

The Sporocyst and Cercaria of *Amblosoma suwaense* (Digenea : Brachylaimidae : Leucochloridiomorphae)

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(Received for publication ; July 24, 1978)

In an earlier paper Shimazu (1974) described a new species of digenetic trematode, *Amblosoma suwaense* Shimazu, 1974 (Brachylaimidae : Leucochloridiomorphae), on gravid adults obtained experimentally by inoculating metacercariae found in the freshwater snail, *Sinotaia quadrata* (Benson) (Viviparidae), onto the chick chorioallantois.

The purpose of this paper is to report the sporocyst and cercarial stages of the trematode found in recent years, together with the results of an experiment carried out to infect the cercariae to *S. quadrata*. Part of this work has been presented in a preliminary note (Shimazu, 1975).

Materials and Methods

A large number of molluscs belonging to several different species were collected along the south-western shore of Lake Suwa, Nagano Prefecture, Japan, where natural infections of *S. quadrata* with *A. suwaense* metacercariae occurred (Shimazu, 1974), at various intervals during 1972 to 1977. They were crushed and examined for the parthenitae and cercaria of *A. suwaense*. Of approximately six thousand snails of *S. quadrata* examined, only two which were taken in the mouth of the river Hannoki in

September 1974 and June 1976 respectively were found to be infected with the sporocysts producing the cercariae described below. The two harboured metacercariae of *A. suwaense* as well.

As experimental second intermediate hosts for the cercariae were used 10 young snails of *S. quadrata*, about 10 mm in shell width, collected in the river Funato in September 1974. They were exposed for 12 hours to a few hundred, fully-formed cercariae dissected out. After the exposure, they were maintained at room temperature for five months from October 1974 to February 1975, and three or four of them at a time were examined monthly for metacercariae. In June 1976, an attempt to repeat a similar infection experiment was given up, for the cercariae obtained appeared still immature.

Sporocysts and cercariae were studied alive or in preserved material. Specimens were fixed in hot 5% formalin; ten mature cercariae fixed thus were measured. Delafield's haematoxylin and eosin were used to stain whole mounts. Cercariae fixed in Schaudinn's solution were stained with 0.05% aqueous toluidine blue to demonstrate the mucoid glands. Fragments of the parasitized digestive gland of the host were fixed in 5% formalin, made into serial paraffin sections, 10 μ m thick, and stained with haematoxylin-eosin or Heidenhain's azocarmine-aniline blue. The metacercariae recovered

Supported in part by Grants-in-Aid for Research from the "Nagano-ken Kagaku Shinkōkai" in 1975 and 1976.

in the experiment were prepared as whole mounts which were stained with alum carmine. Some of the mounted specimens examined are deposited in the collection of the National Science Museum, Tokyo (NSMT-PI-1864~1891).

Results

Sporocyst

Sporocysts were found in the interlobular connective tissue of the digestive gland of *S. quadrata*. They were white in colour, and unbranched or branched in shape. Unbranched ones were sausage-shaped (Fig. 1A). It was impossible to determine what extent the sporocysts had branched out to, because they were easily torn to pieces when dissected out. In branched ones taken out intact (Fig. 1B), some of the terminal tips of outgrowths appeared to open to serve as birth-pores. The sporocysts of both shapes contained only the cercarial generation at various stages of development from germ balls to fully-formed cercariae.

Cercaria

The morphology and measurements of mature cercariae were as follows (Figs. 1-6):

Body proper elongate-oval, slightly wider anteriorly than posteriorly, spinous, 0.203-0.257 mm long by 0.063-0.102 mm wide. Tail forked, well developed, functional, aspinose; stem 0.050-0.084 mm long by 0.021-0.027 mm wide; furcae laterally flattened, 0.063-0.143 mm long. Sensory hairs numbering at least 32, five pairs on lateral margins of anterior part of body, three

pairs on ventral and dorsal mid-lines of tail stem, and four pairs on ventral and dorsal margins of each tail furca. Many minute papillae without sensory hairs scattered around ventral orifice of oral sucker. Eye-spots, stylet, and finfolds absent.

