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Cercaria of *Nanophyetus japonensis* from the Freshwater Snail, *Semisulcospira libertina*, in Japan and Its Experimental Infection

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Key words: *Nanophyetus japonensis*, cercaria, metacercaria, adult, experimental exposure, morphology

Introduction

The freshwater snail, *Semisulcospira libertina*, is widely distributed in the rivers, ponds and lakes of Japan. The search for cercariae parasitizing the snail has been carried out by many parasitologists, because the snail is known as a first intermediate host of important human trematodes, such as *Paragonimus westermani*, *Metagonimus yokogawai*, etc. The author started a survey of the snail in 1975 in the Tohoku District, and found on October 5, 1976 a microcercous xiphidiocercaria from snails collected in the drainage way at Tsunagi-Onsen, Morioka City, Iwate Prefecture. This cercaria was morphologically nearly identical with the cercaria of *Nanophyetus salmincola* harboring in the freshwater snail, *Oxytrema silicula*, from the Pacific Coast of North America (Sinitsin, 1930; Simms *et al.*, 1931) or the cercaria of *N. schikhobalowi* harboring in the freshwater snails, *Semisulcospira laevigata* and *S. cancella*, from the Far East of the USSR (Filimonova, 1965). Therefore, the second intermediate hosts, the brook trout, *Oncorhynchus masou*, and the goldfish, *Carassius carassius auratus*, were experimentally exposed to the cercaria. The metacercariae were recovered from the hosts 16 to 45 days postexposure to the cercariae. The adult worms were obtained from rats, the final hosts, 12 days postadministration of the metacercariae. From the morphological features of the metacercariae and the adult worms, this parasite was identified as *Nanophyetus japonensis*. The present paper will demonstrate the above-mentioned results in detail.

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Materials and Methods

1. Detection of cercariae

Each freshwater snail, *Semisulcospira libertina*, was put into a 4 cm wide petri dish containing dicloried tap water. The cercariae began to come out from the snails within a few minutes. The snails were left in the dishes for 2 days and the cercariae which came out into the water were observed by dissection microscope. Negative snails were crushed in the water and re-examined for cercaria.

2. Experimental exposure of freshwater fishes to cercariae

Two species of freshwater fishes, the brook trout, *Oncorhynchus masou*, and the goldfish, *Carassius carassius auratus*, were used for experimental exposure. The trout were 18-19 cm in body length and were bred for 7 months at Yasuka Fish Farm, Shizukuishi Town, Iwate Prefecture. Fifteen of them were exposed for 30 minutes to cercariae obtained from 5 positive crushed snails in a plastic container, 26 cm long, 38 cm wide, and 23 cm high with water 5 cm in depth. The trout were bred for 45 days in a concrete ditch, 10 m long and 1 m wide, that was divided into two parts with

wire netting. The exposed trout were left at the lower part of the stream, and the control trout at the upper part. Mountain water continuously flowed through the ditch.

Colorless goldfish about 5 cm in body length were obtained for the experimental exposure to cercariae. Three to 5 goldfish and 2-3 cercarial positive crushed snails were placed together for 1 to 2 hours in a plastic container, 21 cm long, 9 cm wide, and 11 cm high with water 3 cm in depth, and then the fish were bred in a plastic container, 33 cm long, 18 cm wide, and 23 cm high.

The water was continuously aerated to create many chances of contact between the cercariae and fishes during the exposure period.

3. Experimental infection in rats

Metacercariae encysted in the fish were liberated from the tissue of the fish at 37°C by placing in artificial gastric juice consisting of 100 μg/ml pepsin and 0.08 N HCl for 1 to 2 hours. The obtained metacercariae were administrated to rats. Adult flukes were recovered from the small intestines of rats one to two weeks postadministration.

4. Procedure for the observation of flukes

For the morphological observations of cercaria and metacercaria, especially of the excretory system, the materials were put into a half diluted physiological saline solution and were pressed by a cover glass till they became very thin, flat and transparent. These materials were preservable for observation for a long time when the margin of the cover glass was sealed with vaseline.

The measurements of redia and cercaria were carried out with materials fixed in 10 % hot formalin and slightly pressed under the cover glass. The observations of excysted metacercariae and adults were mainly carried out with materials fixed in Schaudinn's solution under cover glass pressure, stained with Haidenhain's iron-hematoxylin or borax carmine, and mounted in balsam. All measurements were recorded in μm with the aid of a camera lucida.

