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The Life Cycle and Larval Development of *Fibricola seoulensis* (Trematoda: Diplostomatidae)†

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Abstract: The life cycle of *Fibricola seoulensis* was studied in the laboratory and in the field, with special interests in the larval developments within the eggs and in the intermediate hosts. The first emergence of miracidia after incubation of eggs in 26°C water began on the ninth day. The miracidia, elongate and cylindrical shape, had epidermal plates in the formula of 6, 9, 4 and 3, with two pairs of flame cells and lateral processes. A kind of fresh water snail, *Hippeutis* (*H.*) *cantori*, was found to shed furcocercous cercariae from the 13th day after experimental challenge with miracidia while *Physa acuta* failed to shed. The same kind of snail collected from the field also shed the same cercariae. The cercariae were equipped with 2 pairs of penetration glands and 5 pairs of flame cells. The tadpoles of *Rana nigromaculata* were found susceptible to experimental infection with the cercariae. The same kind of tadpoles collected from various areas were also found naturally infected. The metacercariae in the tadpoles which were infected experimentally became infective to the definitive host in 21 days. The metacercariae were located free in the body cavity of tadpoles, and attained sexual maturity in rats in 7 days. The present study successfully followed the complete life cycle of *F. seoulensis* and found that it is possible to maintain the life cycle in the laboratory.

Key words: *Fibricola seoulensis*, life cycle, biology, larval development, intermediate host

INTRODUCTION

Fibricola seoulensis (Trematoda: Diplostomatidae) was first described by Seo *et al.* (1964) from the small intestine of house rats, *Rattus norvegicus*, in Seoul, Korea. Later an enzootic survey revealed that this fluke is distributed almost nationwidely in house rats (Seo *et al.*, 1981). It was recognized in the field studies that tadpoles and frogs of *Rana nigromaculata* serve as the second intermediate host (Hong *et al.*, 1982 & 1985), while terrestrial snakes play the role for a kind of paratenic host. *Rhabdophis* (=*Natrix*) *tigrina lateralis* was most frequently infected among several species of snakes examined (Hong *et al.*, 1982; Cho *et al.*, 1983).

Various kinds of laboratory animals, *i.e.*,

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albino rats, mice and guinea pigs were found to be suitable final hosts (Hong *et al.*, 1983; Cho *et al.*, 1983). The metacercariae grew to adults within a week in these animals. The main habitat was the duodenum, just distal to the pylorus. As the metacercaria developed, cylindrical hindbody grew out from the conical primordium on the posterodorsal surface of its forebody (Hong, 1982; Hong *et al.*, 1983). The tegumental surface of adult *F. seoulensis* was covered with velvety cytoplasmic processes, and armed with serrated spines and 4 kinds of sensory papillae (Seo *et al.*, 1984; Lee *et al.*, 1985).

While the larval stage of *Alaria* sp. was found to infect humans (Beaver *et al.*, 1984), diplostomatid fluke adults had never been known to infect humans before many human cases infected by *F. seoulensis* adults were reported in Korea (Seo *et al.*, 1982; Hong *et al.*, 1984 & 1986a). They had the history of consuming raw flesh and/or viscera of terrestrial snakes. The flukes responded well to the treatment with praziquantel (Hong *et al.*, 1984 & 1986a; Seo *et al.*, 1985; Lee, 1985) or bithionol (Seo *et al.*, 1982).

Among the species of *Fibricola*, complete life cycle has been reported in 3 species; *F. cratera*, *F. texensis* and *F. lucida* (Sudarikov, 1960; Chandler, 1942). This study was undertaken to extend our knowledge concerning the life history of *F. seoulensis* by laboratory experiments and field surveys.