Oral sucker ellipsoidal, subterminal, 0.042-0.046 mm long by 0.038-0.049 mm wide. Ventral sucker rounded, slightly posterior to middle of body, 0.037-0.046 mm long by 0.034-0.044 mm wide. Prepharynx very short. Pharynx barrel-shaped, 0.020-0.027 mm long by 0.017-0.020 mm wide. Oesophagus short. Intestinal caeca short, extending only to mid-level of ventral sucker.

Penetration glands divided into two distinct groups; anterior one consisting of six or seven pairs of smaller gland cells, more finely granular, acidophilic, distributed between pharynx and ventral sucker; posterior one composed of about five pairs of larger cells, finely granular, basophilic, situated dorsal and posterior to ventral sucker. Fine ducts of penetration glands on each side of body running forward in a bundle, passing through oral sucker, in which the ducts of the anterior group and those of the posterior group lie in an outer or lateral and an inner layer of bundle respectively (Fig. 5, d), opening separately into a small pit on anterior end of body. Many unicellular glands (number unknown) packed densely between two bundles of penetration-gland ducts in dorsal wall of oral sucker, ovoid, large, with acidophilic cytoplasm (Figs. 5 and 6, b). One or more large unicellular glands with basophilic cytoplasm seen in

Explanation of Figures

Figs. 1-6 The sporocyst and cercaria of *Amblosuma suwaense* Shimazu, 1974, found in *Sinotaia quadrata* (Benson).

Fig. 1 An unbranched (A) and a branched (B) sporocyst.

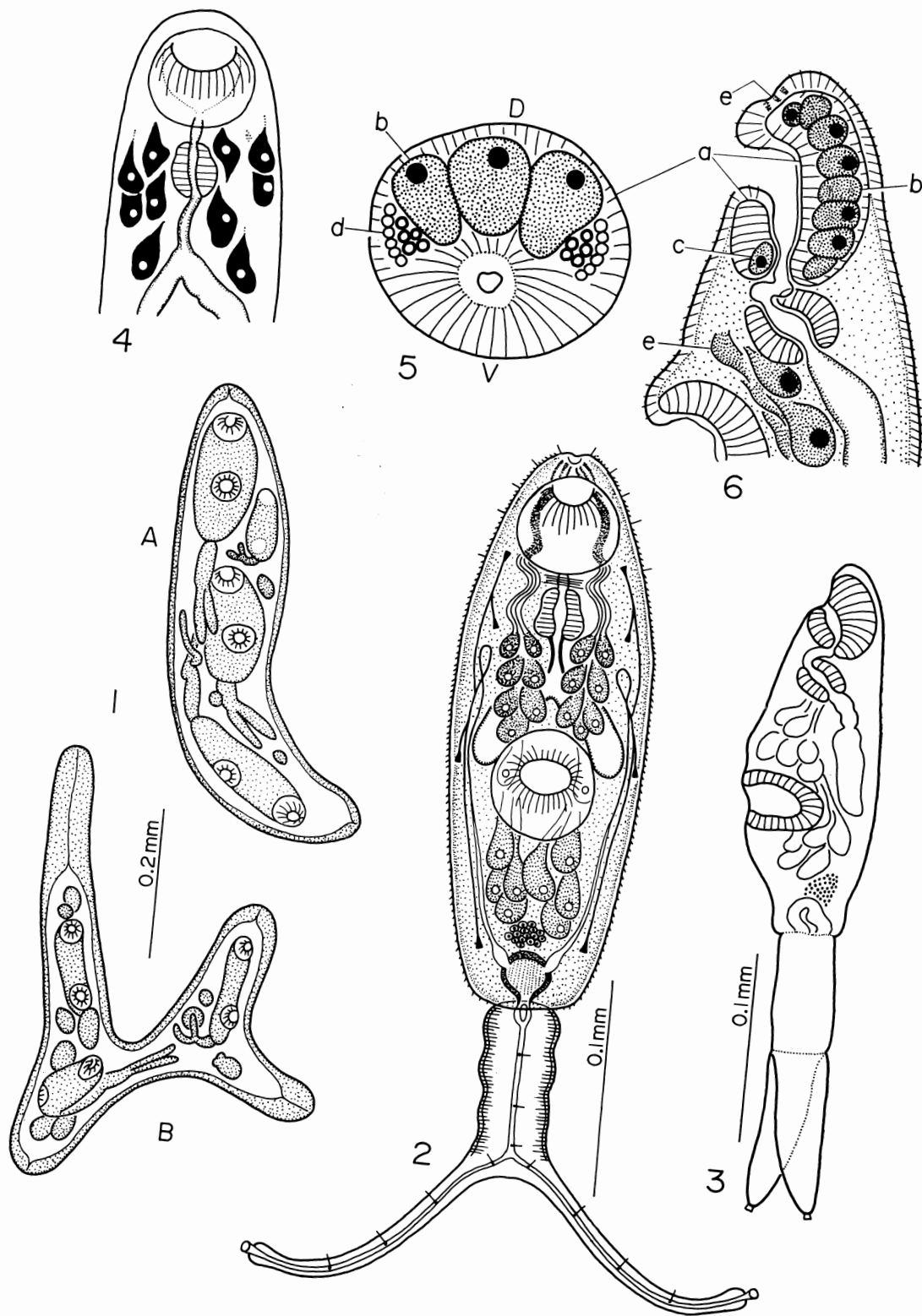
Fig. 2 A fully-formed cercaria, ventral view.