Results

1. Geographical distribution of the cercaria of *Nanophyetus japonensis* in *Semisulcospira libertina*

The cercariae of *N. japonensis* were detected in the freshwater snail, *Semisulcospira libertina*, collected in Iwate, Akita and Yamagata Prefectures (Table 1). In Iwate Prefecture the snails were collected from the drainage way of the hot-springs in Tsunagi-Onsen, Morioka City, 8 times from October 5, 1976 to September 11, 1977. The cercariae were detected in the snails every time except on December 15, 1976. In Akita Prefecture, only one out of 102 snails collected on September 21, 1982 were positive with cercariae. From 1975 to 1983, the snails collected from 16 areas of the Shonai Plains and 12 areas of the inland in Yamagata Prefecture were examined. Snails positive with the cercariae of *N. japonensis* were found in 2 rivers in the inland. In the upper stream of the Sukawa River, Kaminoyama City, 6 out of 94 snails collected on November 27, 1977, 2 out of 87 snails on May 3, 1978 and 6 out of 76 snails on May 28, 1978 were positive with cercariae. In the Daimon River, Yamagata City, the snails were collected 6 times during the period from June 7, 1978 to July 18, 1983, and only 2 out of 80 snails collected on July 18, 1983 were positive with cercariae.

2. Morphology of rediae and cercariae

Rediae and cercariae were obtained by crushing the snail. Numerous rediae and cercariae harbored in the mid-gut of the snail. Mature cercariae came out from the snails when they were left in dicloried tap water for one day.

Redia (Fig. 1-A, Photo. 1-A) : The rediae were found among the cercariae. They greatly varied in size, the length being 200 μm to 1945 μm and the width 50 μm to 444 μm. The shape of the redia was generally cylindrical, but the smaller ones tapered from the anterior end to a point at the posterior end. A spherical pharynx at the anterior end was 42-75×46-70 μm, and led into a sac-like or cylindrical gut which reached from one third to

Table 1 Geographical distribution of the cercaria, *Nanophyetus japonensis*, from the snail host, *Semisulcospira libertina*, in Tohoku District, Japan

Locality		Date			No. exam.	No. infect.	%
Iwate	Tsunagi-Onsen, Morioka	5	Oct.	1976	237	1	0.4
		10	〃	〃	140	2	1.4
		29	〃	〃	460	3	0.7
		15	Dec.	〃	147	0	0
		5	May	1977	188	2	1.1
		20	Jul.	〃	311	3	1.0
		28	〃	〃	650	5	0.8
		11	Sept.	〃	72	1	1.4
Akita	Nishiki-Mura	5	Oct.	1976	55	0	0
		1	Sept.	1982	107	0	0
		21	〃	〃	102	1	1.0
		13	〃	1983	130	0	0
	Other 2 areas	31 Aug. 1975 ～ 8 Aug. 1983			665	0	0
Yamagata	Inland areas	7	Jun.	1978	20	0	0
	Daimon River, Yamagata	19	Oct.	〃	150	0	0
		11	Nov.	〃	300	0	0
		8	Jul.	1979	266	0	0
		7	Nov.	1982	262	0	0
		18	Jul.	1983	80	2	2.5
	Sukawa River, Kaminoyama	27	Nov.	1977	94	6	6.4
		3	May	1978	87	2	2.3
		28	〃	〃	76	6	7.9
	Other 10 areas	3 May 1978 ～ 8 Aug. 1983			1439	0	0
	Shonai Plain 16 areas	9 Jul. 1975 ～ 17 Aug. 1976			2793	0	0

two thirds of the length of the redia. The birth pore was located on the body surface near the side of the pharynx. The redia contained numerous cercariae in various stages of development.

Cercaria (Table 2, Fig. 1-B, Photo. 1-B): The cercaria was microcercous xiphidiocercarial in type. The body was oval in shape and the size of the material fixed with 10 % hot formalin was 229-387μm long by 105-141 μm wide. The body surface, cuticle, was transparent, and was covered with many minute backward directed spines and long sensory hairs. They were distributed aboundantly on the anterior part of the body. The oral sucker was subglobular in shape, 46-75 μm long by 46-68 μm wide, accompanied by a conspicuous 13-17 μm long stylet, which had a round base and sharply pointed tip. The mouth, surrounded by the oral sucker, was followed by the pharynx, but the esophagus and intestine could not be traced. The nerve commissure ran transversely through the somewhat posterior part of the pharynx. The

