THE FIRST INTERMEDIATE HOST OF A FROG TREMATODE, PLEUROGENES JAPONICUS YAMAGUTI

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INTRODUCTION

In the autumn of 1950, K. Okabe and the author determined that a fresh-water shrimp, Neocaridina denticulata (de Haan), served as the second intermediate host of the frog trematode, Pleurogenes japonicus Yamaguti. Since then the author has studied the first intermediate host of this worm and in the spring of 1951, found a new species of cercaria possessing a virgulate organ in Bulimus kiushuensis Hirase collected at Kurume, Kyūshū. In the infection experiments of this cercaria on N. denticulata, it was shown that the cercaria penetrated and encysted in this fresh-water shrimp, and that the newly encysted metacercaria was identical with the metacercaria of P. japonicus.

CERCARIA

Of 76 specimens of B. kiushuensis collected in May, 21.1 percent were infected with colorless, oval-shaped sporocysts, measuring 0.203—0.328 mm in length and 0.108—0.212 mm in breadth (Fig. 1). The sporocyst contained one or two mature and from two to five immature cercariae.

Fig. 1. Sporocyst of P. japonicus
The emerged cercaria is a rapid swimmer and is indifferent to light. The body is elliptical in shape, measured 0.208–0.244 (0.225) mm in length and 0.116–0.156 (0.136) mm in breadth when viable (Fig. 2). The cuticle is entirely covered with minute spines. The posterior half of the body is darkened by numerous fine refractive granules which make the observation of the inside structure of cercaria very difficult. In the living specimen the fully extended tail is almost as long as the body length, but it can be retracted to much less than the body length, measuring 0.088–0.232 (0.129) mm long and 0.022–0.032 (0.029) mm broad.

The oral sucker is located subterminally, measuring 0.048–0.062 (0.055) mm × 0.048–0.060 (0.053) mm. A conspicuous stylet is set in the dorsal wall of the oral sucker. The stylet, 0.024 mm long and 0.008 mm broad at the base, has a sharp point and a ring shaped thickening at the junction of the anterior and the middle third of its length. The virgulate organ is present in the posterior part of the oral sucker and has a width of 0.034–0.040 (0.036) mm. The acetabulum is situated slightly posterior to the middle of body and has a diameter of 0.030–0.036 (0.033) mm.

The digestive system is represented by a mouth cavity, a small muscular pharynx, and a slender esophagus. The intestinal ceca are not at all defined.

On each side of the acetabulum, four pairs of penetration glands are seen. Each gland possesses a large nucleus which is stainable with neutral red in
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living specimens. The gland ducts arise from cell clusters which are massed in a large bundle on each side of the body, and after making a sharp curve on the lateral margin of the oral sucker the ducts open at the dorsal side of stylet.

The genital system cannot be defined. The excretory vesicle is V-shaped, and the excretory pore opens at the posterior end of body. The formula of flame cells cannot be determined.

In 1937, K. Okabe reported a new xiphidiocercaria in B. kiushuensis, the same snail host of the present cercaria, and determined by the infection experiments that the cercaria penetrated and encysted in the nymphs of the dragonfly, Sympetrum darwinianum Selys and Orthetrum albistylum Selys. And he identified the encysted metacercaria as the metacercaria of a frog trematode, Loxogenes liberum Seno.

The cercaria of L. liberum also has the virgulate organ and the general body shape is similar to that of the present cercaria. They are, however, distinguishable by the following characteristics given in Table 1.

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<th>Table 1. Comparative data of the new cercaria and the cercaria of Loxogenes liberum.</th>
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<td><strong>Size of cercaria</strong></td>
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The percentage of infection for both cercariae in the same snail hosts collected in May are as follows: of the 76 snails collected, 16 or 21.1 percent were infected with the new cercaria and 18 or 23.7 percent were with the cercaria of L. liberum.

INFECTION EXPERIMENTS

Many B. kiushuensis were collected and each was placed in a test tube with about 10cc of water to allow for natural emergence of the cercaria from the snails. The naturally emerged cercariae were examined under the microscope and only the new species of cercariae were used for the following experiments.

About forty fresh-water shrimps, N. denticulata were secured from a river and thirty of them were examined for the metacercaria and were proved to be negative, except for the presence of metacercaria of Coitocoeicum plagi-orchis Ozaki. The remaining fresh-water shrimps were put into a small
aquarium together with the naturally emerged new cercariae and it was found that the cercariae penetrated and encysted in the cephalothorax of those shrimps.