MATERIALS AND METHODS

1. Embryonation of the Eggs

A total of 480 gravid *F. seoulensis* was recovered, free of fecal debris, from the small intestine of albino rats 10 days after experimental infection, as described by Hong *et al.* (1982). They were incubated in Tyrode's solution for 12 hours at 36.5°C and the eggs laid were collected. The eggs were rinsed 3 times with distilled water and transferred into petri dishes containing distilled water. The dishes were stored at 26°C in a dark incubator with aeration. The process of embryonation was observed for 11 days with the phase contrast and the bright field photomicroscopes. Some hatched miracidia were stained with neutral red to observe the internal structures, and some were by silver impregnation method to observe the epidermal plates.

2. First Intermediate Host Study

Fresh water snails, *Physa acuta* or *Hippeutis* (*Helicorbis*) *cantori*, which were reared in our laboratory over one year, were used for experimental infection of miracidia. Active miracidia, 10~20 in number, were dropped into petri dishes of 55 mm diameter each containing a snail in tap water. The dishes were laid at windowside for 3~4 hours to facilitate penetration of the miracidia. The exposed snails were kept in an aquarium at 26°C for 41 days. The shedding of cercariae was examined daily from the 5th day after infection by natural emerging method.

In search of the natural first intermediate host, *Lymnaea* sp. and *H. cantori* snails were collected at rice paddies in 5 localities of Namyangju-gun, Kyonggi-do from April to June and July to September 1985, respectively (Fig. 1). One or both kinds were the most dominant snails in the surveyed areas. The snails were examined either by natural emerging or by crushing method. Stained with neutral red after fixation in hot 10% neutral formalin, the cercariae and/or sporocysts from the snails were observed microscopically.

3. Second Intermediate Host Study

To survey their natural infection with metacercariae of *F. seoulensis*, the tadpoles of *Rana nigromaculata* were collected at 5 localities from April to July (Fig. 1). The abdominal cavity of this host was opened and washed vigorously 3 times with 0.43% saline solution. Their flesh was compressed between glass slides and examined under a dissecting microscope.

In order to observe the development of metacercariae in tadpoles, sixteen apparently non-infected tadpoles collected from local areas

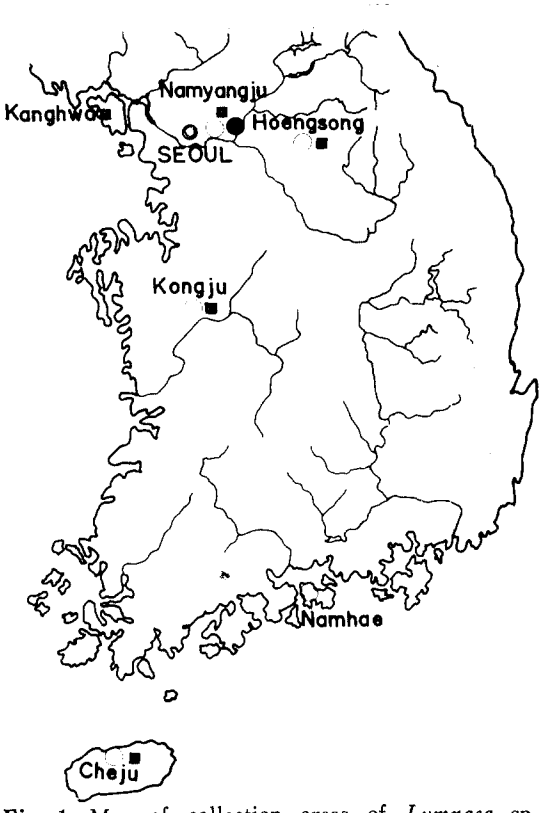

Fig. 1. Map of collection areas of *Lymnaea* sp. (○), *H. cantori* (●) and tadpoles of the frog, *Rana nigromaculata* (■).

were experimentally challenged with the cercariae emerged from naturally infected snails. Two tadpoles each were autopsied at 2, 4, 5, 7 and 21 days after challenge and the metacercariae were recovered from their abdominal cavity. They were fixed in hot 10% formalin, stained with Semichon's acetocarmine, and microscopically examined.