Fig. 3 Another fully-formed cercaria, lateral view.

Fig. 4 Mucoïd glands in a mature cercaria in a sporocyst.

Fig. 5 A transverse section of the oral sucker (a) of a mature cercaria, showing acidophilic, unicellular gland cells (b) located between the bundles of the penetration-gland ducts (d) in the dorsal wall. D: dorsal, V: ventral.

Fig. 6 A sagittal section of a mature cercaria through the oral sucker (a), showing acidophilic, unicellular cells (b) packed densely in the dorsal wall of the oral sucker, a basophilic, unicellular gland cell (c) in the bottom of the ventral wall of the oral sucker, and the penetration glands and their ducts (e).



bottom of ventral wall of oral sucker (Fig. 6, c). Mucoïd glands consisting of five pairs of cells, three in inner longitudinal rows and two in outer, located between oral sucker and intestinal bifurcation (Fig. 4).

Primordium of reproductive organs forming a mass of cells situated immediately anterior to excretory bladder. Excretory system stenostomate. Excretory bladder not epithelial, thick-walled, globular, small, in posteriormost part of body, with spined internal surface, discharging with a median caudal tube, which runs down through the tail stem, bifurcates at the furcae, and opens through a cup on each furcal tip. Lateral main collecting tube on each side of body reaching to oesophageal level, there turning backward to near ventral sucker; ascending limb short, bearing three cilia; descending limb non-ciliated, forming a small dilation just before entering bladder. Flame-cell formula $2[(1+1)+(1+1)]=8$; no flame-cells in tail. Cort's island present.

Results of experimental infection

Of the 40 experimental snails used, 19 survived and were examined for metacercarial infections. Each of them harboured 3 to 24 (mean 12.6) unencysted metacercariae in the bottom of the space between the digestive gland and shell. All the metacercariae recovered were identified as *A. suwaense*.

One month after exposure 34 metacercariae were recovered. They were closely similar in morphology to the previously-described cercaria except for the following minor differences: The tailless body was slightly larger in size, measuring about 0.25 mm long by 0.13 mm wide; the intestinal caeca were a little longer and more largely distended; and the penetration glands had disappeared. The genital primordium still remained a cellular mass. The excretory system was the same as that of the cercarial body proper. Afterwards, the growth and development of the metacercariae were slowly progressive. Five months after exposure 45 worms were recovered. They were still immature, and fourteen of them were meas-

ured as follows: body 0.816–1.550 mm long by 0.265–0.448 mm wide; oral sucker 0.085–0.140 mm long by 0.097–0.157 mm wide; ventral sucker 0.097–0.170 mm long by 0.110–0.208 mm wide; and pharynx 0.055–0.085 mm long by 0.055–0.072 mm wide. The major genitalia had already been feebly differentiated except the vitelline glands which were not yet recognizable.