The development of the encysted metacercaria in the fresh-water shrimp was observed from twenty hours to thirty-one days after infection and the summary of the observations are as follows.

Twenty hours after penetration the encystment of the metacercaria was completed. The cyst was round or oval in shape, measuring 0.104–0.116 (0.109) mm x 0.096–0.112 (0.103) mm, and had a very thin wall easily ruptured by slight pressure. The size of metacercaria was 0.216–0.224 (0.219) mm long and 0.120–0.132 (0.124) mm broad. The virgulate organ was present, but rather retracted in shape. The stylet was set in the oral sucker. The rudiments of penetration glands were seen. Concretions in the excretory bladder were not yet found. The encysted metacercaria was found in the muscles of the cephalothorax, but none was found in the gonads and the liver of the shrimp 20 hours after infection. The testes were found just in front of the arms of the excretory bladder, but ovary was not yet found.

Five days after penetration, the virgulate organ and penetration glands had disappeared, but the stylet was still in the oral sucker. The testes were distinctly visible. A small number of concretions were seen in the V-shaped excretory bladder. The ovary was found in front of the right cecum. The anlage of the cirrus pouch could not be differentiated. The cyst measures 0.136–0.160 (0.142) mm x 0.120–0.160 (0.137) mm. The size of metacercaria was 0.268–0.332 (0.294) mm in length and 0.124–0.156 (0.142) mm in breadth (Fig. 3).

Ten days after penetration, the acetabulum was distinctly developed and it was almost the same size as the oral sucker. The stylet had lost its position
and situated between the metacercaria and the wall of the cyst. The anlage of the cirrus pouch was found obliquely between the acetabulum and the left margin of the body, but the development of the seminal vesicle was poor. No eggs were found in the uterus. Numerous concretions were present in the excretory bladder and it appeared U-shape. The cyst was round in shape, measuring 0.176–0.188 (0.180) mm × 0.176–0.188 (0.178) mm. The size of metacercaria was 0.424–0.508 (0.469) mm long and 0.196–0.268 (0.222) mm broad.

The uterus of the 15-day old metacercaria contained a small number of eggs, but all were colorless. The vitelline follicles were found on each side of the esophagus. The cyst had a diameter of 0.264–0.280 (0.271) mm. The encysted metacercaria were found in the liver, gonads, and muscles of the cephalothorax of the fresh-water shrimp. The size of the metacercaria was 0.616–0.680 (0.649) mm in length and 0.320–0.364 (0.336) mm in breadth (Fig. 4).

![Fig. 4. 15-day-old metacercaria of P. japonicus](image)

The structure of the 20-day old metacercaria was almost the same as that of the 15-day old one, except for an increase in uterine convolutions as well as the size of body. Some eggs in the uterus were light brown in color, while others were colorless. The cyst measured 0.356–0.408 (0.381) mm in diameter. The wall of the cyst was about 0.004 mm in thickness. The size of the metacercaria was 0.735–0.780 (0.765) mm in length and 0.376–0.452 (0.386) mm in breadth.

Thirty-one days after penetration, many metacercariae were encysted in the liver, gonads, and muscles of the cephalothorax of the fresh-water shrimp.
There were numerous eggs in the uterus and the free space within the cyst was filled with many eggs. The cyst had a diameter of 0.600–0.645 (0.624)mm. The wall of cyst was transparent and thin, measuring about 0.006mm in thickness.

The following description and measurements of liberated metacercaria were based on five living specimens of 31-day old metacercaria examined under a small cover slip.

The body is elongated, elliptical in shape, measuring 1.110–1.200 (1.158)mm long and 0.555–0.600 (0.585)mm broad at the middle of body and covered entirely with minute spines. The subterminal oral sucker measures 0.112–0.120 (0.115)mm × 0.100–0.108 (0.103)mm. The prepharynx is very short and is scarcely visible in extended specimens. The pharynx is subgrobular in shape, measuring 0.048–0.056 (0.050)mm × 0.044–0.048 (0.047)mm. The esophagus is 0.092–0.168 (0.156)mm long and bifurcates at about midway between the oral sucker and the acetabulum. The ceca are 0.156–0.180 (0.173)mm long and terminate just in front of the testes. The acetabulum almost in the middle of body and has a diameter of 0.108–0.112 (0.109)mm.