4. Infection of Final Host

Metacercariae collected from a tadpole 21 days after the cercarial challenge were fed orally to an albino rat. The rat was autopsied at the 7th day of infection and examined to recover the adult diplostome.

RESULTS

1. Embryogenesis in the Eggs

On the first day of embryonation, the intraoval cell divided into a 4-cell stage, on the second day to morular stage, and morular or blastular stage on the third day. On the fourth day, all eggs developed into embryos. On this day the eye spots appeared in half of them and short cilia were found on the surface of the embryos. On the fifth day all embryos had eye spots of conical shape. The length of the embryos approximately reached to that of the egg shell. At that time the early miracidia began to contract and stretch. On the sixth day miracidia moved actively, their eye spots became crescentic, and the terebratorium appeared at the anterior end. On the seventh day miracidia grew to be $1\frac{1}{3}$ to $1\frac{1}{2}$ longer

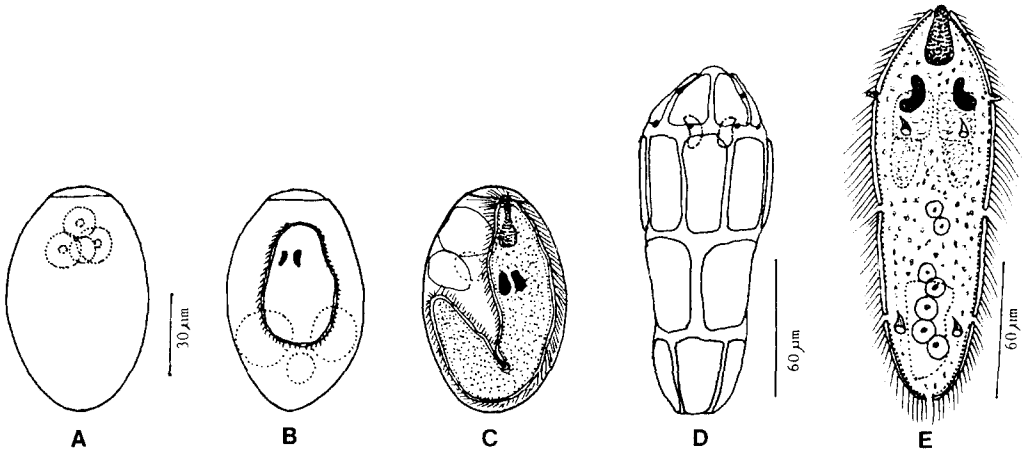

Fig. 2. Embryogenesis of *F. seoulensis* eggs. A : 4-cell stage, B : immature miracidium (embryo), C : mature miracidium, D : epidermal plate, E : internal structures.

than the egg shell (Fig. 2A, 2B & 2C).

When the petri dishes containing embryonated eggs were placed under the light on the ninth day, almost all miracidia hatched out at a time. The hatching rate of the eggs collected from fecal pellets of experimental rats was much lower, about 50%, than that of the eggs collected after incubation of gravid worms, when the eggs were incubated under the same condition. As embryogenesis progressed, the amount of mucoid substance increased on the egg shell and the eggs seemed to become sticky to each other or to the bottom of the petri dish.

2. Morphology of the Miracidia

The miracidia, elongate-cylindrical, infravitally stained with neutral red measured 119.4 μm in length (106.6~123.0 μm) and 34.3 μm (29.5~41.0 μm) in maximum diameter. Cilia covering the surface were 7.2 μm in average and became shorter nearby the terebratorium. The terebratorium was protrusible at the anterior end and measured 16.7 μm long and 8.2 μm wide. There were 4 rows of ciliated epithelial cells containing 6, 9, 4 and 3 epidermal plates respectively. The large crescent-shaped eye spots were at the level of the first junction. There were eight sensory papillae, six of which were located at the center of the posterior margins and two at the middle interface of epidermal plates in the first row. Two pairs of flame cells; one pair posterior to the eye spots and another behind the third junction of the rows of epidermal plates. It was not easy to follow the collecting tubules and to find out the excretory pore. The apical gland consisted of 4 glandular cells, two large anterior and two small posterior ones, which were at the level of the second row. A few germ cells were observed in the posterior portion of the miracidium (Fig. 2D & 2E; Fig. 4A & 4B).

