A. MALEK
Department of Tropical Medicine, Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana 70112

ABSTRACT

The tropicorbid snail *Biomphalaria obstructa* was found to be naturally infected with the trematode *Ribeiroia ondatrae* (Price, 1931) and with the metacercariae of *Echinoparyphium flexum*, in southeastern Louisiana. The systematic status of *Ribeiroia ondatrae* in the Americas is discussed.

Records of tropicorbid snails, *Biomphalaria obstructa* (Morelet) from the United States are from Florida near Miami, several localities in southeastern Louisiana, and from Texas. This area of the United States where tropicorbid snails are found represents the northernmost zone of distribution of these and other neotropical planorbids. *B. obstructa* and several other related species are referred to as "tropicorbids" because they used to belong to the genus *Tropicorbis*, which has been brought into synonymy with *Biomphalaria* by the International Commission on Zoological Nomenclature.

In a study of the susceptibility of Louisiana tropicorbids to infection with *Schistosoma mansoni*, and their morphology, distribution and ecology (Malek, 1967, 1969), their natural infection with trematodes was also determined based on the examination of a large number of specimens from several localities. A comparison was made of reported trematodes in the same or related species in the neotropics, and in other planorbids in areas of the United States north of the zone of distribution of the tropicorbids.

MATERIALS AND METHODS

Tropicorbid snails were collected from 24 localities, mostly in southeastern Louisiana. The snails were isolated to check on their cercarial shedding. They were kept under observation for at least a month from the time they were collected from the field to allow immature infections, if any, to reach maturity and the shedding of cercariae. Some of the snails which did not shed cercariae were dissected in order to determine the occurrence of larval stages in their tissues. Associate snails in the habitat were also examined for their trematode infections. Life cycle studies were conducted to determine the identity of some of the larval forms. Trematode eggs from the feces of definitive hosts, which had been force-fed with infective

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larval forms, were incubated in water in petri dishes. Water in the dishes was changed every other day. In addition, fecal sediment was also placed on strips of filter paper in test tubes with about an inch of water following the method which had been advocated by Harada and Mori (1955), and which was adapted for the incubation of helminth eggs (Beaver et al., 1964).

RESULTS

The survey revealed the infection of tropicorbid snails in one population at Willswood Pond, Jefferson Parish, southwest of New Orleans with cercariae proved to be those of *Ribeiroia ondatrae* (Price, 1931), when the experimental life cycle was carried out. In four other populations (Clayton Pond; lake at auditorium, Louisiana State University campus in Baton Rouge; at Bonnet Carre Spillway near Norco, St. Charles Parish, and at Willswood Pond) echinostome metacercariae were found in the tissues of the snails. These proved to be the metacercariae of *Echinoparyphium flexum* (Linton, 1892) Dietz, 1910 when fed to a pigeon. A plagiorchid metacercaria was found in two populations at Blind River, Ascension Parish, and at a bayou along Highway 51, St. John Parish, while in three populations an undetermined strigeid cercaria was found (bayou along Highway 51 near Lake Maurepas; Mississippi Delta at Pass a Loutre, Plaquemines Parish; and Bonnet Carre Spillway near Norco).

1. *Ribeiroia ondatrae* (Price, 1931).