The testes are oval in shape, 0.172–0.204 (0.193)mm × 0.124–0.148 (0.140)mm and lie symmetrically lateral to the acetabulum. The cirrus pouch is club-shaped, 0.260–0.324 (0.282)mm in length and 0.076–0.088 (0.083)mm at its maximum diameter. It lies obliquely between the anterior margin or a little in front of the acetabulum and the left margin of the body, overlapping the left cecum ventrally. The cirrus pouch contains a looped seminal vesicle, a pars prostatica surrounded by prostatic cells, and a straight ejaculatory duct. The cirrus opens on the left body wall at a level halfway of the esophagus.

The ovary is globular in shape, 0.180–0.220 (0.192)mm × 0.136–0.160 (0.145)mm, lies directly laterally outside of the right cecum. The oviduct arises from the postero-medical part of ovary, proceeds backward across the right cecum dorsally, then receives the voluminous seminal receptacle, and then curves around the latter to receive the common vitelline duct and finally passes into the ootype. The Laurer's canal runs backward from the seminal receptacle to open on the middorsal surface at the level of the acetabulum. The uterus occupies the posttesticular area and contains numerous eggs. The metraterum crosses over the cirrus pouch and then curves towards the genital atrium, surrounded by "Bagleit-zellen". The eggs are fertilized, elliptical in shape, light brown in color, and thick-shelled, measuring 0.024–0.028mm×0.014–0.016mm when viable. The vitellaria form grape-like bunches on each side of esophagus.

The excretory bladder is Y-shaped with a short stem and long lateral arms which extend up near the testes. The excretory pore opens at the posterior end of body. The liberated 31-day old metacercaria is shown in Fig. 5.
In view of these structure and measurements, the 31-day old metacercaria is quite similar to the naturally infected metacercaria found in the fresh-water shrimp, *N. denticulata*.

In a series of these experiments, a total of 26 metacercaria encysted in *N. denticulata* (34 days after the exposure to the new cercaria) were given to a laboratory-raised frog, *Rana nigromaculata nigromaculata* (Hallowell) and 6 days after feeding, 23 adults were found in the upper portion of the small intestine of the frog and were identified as *P. japonicus*.

The measurements of 6-day old adults based on ten living specimens under a small cover slip are as follows; body, 1.110–1.230 (1.158)mm in length and 0.495–0.675 (0.533)mm in breadth; oral sucker, 0.120–0.128 (0.124)mm × 0.104–0.116 (0.109)mm; acetabulum, 0.114–0.122 (0.117)mm in diameter; pharynx, 0.056–0.064 (0.061)mm × 0.040–0.044 (0.042)mm; esophagus, 0.084–0.120 (0.105)mm in length; testes, 0.136–0.160 (0.146)mm × 0.088–0.112 (0.101)mm; ovary, 0.144–0.172 (0.163)mm × 0.108–0.132 (0.124)mm; cirrus pouch, 0.312–0.364 (0.339)mm in length and 0.068–0.176 (0.072)mm in maximum diameter; receptaculum seminis, 0.068–0.112 (0.095)mm × 0.052–0.080 (0.070)mm; eggs, 0.024–0.028mm × 0.016mm.

The results from the above experiments demonstrate that the metacercaria developed from a new xiphidiocercaria harbored in *B. kiushuensis* and experimentally encysted in *N. denticulata* is identical with the metacercaria of *P. japonicus* and that, therefore, the first intermediate host of this parasite is *B. kiushuensis*.
SUMMARY

(1) A new xiphidiocercaria possessing a virgulate organ is described from Bulimus kiushuensis Hirase.

(2) The cercaria penetrates and encysts in fresh-water shrimp, Neo-
caridina denticulata (de Haan) and develops into the metacercaria which is identified as the metacercaria of Pleurogenes japonicus Yamaguti.

(3) The metacercaria of P. japonicus becomes sexually mature about 20 days after infection.

(4) Thirty-four-day old metacercaria encysted in N. denticulata were given to a laboratory-raised frog, Rana nigromaculata nigromaculata (Hallowell), and 6 days after feeding the adults of P. japonicus were recovered from the upper portion of the small intestine of the infected frog.

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