3. Development of Miracidia to Cercariae in the Snail Host

Penetration of the miracidia into the snail body was, though hardly seen under stereomicroscope, confirmed by the disappearance of many miracidia from the water and by their casted-off epithelia on the bottom of the petri dishes.

Neither cercariae nor sporocysts developed in 448 *P. acuta* challenged with the miracidia. Out of 445 *H. cantori* challenged, 26 snails (5.8%) shed a large number of furcocercous cercariae. The first appearance of cercariae was

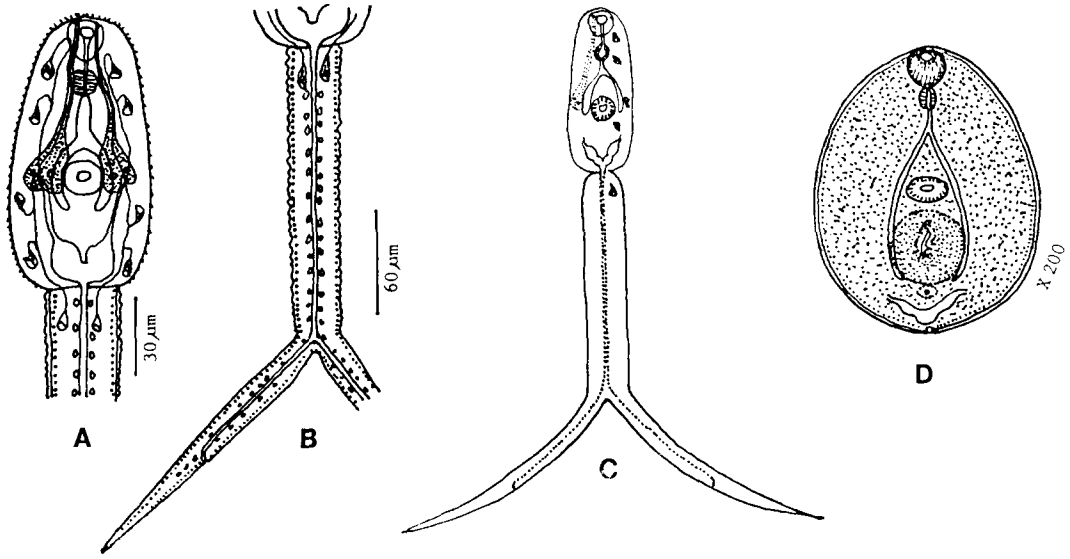

Fig. 3. Cercaria and metacercaria of *F. seoulensis*. A: cercarial body, B: tail of cercaria, C: whole view of cercaria, D: metacercaria recovered from experimental tadpole.

detected from the 13th to 33rd day from each snail after the miracidial challenge. The infected snails shed cercariae for 4～5 days, then died. The snails which naturally shed the cercariae were examined by crushing method to reveal a number of tubular sporocysts that threaded in and out of the snail liver, ovotestis, genital tract, digestive gland and adjacent tissues.

The long complete sporocysts, *i.e.*, daughter sporocysts, were 3,128μm (2,217～4,001 μm) long and 130 μm (102～164 μm) in diameter and