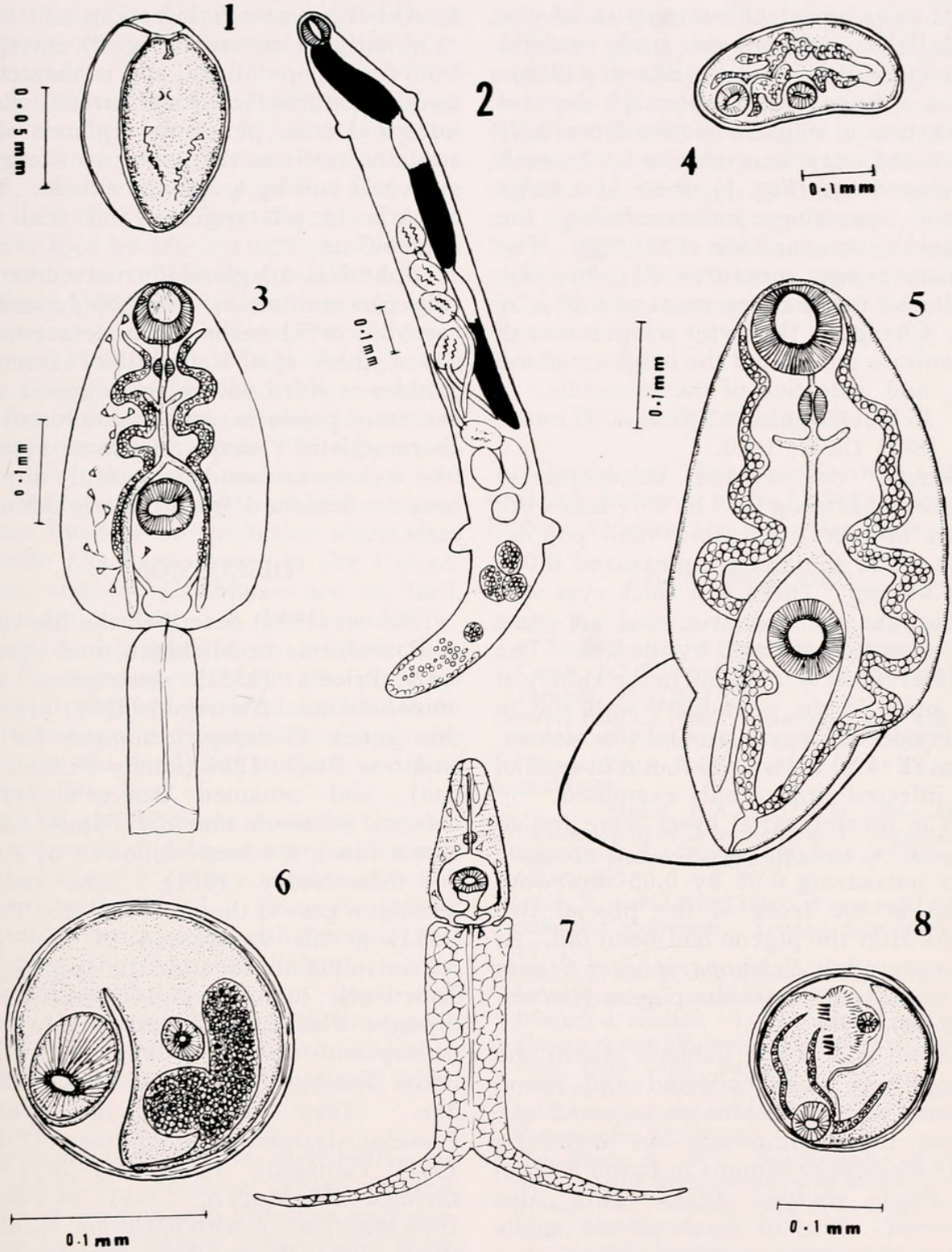
a. *Redia* and *Cercaria*: Daughter rediae (Fig. 2), were recovered from infected snails; mature rediae are orange in color, but young rediae are colorless except for the gut. The gut is about half the length of mature rediae, but more than half the length of immature ones. The redia possesses a "collar" in the anterior region, and two "appendages" in the posterior third of the body. The birth pore is situated immediately behind the collar. An average of 68 daughter rediae were collected from each of snails 7 to 9 mm in greatest diameter.

The cercaria (Fig. 3), emerged from the snails in the early hours of the evening. Morphological details of the cercaria conform with those described by Beaver (1939) for *Psilostomum ondatrae*. Prevalence of infection among *B. obstructa* was four snails out of 62 or 6.5%. Forty-seven *Helisoma trivolvis lentum* from the same habitat were not infected with this fluke.