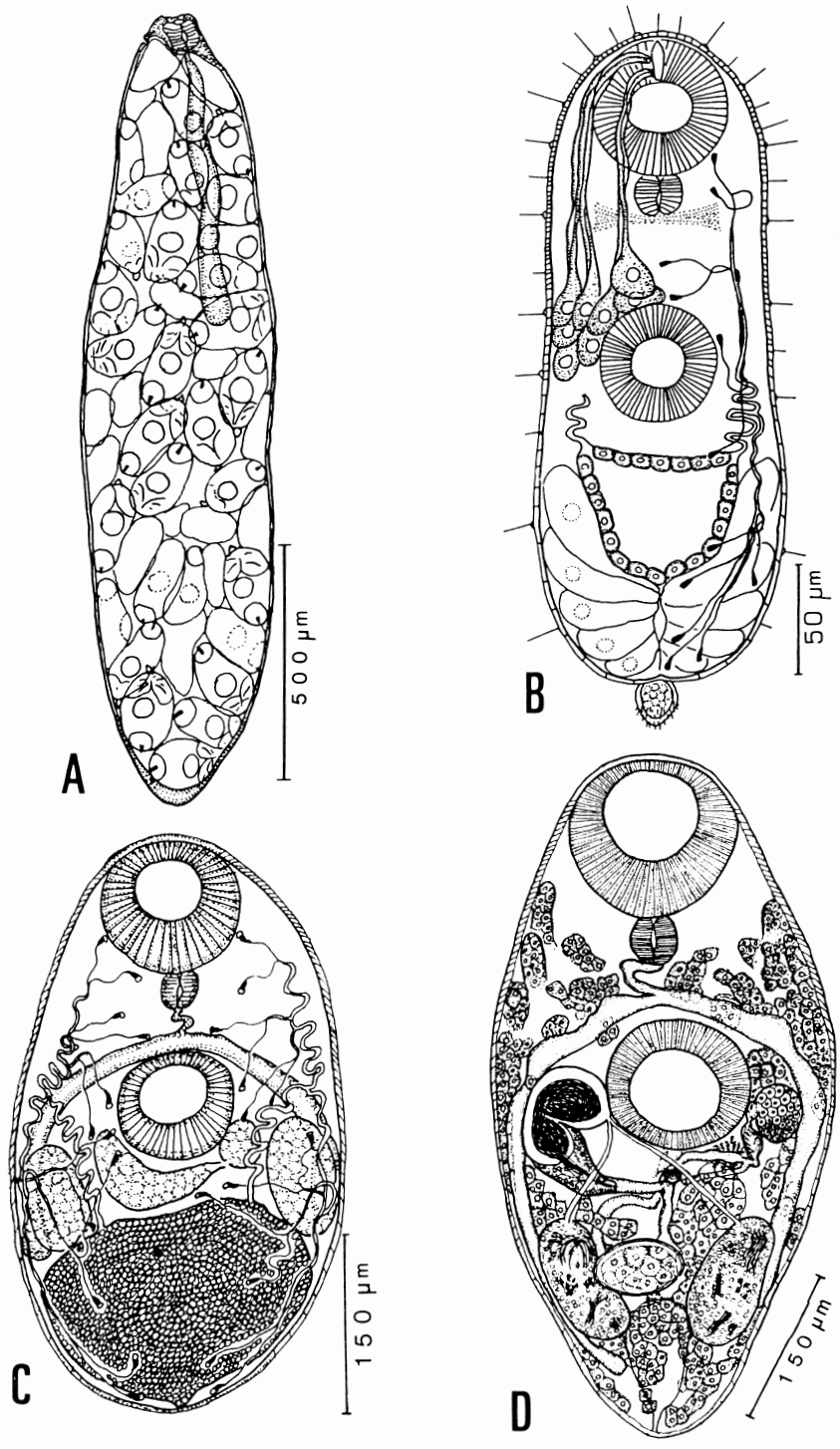

Fig. 1

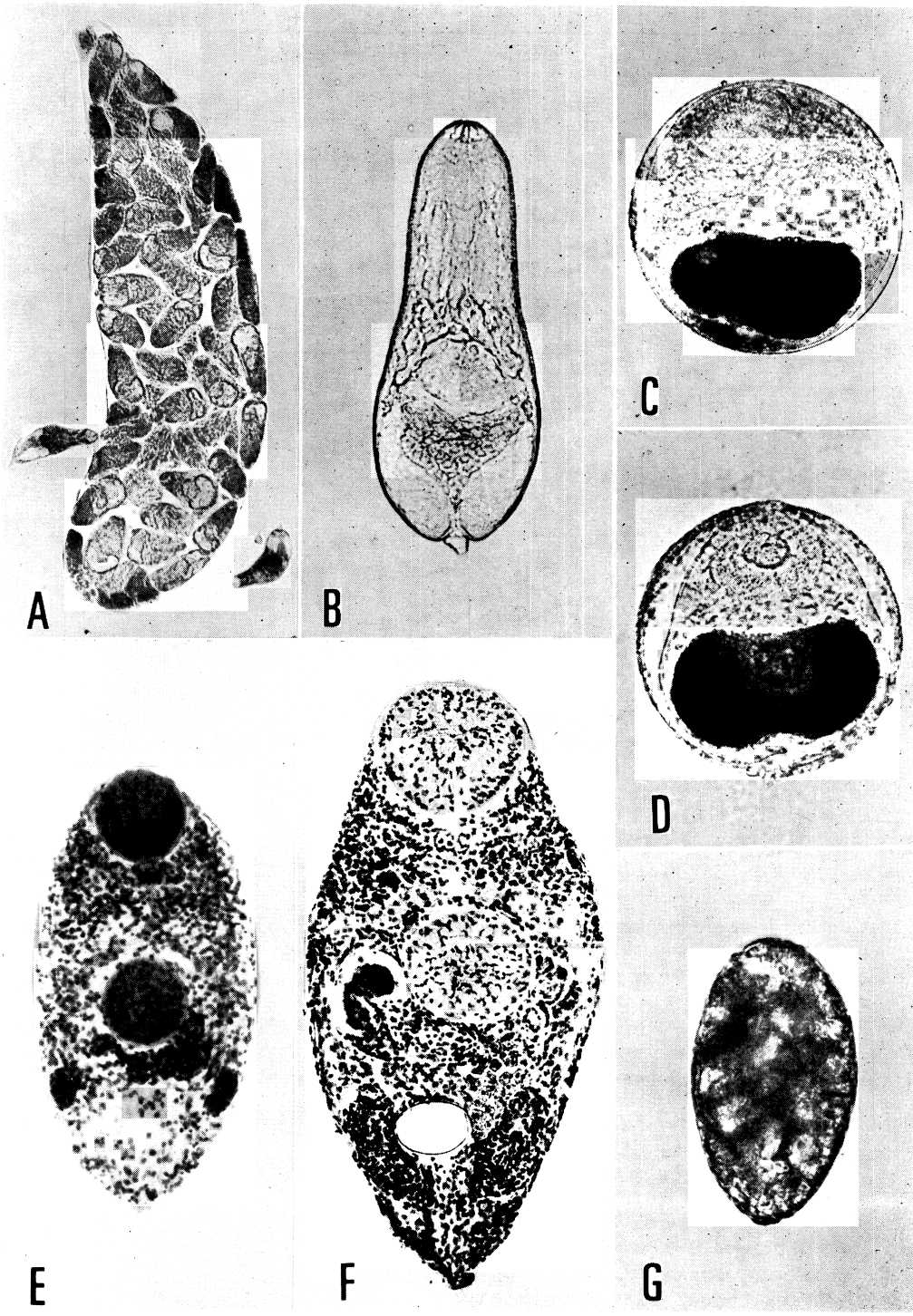

Photo. 1

Table 2 Measurements of various stages of *Nanophyetus japonensis* in Japan (in μm)

	Cercaria	Metacercaria	Adult
Body length	229-387(305)*	253-460(342)	560-690(640)
Body width	105-141(125)	207-298(247)	245-340(311)
Oral sucker length	46- 75(54)	83-103(95)	113-150(138)
Oral sucker width	46- 68(57)	103-130(115)	128-170(155)
Acetabulum length	44- 56(50)	63- 83(69)	108-125(119)
Acetabulum width	47- 58(53)	83-112(96)	103-138(129)
Pharynx length	18- 26(22)	30- 35(31)	34- 45(38)
Pharynx width	14- 22(18)	25- 30(27)	34- 45(40)
Ovary length		38 (38)	50- 75(66)
Ovary width		25- 38(28)	23- 50(36)
Right testis length		69- 90(75)	105-140(122)
Right testis width		48- 63(54)	53- 63(58)
Left testis length		60-100(78)	110-135(125)
Left testis width		43- 73(52)	63- 78(67)
Seminal vesicle length			96-135(113)
Seminal vesicle width			71-126(99)
Tail length	15- 19(18)		
Tail width	12- 17(14)		
Stylet length	13- 17(15)		
Stylet width	4- 5(5)		
Cyst length		181-345(236)	
Cyst width		165-338(226)	
Excretory bladder length		70-125(108)	
Excretory bladder width		138-233(192)	

* range (average)

Fig. 1 Redia and cercaria of *Nanophyetus japonensis* discovered in Japan, and metacercaria and adult obtained from the experimental infections.

A. Redia pressed under cover glass, fixed in Schaudinn's solution, stained with borax carmine and mounted in balsam.
B. Living cercaria from the freshwater snail.
C. Living excysted metacercaria from the trout 45 days postexposure.
D. Adult from the rat 12 days postadministration, treated with the same procedure as redia.

Photo. 1 Redia and cercaria of *Nanophyetus japonensis* discovered in Japan, and metacercariae and adult obtained from the experimental infections.