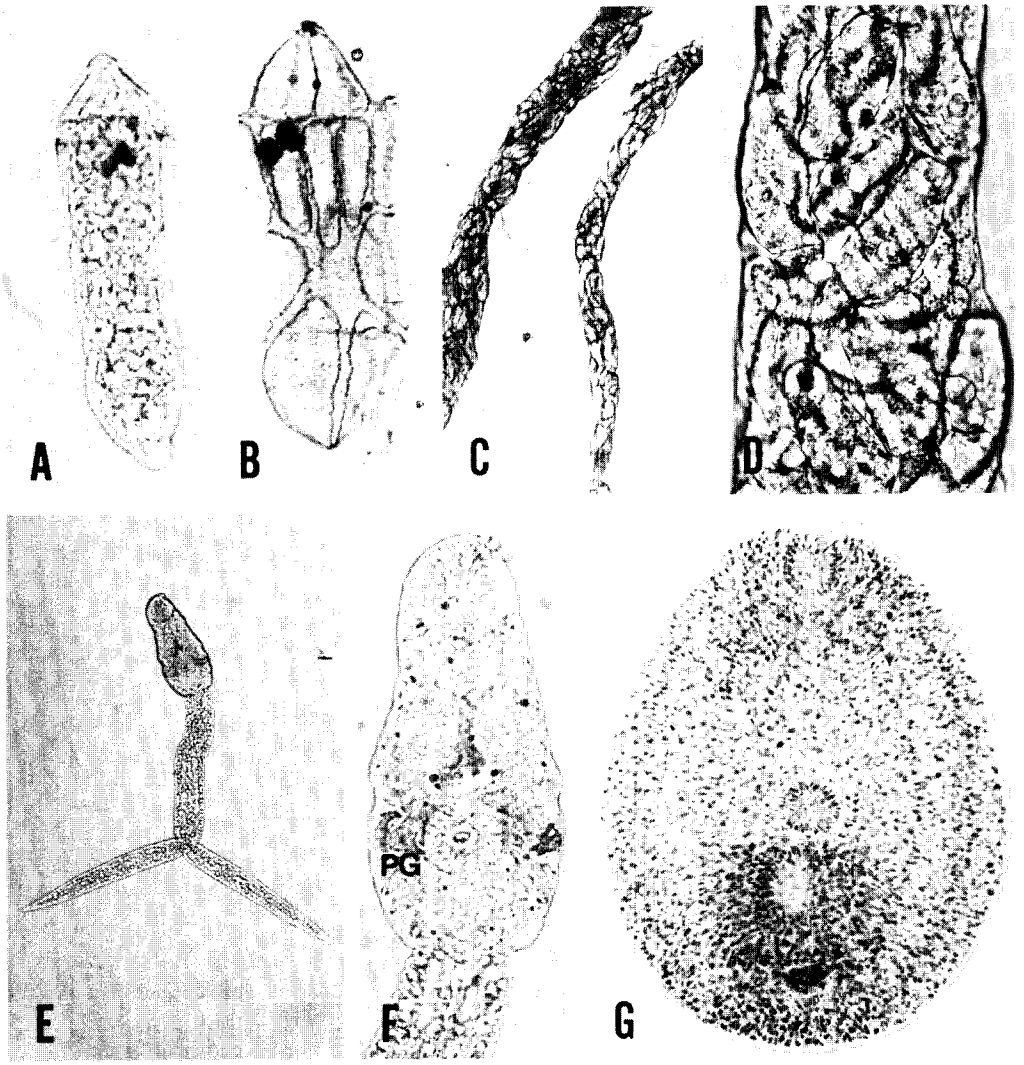

Fig. 4. A: miracidium infravitally stained with neutral red (×400), B: epidermal plate of miracidium stained by silver impregnation method, C & D: daughter sporocyst from experimentally infected *Hippeutis* snail showing numerous cercariae (×40 & ×200), E: cercaria from experimental snail stained with neutral red (×100), F: higher magnification of cercarial body of Fig. 4C showing penetration glands (PG) (×400), G: metacercaria recovered from the tadpole 21 days after experimental infection, acetocarmine stained (×200).

contained numerous developing furcocercous cercariae. The birth pore was not seen. The long sporocysts revealed their sluggish movements (Fig. 4C & 4D).

Total 3,936 *Lymnaea* sp. snails collected from five localities were all negative for furcocercous cercariae but some of them harbored echinostome, gymnocephalous and/or xiphidiocercariae. Out of 1,410 *H. cantori* collected at Namyangju-gun, 12 snails (0.85%) shed furcocercous cercariae.