Apparently cercariae leave the rediae and spend a maturation period in various tissues of the snail, the majority being in the ovotestis and digestive gland region. After leaving the snail the cercariae are active; they swim continuously and they do not tend to concentrate.

b. *Metacercaria*: The cercaria encysted readily in guppies (*Lebisthes reticulatus*) and goldfish (*Carassius auratus*). Fifty cercariae were placed with one guppy, and 18 metacercariae were later recovered from the guppy. The metacercariae (Fig. 4,5) were located in the internal nares and roof of the pharynx; some were found around the eyes; one was found externally at the junction of body and pectoral fin. Inside its kidney-shaped cyst the metacercariae seemed to be highly developed morphologically, and it took a short period in the definitive host for them to develop to adult flukes.

c. *Adult Fluke:* Thirty metacercariae were fed to each of two 3-week old chicks, and 36 cysts were fed to a canary (*Serinus canarius*) but they failed to develop in these hosts. However, when 30 cysts were fed to each of two white mice (*Mus musculus*), eggs were found in the feces eight days later. When the mice were sacrificed two weeks after they were fed the metacercariae an average of 22 adult worms were recovered from the duodenum immediately posterior to the pyloric sphincter. There were large granulomas with areas of hemorrhage and central areas of necrosis. Many eggs were evident in the lesion. The worms were massed within the granulomas in separate pouches or tunnels, and they were very firmly lodged in the tissues with both suckers. The muscularis propria did not seem to be invaded but



Figs. 1-8. Trematode infections of the snail *Biomphalaria obstructa* in Louisiana. 1. Egg of *Ribeiroia ondatrae* from mouse feces, with a fully developed miracidium. 2. Redia of *R. ondatrae* with cercariae, cercarial embryos, germ cells and germ balls. 3. Cercaria of *R. ondatrae* shed by *B. obstructa*. 4. Metacercaria of *R. ondatrae* from a guppy. 5. Excysted metacercaria of *R. ondatrae*. 6. Plagiorchid metacercaria from tissues of *B. obstructa*. 7. Strigeid cercaria shed by *B. obstructa*. 8. Metacercaria of *Echinoparyphium flexum* from tissues of *B. obstructa*.

the mucosa was completely disrupted.

Morphological characteristics of the adult flukes in the present study conform with those described by Beaver (1939).

d. *Egg:* After about 16 days of incubation of eggs in mouse feces fully developed ones were obtained. In each developed egg (Fig. 1), there is a large vacuole pressing and confining the miracidium to one side of the egg. The immature egg measures 84μ by 48μ, while the mature egg measures 98μ by 58μ. Change of the water temperature of the culture stimulated the hatching of the egg, and collection of the miracidia.

2. *Echinoparyphium flexum* (Linton, 1892) Dietz, 1910.

Round echinostome metacercariae (Fig. 8) measuring 0.17 by 0.17 mm were found in four tropicorbid snail populations. The acetabulum measured 0.035 by 0.04 mm. There is a thick cyst wall around the metacercaria, and an outer cyst apparently formed by the host. The metacercariae were found in the kidney of the snail, in the pulmonary wall and in the connective tissue around the rectum. From 22 to 30 cysts were found in each of the infected tropicorbids examined.

The metacercarial cysts were fed to two chicks, and one pigeon. Echinostome eggs measuring 0.08 by 0.05 mm were found in the feces of the pigeon two weeks after the pigeon had been fed. At necropsy adult *Echinoparyphium flexum* were recovered from the pigeon (*Columba livia*).

From one of the habitats where the tropicorbids were collected and found infected with this echinostome (small lake across from auditorium on Louisiana State University campus in Baton Rouge) 31 *Physa anatina* snails were also collected. Two of these physid snails contained metacercarial cysts identical to those found in the tropicorbid snails. Moreover, one physid snail was shedding an echinostome cercaria. When placed in the same container with laboratory-reared tropicorbids and physids the cercariae encysted readily in both species of snails, and metacercarial cysts were recovered from them and were identical to those found naturally in both species of snails.

3. Other trematode infections.

A strigeid cercaria (Fig. 7) emerged from three populations, and is characterized by attenuated long furcae, stout intestinal ceca, penetration glands situated anteriorly at the penetration organ, stout tail and by a spinous cuticle. The cercaria is pharyngeate and with an acetabulum.