A. Redia and free cercariae pressed under cover glass, fixed in Schaudinn's solution, stained with borax carmine and mounted in balsam.
B. Living cercaria from the freshwater snail.
C. and D. Living metacercariae encysted in the goldfish.
E. Excysted metacercaria from the goldfish 20 days postexposure.
F. Adult from the rat 12 days postadministration.
E and F were also treated with the same procedure as A.
G. Egg in the feces of the rat 12 days postadministration.

acetabulum, 44-56 μm long by 47-58 μm wide, being subglobular in shape, was smaller than the oral sucker, and was located approximately in the middle part of the body. Eight pairs of homogeneous penetration gland cells were present on both the lateral and upper sides of the acetabulum. Individual ducts from the gland cells were arranged into 4 groups of 4 ducts each, running together anteriorly along the dorsal side of the body, around the oral sucker, and then opening laterally beside the stylet in the vicinity of the anterior margin of the oral sucker. The oblong cell mass deeply stained with carmine lay dorsally to the acetabulum and just anteriorly to the excretory bladder. The posterior body was occupied by a large U- or V- shaped excretory bladder extending behind the acetabulum. Its wall was lined with a thick layer of cuboid epithelia containing coarse reflective granules. From both of its antero-corners, the main collecting tubes ran upward in a zigzag course, making a complicated convolution, and then were divided into anterior and posterior collecting tubes at the level of the acetabulum. The anterior tube ran upward, extending 2 branches on the way, and terminated in 2 capillaries at the side of the oral sucker. The above-mentioned 2 branches also bore 2 capillaries and each capillary had one flame cell (Fig. 1-B). As mentioned above in the case of the anterior collecting tube, the posterior collecting tube branched twice at intervals and finally divided into 2 capillaries at the posterior end of the body. These 2 branches also ended in 2 capillaries bearing one flame cell. The flame cell formula was therefore constructed as 2 [(2+2+2)+(2+2+2)] = 24. There was a pronounced median groove on the ventral side of the hind body. Several pairs of large crescent adhesive gland cells lay in the ventral part from the posterior end of the acetabulum to the posterior extremity of the body. Many cystogenous gland cells stained by PAS were scattered in the dorsal side of the body from the level of the pharynx to the posterior extremity of the body. By staining with metachromatic dyes, such as toluidin blue or thionin, 6 pairs of mucoid gland cells were detected in the ventral side of the body, and the ducts from the cells reached to the dorsal parts of the oral sucker and the acetabulum, and both of them opened in the dorsal surface of the body.

Table 3 Experimental exposure of the cercaria, *Nanophyetus japonensis* from Japan in brook trout, *Oncorhynchus masou*

Group	Exposed	Control
No. trout examined	15	15
No. trout encysted	15	4
Range of No. of metacercariae per trout	2-5	0-1
Total No. of metacercariae detected in		
Gills	33	1
Muscles		
near the dorsal fins	12	0
near the pectoral fins	5	0
Kidneys	5	3
Intestine	1	1

The tail, measuring 15-19 μm long by 12-17μm wide on the ventral end of the body, was short and smooth except for the tip, where hair-like spines grew.

3. Experimental exposure in fishes

Fifteen cultured trout, *Oncorhynchus masou*, 18-19 cm in body length, were exposed for 30 minutes to all cercariae obtained from 5 snails by the crush method. The trout 1 day postexposure had no visually recognizable symptoms. The trout 45 days postexposure were killed and divided into separate organs such as the head, fins, scales, epidermis, muscles and internal organs. Small amounts of tissues were sandwiched between 2 glass slides, pressed and examined for encysted metacercariae under the dissection microscope. Metacercariae were detected in all the exposed fish. The total number of metacercariae detected from 15 trout was 33 in the gills, 17 in the muscles, 5 in the kidneys and 1 in the intestine. The encysted sites in the muscles were only near the basal parts of the dorsal and pectoral fins. On the other hand, one metacercaria was also detected in 4 out of 15 trout of the control group, but their number was much less than the number in the exposed group (Table 3).

Three goldfish were exposed for 2 hours to the cercariae obtained from a crushed snail. The fins 1 day postexposure showed hemorrhages caused by the penetration of the cercariae. After 5 days, the goldfish recovered from the illness. The number of metacercariae encysted in one fish 20 days postexposure was 555 in the fins, 31 in the gill, 8 in the kidney and 6 in the head. In the muscles, scales and epidermis no metacercaria was detected. Of all the fins, the tail fin, especilly the insides of the rays, harbored the largest number (491) of metacercariae and in the other fins 10-20 metacercariae were observed. In the goldfish 60 days postexposure, the metacercariae were detected only in the fins; 128 in the tail fin, 4 in the caudal fin and 2 in the pectoral fins. The life span of the metacercariae encysted in the goldfish was generally short, that is, 2/5 of the number of metacercariae were dead after 71 days, 3/5 after 85 days and 4/5 after 94 days, respectively, while most of the metacercariae 20 days postexposure were living. Although a very small number of metacercariae still survived 430 days after infection, all the metacercariae were dead at 532 days.