4. Morphology and Behavior of the Cercariae

Body elongate ovoid, 93.2 μm (80.6～121.0 μm) long and 45.0 μm (33.6～57.1 μm) wide. Oral sucker oval 29.4 μm (26.9～33.6 μm) long and 26.4 μm (25.2～30.2 μm) wide, bearing oral and penetration duct openings. Pharynx oval, connected to oral sucker without prepharynx and leading to a short esophagus which bifurcated into ceca terminating at the level of posterior margin of ventral sucker (Fig. 3A). Ventral sucker 23.0 μm (20.2～25.2 μm) long and 24.2 μm (20.2～26.9 μm) wide, lip aspinous, however, had inwardly directed spines on its inner surface. Two pairs of penetration gland cells, pear-shape, located lateral to ventral sucker. Ducts arising from penetration gland cells ran anteriorly and bulging in oral sucker and opened anterodorsally to oral opening. Tail stem 157.4 μm (137.8～174.7 μm) long and 32.9 μm (25.2～42.0 μm) wide, its furcae 155.6 μm (139.4～148.8 μm) long. There were ten flame cells observed; one pair posterolateral to oral sucker, one pair anterior to ventral sucker, two pairs posterior to ventral sucker, and last one the largest and most conspicuous in proximal portion of tail stem. The flame cell capillaries and collecting tubules were very hard to follow. Excretory bladder small and Y-shaped. Excretory canal passed along the axis of tail stem, bifurcated at the junction of tail stem and furcae, and its branches opened halfway of the ventral margin of each furca (Fig. 3A, 3B, & 3C; Fig. 4E & 4F).

When undisturbed the cercariae hang down quietly with their body either contracted or stretched, with the tail stem straight, and furcae separated by a right angle. The cercaria showed positive phototaxis and rested beneath the surface of water even in shallow containers. Cercariae sank slowly and from time to time swam upwards. While the cercariae swam, the body and tail stem extened, and the tail stem bent first to one side then to the other, and furcae whipped back and forth. The cercaria, therefore, was propelled by the vibratory motion and pulled by the furcae, tail foremost.

The morphological characteristics and behavior of the furcocercous cercariae emerging from naturally infected *H. cantori* collected at Namyangju-gun were identical to those of the cercariae obtained from experimentally infected snails except for slightly larger measurements.

5. Natural Infection Rate and Development of the Metacercariae in Tadpoles

From 400 tadpoles of *R. nigromaculata* collected from 5 localities, the incidence rate of diplostome metacercariae was 75.1% in average, and the mean number of metacercariae per tadpole ranged from 2.3 to 119.4 (Table 1).

In the experimental infection of tadpoles by the cercariae, almost all cercariae attached to the skin of tadpoles by their suckers, creeped about and finally penetrated into the junction of their abdominal wall and tail. Their penetration was aided by vigorous zigzag motion of the tail which was casted off after completion of the penetration. The penetration took about 5～6 minutes.

At the second day after the challenge, the cercarial bodies appeared in the abdominal cavity of tadpoles. The metacercariae of *F. seoulensis* were located free in the abdominal cavity of tadpoles without formation of cyst wall throughout the period of observation. The length of metacercariae recovered from tadpoles at the second day was 162.1 μm which was slightly larger than the body of cercariae. Penetration glands and leading ducts were

Table 1. The incidence of diplostomatid metacercariae from tadpoles of *R. nigromaculata* in several areas

Locality	Date	No. tadpoles		No. metacercariae	
		examined	posit. (%)	range	(average)
Cheju city	April 27	38	0	—	
Kangwha-gun	May 25	181	134 (74.0)	1~ 40	(2.3)
Namyangju-gun	May 26	45	45(100.0)	1~100	(29.8)
	July 31	10	10(100.0)	47~202	(119.4)
Kongju-gun	June 2	100	92 (92.0)	1~ 32	(5.5)
Hoengsong-gun	June 6	26	24 (92.3)	1~ 30	(9.6)
Total		400	305 (76.3)	2.3~119.4	