Spherical plagiorchid metacercariae (Fig. 6) were found in two populations of the tropicorbid snails. The metacercaria has a thick cyst wall. The excretory bladder is filled with black pigment and excretory products, and has retained its characteristic Y-shape. In some cysts a free stylet was found, apparently after it became detached from the xiphidiocercaria.

DISCUSSION

Beaver (1939) described the life cycle of *P. ondatrae* in Michigan, and agreed with Price's (1931) description and nomenclature. Yamaguti (1958) erected the genus *Pseudopsilostomum* for *P. ondatrae* Price, 1931 (family Psilostomidae), and renamed Beaver's (1939) material *Ribeiroia thomasi* (Family Cathaemasiidae), a scheme followed by Pratt and MacCauley (1961). The reason Yamaguti gave is that he noted that Price (1931) in his description of the type specimen did not mention the esophageal diverticula. Lumsden and Zischke (1963) brought *Pseudopsilostomum* into synonymy with *Ribeiroia* Travassos, 1939, under another family, the Cathaemasiidae. They considered the species *Pseudopsilostomum ondatrae* (Price, 1931) Yamaguti, 1958, and *Ribeiroia thomasi* (McMullen, 1938) Yamaguti, 1958 synonymous with *Ribeiroia ondatrae* (Price, 1931) Price, 1942. Beaver (1939) examined 8 paratypes from USNM Helminth. Coll. No. 29750, and did not overlook the esophageal diverticula in his description and illustrations. Moreover, Basch and Sturrock's description (1969) of a new species, *Ribeiroia marini* (family Cathaemasiidae) from the snail *Biomphalaria glabrata* on St. Lucia is not justified on account of the very little differences in

the number and arrangement of the miracidial plates, and minor differences in the cercaria of *R. marini* and that of *R. ondatrae*.

Basch and Sturrock (1969) considered their cercaria from St. Lucia to be identical with the cercaria from Puerto Rico, also from *Biomphalaria glabrata*. The cercaria was first described from Puerto Rico by Marin (1928) as Cercaria III, and was redescribed by Faust and Hoffman (1934) as a psilostome cercaria, *Cercaria marini*. I believe that *Cercaria thomasi* McMullen, 1938 is synonymous with *Cercaria marini*, and that the same species of fluke *Ribeiroia ondatrae* occurs throughout the Americas. In support of my view that the same species *R. ondatrae* occurs throughout the Americas is that most of the localities where the parasite has been found in the United States and the Caribbean are on bird migratory routes. It is very likely, therefore, that birds carry the infection with the same species from one place to the other, and whichever planorbid snails (*Helisoma* spp. or *Biomphalaria* spp.) are found in the area are utilized as first intermediate host.

Although *Ribeiroia ondatrae* in the present study developed in mice but not in chicks and a canary, birds as hosts cannot be excluded. Beaver's (1939) laboratory experiments with *Psilostomum ondatrae* showed that infection was obtained in chicks, pigeon, duck and canary. Moreover, Price (1931) described the species from the muskrat in Canada, as well as from the California gull from Oregon.

Outside the zone of distribution of *Biomphalaria obstructa* in the United States, infections with *Ribeiroia ondatrae* and *Echinoparyphium flexum* have been reported from other snail hosts. In the midwest Beaver (1939) obtained natural infections with the cercariae of *R. ondatrae* (= *P. ondatrae*) in the planorbid snail *Helisoma antrosum percarinatum* in Michigan, and McCoy (1928) reported that *E. flexum* cercariae encysted in *Physa integra*, and *Helisoma trivolvis* in Missouri. Najarian (1954) found natural infections with *E. flexum* cercariae in *Stagnicola palustris* in Michigan, and showed that the cercariae encysted naturally in *S. palustris*, *Physa gyrina*, and the planorbid *Gyraulus parvus*. Najarian described the life cycle of *E. flexum*, and enumerated later (1961) the differences between the latter species and *E. recurvatum* (von Linstow, 1873) Luhe, 1909. On the bases of the adult morphology, which has a seminal receptacle, the specimens obtained from the pigeon in the present study are *E. flexum*.

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