4. Morphology of metacercariae experimentally obtained (Table 2, Fig. 1-C, Photo. 1-C, D and E)

The cysts were spherical or ovoid, measuring from 165 to 345 μm in diameter. The cyst wall was smooth and clear, 2-5 μm thick. The larval flukes lay in various positions in the cysts and moved frequently. Usually the larva was contracted so that the oral sucker and the acetabulum almost touched each other. Sometimes, the body was elongated and found a ring shape along the inside of the cyst wall. The most conspicuous feature of the encysted fluke was the large excretory bladder which occupied one half to one third of the cyst. The excretory bladder was filled with highly refractive droplets which appeared black with transmitted light or white with reflected light. The excretory bladder was usually reniform or heart-shaped.

The excysted metacercariae were ellipsoidal or oval, measuring 253-460 μm in length and 207-298 μm in width. The body surface was covered with minute spines except for the posterior part. The oral sucker was located at the subterminal anterior part of the body, and was followed by the pharynx and esophagus. The esophagus was divided into two ceca in front of the acetabulum. The ceca extended to the anterior corner or the middle level of the testes, and sometimes it crossed testes. The acetabulum was located in the mid-part of the body, being round in shape and smaller than the oral sucker. The ratio of the acetabulum to the oral sucker was 0.67-0.85; 0.77 on the average. The anlagen of the testes ranged symmetrically in the posterior quarter of body. The vasa efferentia arose from the testes and combined immediately in front of the so-called false cirrus pouch which was located below the acetabulum. The anlage of the round ovary was located on the dorsal side of the acetabulum. In the pressed specimen, the ovary was observed on the left or right side of the acetabulum. The oviduct still remained tubular, and Laurer's canal, the uterus, the vitelline duct and vitelline reservoir were already recognizable. The metraterm which connected with the false cirrus pouch opened into the genital pore which was located at the somewhat posterior part of the acetabulum. The excretory bladder occupied the posterior one third to one fourth part of the body. The flame cell formula was 2 $[(2+2+2)+(2+2+2)]=24$, identical to the formula of the cercaria (Fig. 1-C).

5. Morphology of adults experimentally obtained (Table 2, Fig. 1-D, Photo. 1-F)

Four adults were obtained from a rat 12 days postadministration of 40 metacercariae from *Oncorhynchus masou* experimentally infected with the cercariae. On the other hand, one adult was collected from a rat 12 days postadministration of 65 metacercariae from the goldfish experimentally infected.

The body of the adult worm was spindlical, oval or ellipsoidal, often tapering at the posterior end, and measuring 560-690 × 245-340 μm. The body surface was covered with minute spines which were numerous on the anterior part of body, and the number of

spines gradually decreased on the posterior part. No spines were detected in the area from the posterior one fifth of the body to the posterior end. The oral sucker existing on the anterior end of the body was circular, measuring 113-150×128-170 μm. The pharynx was subspherical, 34-45×34-45 μm, followed by a winding esophagus 1-2 times the length of the pharynx. The esophagus was divided into two ceca which were much wider than the esophagus. The ceca, extending up to the posterior part of the testes, ended between the testes and dorsal surface of the body. The acetabulum was located somewhat behind the diverging point of the ceca. The ovary was on the left side of the acetabulum, and the false cirrus pouch was on the right side of the acetabulum or vice versa. The false cirrus pouch had a thin wall and contained the ejaculatory duct, a pars prostatica and a large seminal vesicle divided into two parts by a constriction. The ovary was almost round, measuring 50-75×23-50 μm and was connected with the ootype by the oviduct. The oviduct had two bulges. The bulge nearer the ovary contained active sperm. The inner surface of its wall was lined with fine cilia. This portion may be identical with the so-called fertilization chamber named by Bennington and Pratt (1960). Laurer's canal arose from the other bulge near the ootype, and opened to the medio-dorsal part of the body. The common vitelline duct opened into the oviduct immediately before the ootype. The vitelline glands were composed of large follicles which were scattered irregularly beneath the dorsal surface and extended toward the ventral surface. The yolk cells were detected at the junction of the two vitelline ducts situated at the posterior part of the acetabulum. The junction formed a relatevely wide chamber called the vitelline reservoir. The ootype was located behind the acetabulum in a central position. The uterus was a simple U- or W-shaped tube which extended backward from the ootype and then forward toward the common genital duct. The genital pore opened slightly behind the acetabulum in the medio-ventral position. There were usually a few eggs in the uterus. The eggs (Photo. 1-G) were ovoid in shape, 70.7-90.0 μm long by 42.4-54.0 μm wide, light yellowish brown in color, and they were poorly developed at the time of egg-laying. The egg shell was smooth and operculated at one end and had a very small blunt point at the other. The two large oblong testes lay in the posterior half of the body, and were arranged obliquely or symmetrically. The two tiny vasa efferentia joined to form a common duct, the vas deferens, before entering the false cirrus pouch. The excretory bladder was saccular.