Discussion

There was a fair possibility of the present cercaria being *A. suwaense*, because it was morphologically similar to that of *Leucochloridiomorpha constantiae* (Mueller, 1935) Allison, 1943, the only member with its known cercarial stage (Allison, 1943) in the subfamily Leucochloridiomorphae Yamaguti, 1958. In order to prove the possibility, the present experiment was carried out.

The results obtained showed that all the metacercariae recovered were *A. suwaense*, and the rate and intensity of infection of the experimental snails with them were 100% and 3 to 24 (mean 12.6) worms per infected snail respectively. This is in contrast with the following fact: The rate and intensity of infection ranged from 0 to 8.7% (mean 3.5%) and from one to two worms, respectively, in the case of natural infection with *A. suwaense* metacercariae in seven samples of *S. quadrata*, each of which was composed of 22 to 69 young snails, 9 to 13 mm in shell width, collected monthly from September 1974 to March 1975 at the station in the river Funato from which the experimental snails came (the author's unpublished data). There are striking differences between the experimental and the wild snails in both rate and intensity of infection, though it may be undeniable the possibility that the experimental snails may have received a very light natural infection with metacercariae while still in the river. Moreover, the experimental metacercariae obtained one month after exposure closely resembled the present cercaria in all essential morphological features, as noted previously. Consequently, it may be concluded that the great

majority, if not all, of the metacercariae found in the experimental snails were of experimental infection. This indicates that the present cercaria is *A. suwaense*.

Summary

Furcocercous cercariae of *Amblosoma suwaense* Shimazu, 1974 (Brachylaimidae: Leucochloridiomorphae), were found to be produced in branched or unbranched sporocysts parasitizing the freshwater snail, *Sinotaia quadrata* (Benson) (Viviparidae), in Lake Suwa, Nagano Prefecture, Japan. In an experiment made to infect young snails of *S. quadrata* with mature cercariae dissected out, developing metacercariae were recovered at a high rate and intensity of infection.

Acknowledgement

The author is grateful to Professor Jiro Ito of Shizuoka University, Shizuoka, and Professor Somei Kojima of Shinshu University, Matsuoto, for critically reading the manuscript.

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Amblosoma suwaense (Digenea: Brachylaimidae: Leucochloridiomorphae)

のスポロシストおよびセルカリアについて

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二生吸虫 *Amblosoma suwaense* Shimazu, 1974 (Brachylaimidae: Leucochloridiomorphae) の生活史を解明するために、1972-1977年、諏訪湖の西南岸で貝類を多数採集し、その寄生虫を調べた。その結果、およそ6,000個体のヒメタニシ *Sinotaia quadrata* (Benson) (Viviparidae) のうち、半之木川河口で採集した2個体から岐尾型セルカリアを見出した。単性虫はスポロシストで、ソーセージ形かまたは樹枝状に分岐した形をしていた。

本セルカリアが *A. suwaense* のものであるかどうかを確かめるために、同吸虫の第二中間宿主であるヒメタニシへセルカリアを感染させる実験を行なった。すなわち、感染貝を解剖してえた成熟セルカリアを、舟渡川で採集した幼若ヒメタニシに接触させ、以後5カ月間、実

験貝を月1回剖検して、メタセルカリアの感染の有無と発育を調べた。その結果、どの実験貝からも *A. suwaense* のメタセルカリアを検出した。このときの感染率と感染数は、同時期の舟渡川産幼若ヒメタニシにおける同吸虫メタセルカリアの自然感染例での感染率と感染数に比べて、著しく高く、かつ数も多かつた。更に、感染1カ月後に回収したメタセルカリアは、実験に用いたセルカリアに形態的によく似ていた。以上のことから、実験貝から見出したメタセルカリアは、大部分が実験に用いたセルカリアに由来したと考えられた。故に、本セルカリアを *A. suwaense* と同定した。

本報では、スポロシストとセルカリアを記載し、セルカリアのヒメタニシへの感染実験の結果も簡単に述べた。