Table 2. Measurements of *F. seoulensis* metacercariae collected from the experimentally infected tadpoles (*R. nigromaculata*) by post-infection days

Organs	Average measurements(μm) at days after infection				
	2	4	5	7	21
No. worms measured	3	10	5	4	10
Body length	162.1	164.9	215.8	312.5	336.9
width	76.5	84.5	118.2	204.3	241.9
Oral sucker					
length	36.3	24.5	34.2	44.2	45.9
width	38.0	33.0	43.9	46.3	41.0
Pharynx					
length	17.0	15.5	17.3	—	27.7
width	17.0	14.3	20.5	—	20.3
Ventral sucker					
length	25.5	22.8	31.2	32.3	39.1
width	25.5	22.8	30.2	37.2	44.0
Tribocytic organ					
length	—	—	—	—	72.7
width	—	—	—	—	95.6
Germinative mass					
length	22.7	21.0	—	—	21.0
width	17.6	22.4	—	—	33.6

remained in part. A germinative mass appeared in the posterior portion. The posterior margin of the larvae still had a median notch which was a residue of the tail. At the fourth day the metacercariae enlarged to some degree and became ovoidal in shape. The penetration glands and ducts disappeared completely (Fig. 3D). Thereafter the metacercariae grew so rapidly that the growth curve showed a steep sigmoid slope during the fifth to seventh day and reached 312.0 μm in length. The metacercariae grew steadily, to become pear-shape, 336.9 μm long at the 21th day, and the appearance of tribocytic organ was already recognized. The excretory bladder, biconical in shape, was at the posteriormost portion (Table 2 and Figs. 3D & 4G).

A total of 252 metacercariae was fed to an albino rat and 52 diplostomes (20.9%) identified to be *F. seoulensis* (Seo *et al.*, 1964; Hong, 1982) were recovered from the upper part of the small intestine at 7 days after the infection.

DISCUSSION

As for the life history of *Fibricola seoulensis*, the house rat is known as the definitive host, the second intermediate host is the tadpoles or frogs, and the snake serves as a kind of paratenic host. However, the snail intermediate host was not known. This study confirmed that the planorbid snail, *H. cantori*, is both naturally and experimentally the first intermediate host of *F. seoulensis*. It is one of common fresh water snails in rice paddies or irrigation channels in Korea (Kim *et al.*, 1983). On the other hand, *Physa acuta* appeared not a suitable snail for *F. seoulensis*, although *Physa* sp. was recorded as one of the first intermediate hosts of other *Fibricola* spp. (Chandler, 1942; Sudarikov, 1960). Other snails, such as lymnaeid ones, are also considered unsuitable for *F. seoulensis*, although they dwell in the same ecological conditions of rice paddies in Korea. It is certain that they do have an equal opportunity to become a snail host in the field, however, only *H. cantori* played the role in this study.

After experimental incubation of the eggs at 26°C, the immature eggs continued their cell division to form embryos at the 4th day. Mature miracidia hatched out on the 9th day, when they were exposed to the light. The developmental process of the egg to the miracidium was largely similar to that of *F. cratera* (Cuckler, 1940) or of *F. texensis* (Chandler, 1942). However, hatching of the miracidia took 20~30 days in case of *F. cratera* at room temperature (Cuckler, 1940). The temperature seems to be one of the important factors for the development and hatching of the miracidia. Lee *et al.* (1986) observed that the eggs did not develop at 4°C, and miracidia appeared in more than 90% of eggs at room (11~26°C) or higher temperature (28°C) after 8 days. They further observed that the hatching rate of eggs at room temperature was only 2~6% at the 18th day which became 34% at the 30th day, whereas the rate at 28°C was over 90% after 10 days. The hatching rate of eggs, though, seems to be influenced by the method of egg collection, *i.e.*, the rate was low among the eggs collected from fecal pellets, while almost all of the eggs obtained directly from the flukes incubated in Tyrode's solution hatched out. The lower hatching rate in the former was regarded due either to contamination of microorganisms or to other reasons. Chandler (1942) also reported, in *F. texensis*, a lower hatching rate of the eggs collected from feces.