Discussion

In Japan six species of microcercous xiphidiocercariae parasitizing in the freshwater snail, *Semisulcospira libertina*, as the first intermediate host have been reported. They are: *Paragonimus westermani*, *Cercaria incerta*, *C. libertina*, *C. distyloides*, the unknown species of Nakade (1972) and the unknown species of Saito *et al.* (1983). The cercaria presented in this paper is closest to *P. westermani* from the morphological characteristic of the tail; that is, the tails of both species are conical in shape and bear fine hair-like spines on the tip. However, the cercaria presented in this paper can be readily distinguished from *P. westermani* by many features. The present observations of cercaria include the following: the prepharynx is unrecognizable, the nerve commissure crosses behind the pharynx, the penetration gland cells consist of homogeneous cells; there are adhesive gland cells on the ventral side of the excretory bladder, etc. These morphological features are not observable in *P. westermani*.

In regard to the species of the microcercous xiphidiocercariae with adhesive gland cells, *Nanophyetus salmincola* has been detected from *Oxytrema silicula* in the north-western Pacific area of the USA (Sinitsin, 1930; Simms *et al.*, 1931), *Nanophyetus shikhobalowi* from *Semisulcospira laevigata* and *S. cancellata* in Eastern Siberia (Filimonova, 1965), *Sellacotyle mustelae* from *Campeloma rufum* in the USA (Wallace, 1935), *Skrjabinophyedus repens* from *Bythinella com-*

pressa in Germany (Brendow, 1970), and unknown species from *Bythinella nipponica* (Gyoten, 1981) and *Semisulcospira libertina* (Saito *et al.*, 1983) in Japan. *S. mustelae* lacks the hair like spines on the tail. Two species from *Bythinella* spp. possess the long prepharynx. The cercaria of Saito *et al.* (1983) has the Y-shaped excretory bladder. From the facts mentioned above, the present species also differs clearly from *S. mustelae*, 2 species from *Bythinella* spp. (Brendow, 1970; Gyoten, 1981) and unknown cercaria from the snail, *S. libertina* (Saito *et al.*, 1983)

The present cercaria was morphologically almost identical with both *N. salmincola* (Bennington and Pratt, 1960) and *N. schikhobalowi* (Filimonova, 1963), except for the fact that the body length of the present cercaria was somewhat smaller and the number of flame cells was smaller. Both the flame cell formulae of the cercaria and metacercaria in *N. schikhobalowi* were reported to be 2 [(2+2+2)+(2+2+2)]=24 by Filimonova (1963). These formulae were revised to 2 [(3+3+3)+(3+3)]=30 in the marginal notes of the same paper. The formulae of the present specimens were 2 [(2+2+2)+(2+2+2)]=24 like her first report on them. A mistake has often been made as if 3 capillaries arise from one branch, inasmuch as the sites of the 2 branches arising from the collecting tube are very near each other. The taxonomic problem of the Siberian form, *N. schikhobalowi*, and the Japanese form, the present *Nanophyetus*, may be solved with ease if there is a true difference in the formulae of the 2 forms.

In the redia, the present specimens are very similar to *N. salmincola* (Bennington and Pratt, 1960). The cecum of the redia of *N. schikhobalowi* extends to near the end of the body (Filimonova, 1963), while the lengths of the ceca of both the present specimens and *N. salmincola* are one third to two thirds of the body length.

The genus *Nanophyetus* was first found by Donham (1925), who obtained small flukes from the intestine of a dog that died after eating sore-back salmon from the Pacific Coast of North America. The flukes found by him were described by Chapin (1926) as a new genus and species, *Nanophyes salmincola*, of the family Heterophyidae. The generic name was preoccupied and so it was revised to *Nanophyetus* (Chapin, 1928). After that, Skrjabin and Podjapolskaja (1931) described flukes from natives in Far Eastern Siberia as a new species of *Nanophyetus*, *N. schikhobalowi*, which differs from *N. salmincola* principally in the smaller size of the eggs and the opening of the genital pore. However, Witenberg (1932) considered the description of Skrjabin and Podjapolskaja incomplete and concluded that *N. schikhobalowi* was a synonym for *N. salmincola*. Bennington and Pratt (1960) and Gebhart *et al.* (1966) also agreed with his opinion. From the result of the comparison of the Siberian form, *N. schikhobalowi*, from natives and dogs in Siberia and the US form, *N. salmincola*, from dogs in Oregon, Filimonova (1968) stated that these showed morphologically no significant difference. She (1966; 1968), however, regarded *N. schikhobalowi* as a subspecific status. The main differences between the two subspecies are that the Siberian form does not apparently carry a rickettsia and that natural human infections with the US form have never been reported. However, it seems very difficult to identify the two forms as subspecies from the above mentioned differences.

Recently, it became clear that the genus *Nanophyetus* is distributed in Japan (S. Saito *et al.*, 1977; S. Saito, 1978; Y. Saito, 1977; Y. Saito *et al.*, 1979). Y. Saito *et al.* (1982) observed the morphology of 50 adults from *Meles meles anakuma*, 50 from *Chimarrogale platycephala platycephala*, and 191 from a dog 7 days postinfection. As a result, we described the adult flukes as a new species, *Nanophyetus japonensis*, characterized by a conical projection at the posterior end of the body, the asymmetric position of the oval testes as the one lies anterior to the other in stained preparations as well as in live specimens, and the digestive tracts are usually lateral to the testes or above them, but never mesial. We also added as a characteristic feature of *N. japonensis* the fact that the acet-

abulum of the metacercaria is eminently smaller than the oral sucker.

The adult worms experimentally obtained from the present cercariae were almost identical with *N. japonensis* from the point of the appearance of the posterior part of the body and the relative position of the ceca and the testes, though the two testes were almost symmetrically arranged in the same manner as those of *N. salmincola* and *N. schikhobalowi*. The arrangement of the testes was considered to vary because of many factors such as the freshness of the specimens and the period of the development of the adult, the specific difference of the final host, the preparation method of specimens for morphological observation, etc. For the reasons mentioned above, the author came to the conclusion that the present cercaria was identical with *Nanophyetus japonensis*. However, it was difficult to distinguish the present cercaria from the cercariae of *N. salmincola* and *N. schikhobalowi*.

In Japan, Hatsushika and Maejima (1978) described a cercaria from the snail, *Bythinella nipponica*, as the cercaria of the family Nanophyetidae. The author presumed this cercaria to be identical with the cercaria of Gyoten (1981), and not the cercaria of the genus *Nanophyetus*. Moreover, there was no description about the adhesive gland cell in the cercaria of Hatsushika and Maejima (1978), so their cercaria was omitted from the discussion in this paper.

Summary

During the past 9 years from 1975 to 1983, one species of microcercous xiphidiocercariae was detected from the freshwater snail, *Semisulcospira libertina* in Iwate, Akita and Yamagata Prefectures, Japan. From the feature of the tail with fine hair-like spines on the tip, this cercaria is close to that of *Paragonimus westermani* in appearance. But it can be clearly distinguished from *P. westermani* by many features as follows: the prepharynx is not recognizable, the nerve commissure crosses behind the pharynx, the penetration gland cells consist of eight pairs of homologeneous cells, there are adhesive gland cells on the ventral side of the excretory bladder, etc. On the other hand, 6 species of microcercous xiphidiocercariae with adhesive gland cells have been reported. They are: *Nanophyetus salmincola*, *N. schikhobalowi*, *Sellacotyle mustelae*, *Skrjabinophyedus repens* and 2 unknown species up to date. The present cercaria was identified with that of *Nanophyetus japonensis* from its lack of a prepharynx, the presence of the spines on the tail tip and the U- or V- shaped excretory bladder, and also from the morphological and biological observations of the metacercariae and adults obtained experimental infections.

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日本のカワニナから検出した *Nanophyetus japonensis* のセルカリアとその感染実験

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1975年から1983年の間に，岩手，秋田，山形3県のカワニナから短尾セルカリアの一種を検出した．このセルカリアは尾端に微毛を生じている点で，一見 *Paragonimus westermani* に似ているが，前咽頭を欠く，神経連合が食道を横切る，侵入腺細胞は等質の8対からなる，adhesive gland cells が排泄嚢の腹側にある，などから明らかに *P. westermani* と区別できた．一方，adhesive gland cell を持つ短尾セルカリアは現在までに *Nanophyetus salmincola*, *N. schikhobalowi*, *Sellacotyle mustelae*, *Skrjabinophyedus repens* および不明種2種の合計6種が知られている．今回のセルカリアは尾端に微毛を有すること，前咽頭を欠くこと，排泄嚢がU又はV字型であること，セルカリアの感染実験で得たメタセルカリアと成虫が *Nanophyetus japonensis* にほぼ一致したこと，などから本種のセルカリアと同定したが，*N. salmincola* および *N. schikhobalowi* のセルカリアとの区別点は見出せなかった．