The size of structure of mature miracidia of *F. seoulensis* was similar to those of other *Fibricola* spp. (Sudarikov, 1960). The lateral processes, one of the characteristic structures of strigeid miracidia, have been known to be present only on the first junction of epidermal plates in *F. texensis* or *F. cratera*. However, they were observed on every epidermal junction in *F. seoulensis* miracidia.

The cercariae of *F. seoulensis* were similar to those of *F. texensis* and *F. lucida* in their size and structure. Cort and Brooks (1928) stated that the fundamental flame cell formula for the cercariae of strigeids was [(1+1+1)+(1+1+{1})], which corresponded well to that of *F. cratera* or *F. texensis*. However, it differed from that of *F. lucida*, which has 9 pairs. The cercaria of *F. seoulensis* revealed no forwardly-directed spines which would aid the cercarial penetration into the second intermediate host.

After penetration of the cercariae, the bodies appeared in the body cavity of tadpoles and remained free in there until the tadpoles metamorphosed into frogs (Cook, 1978). The metacercariae of *F. seoulensis* in the body cavity of tadpoles revealed an outline of the tribocytic organ on the 8th day. They became infective to experimental rats at the 14th day after infection. The recovery rate of adult flukes increased as the duration of infection in tadpoles was prolonged (Hong *et al.*, 1986b). The hindbody of *F. seoulensis* grew rapidly to become the same length or even larger than the forebody and attained sexual maturity in experi-

mental rats and mice 7 days after the metacercarial infection (Hong *et al.*, 1982).

By these experimental and field studies, the whole life cycle of *F. seoulensis* has been completely elucidated. The flukes were shown to continue their life cycle just the same way as in other diplostomatid flukes, especially in areas where *H. cantori*, tadpoles or frogs (*R. nigromaculata*), and rats, (*Rattus norvegicus* or *Apodemus* sp.), live together in the same ecosystems.

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=국문초록=

*Fibricola seoulensis*의 생활사 및 유충의 발육

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실험실내에서 충란 및 제 1 중간숙주와 제 2 중간숙주내에서 *F. seoulensis* 유충의 발육과정을 관찰하고 자연 상태에서의 생활사를 연구하고자 하였다. 충란을 26°C 물에서 배양한 제 9 일에 miracidium이 난개를 열고 나왔다. Miracidium은 원통 세장형으로 2쌍의 flame cell과 측방돌기가 있었고 epidermal plate의 배열식은 6, 9, 4 및 3이었다. *Hippeutis* (*Helicorbis*) *cantori* 패류에서는 실험감염 후 제13일에 furcocercous cercaria가 유출되었으나 *Physa acuta*에서는 cercaria가 유출되지 않았다. 논에서 채집한 *H. cantori*에서는 같은 형태의 furcocercous cercaria가 유출되었다. Cercaria에는 2쌍의 침입선과 5쌍의 화염세포가 있었다. 참개구리의 올챙이(*Rana nigromaculata*)는 cercaria 실험감염에 대하여 감수성이 있었으며 채집된 참개구리의 올챙이들은 *F. seoulensis*의 피낭유충에 자연감염되어 있었다. 실험감염 후 올챙이에서 피낭유충은 21일 이내에 종숙주에 대하여 감염능력을 갖게 되었다. 대부분의 피낭유충은 올챙이의 체강내에서 피낭을 형성하지 않고 있었으며 쥐의 소장에서 7일만에 성충이 되었다. 본 연구를 통하여 *F. seoulensis*의 모든 생활사가 밝혀지게 되었으며 또 실험실내에서 전 생활사를 완료시킬 수 있다는 것을 알게 되었다.