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Experimental life history of *Spirometra erinacei*†

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Abstract: The complete life cycle of *Spirometra erinacei* has been experimentally maintained in the laboratory. The cyclops were reared as the first intermediate host, and the tadpoles of *Rana nigromaculata* as the second intermediate host. ICR mice were used as another second host. The experimental definitive hosts were dogs and cats. Maturation and hatching of the eggs took 8 to 14 days by incubation at 29°C. The coracidium measured 43.8×36.9 μm. *Mesocyclops leuckarti* and *Eucyclops serrulatus* were susceptible to the coracidial infection. The procercoids older than 5 days in the cyclops had minute spines at the anterior end, calcium corpuscles in the body parenchyme and the cercomer at the posterior end. Procercoids 10 to 20 days old were infective to tadpoles, and 15 or 21 day old worms could infect the mice. The plerocercoids from the tadpoles at 15 days after experimental infection were pear-shaped and shorter than 1 mm in the length and were infective to mice. Fifteen to 18 days after experimental inoculation of plerocercoids to dogs or cats, the adult worms began to produce eggs. One life cycle from egg to egg needed 48 to 67 days in the laboratory. The morphology of larval or adult worms was compatible with the description of *Spirometra erinacei*.

Key words: *Spirometra erinacei*, life history, egg, coracidium, procercoid, plerocercoid, adult, cyclops, tadpole

INTRODUCTION

Spirometra erinacei is a pseudophyllidean tapeworm whose plerocercoid larva named sparganum infects humans causing sparganosis (Iwata, 1972). In rare occasions, adults were found from humans (Iwata and Matsuda, 1967; Suzuki *et al.*, 1982; Lee *et al.*, 1984). Its natural definitive host is carnivorous mammals such as dogs, cats, hyenas, *etc.* It requires two different intermediate hosts. The first one is cyclops, and the second one is amphibians, reptiles, birds or mammals(Kobayashi, 1931; Mueller, 1938; Iwata, 1972).

In Korea, sparganosis is not an uncommon disease, of which the major infection source is the snake (Weinstein *et al.*, 1954; Cho *et al.*, 1975). It has been speculated that common species of freshwater crustacea may be the first intermediate host. However, the first intermediate host has not been elucidated yet. Also, each developmental stage of sparganum has not yet been described in Korea. In the present study, the experimental life history of *Spirometra*

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erinacei was completed from cultivation of eggs to detection of eggs from adults. The morphology of the larvae and kinds of experimental intermediate hosts are presented in this paper.

MATERIALS AND METHODS

1. Incubation of eggs

Spargana obtained from naturally infected snakes, *Rhabdophis tigrina*, were introduced orally into dogs. After 20 days from the infection, the eggs of *Spirometra* were collected from their feces. The eggs were rinsed and stored in distilled water, and were incubated at 29°C. The process of embryonation of the eggs was observed through a microscope. The hatched larva, coracidium, was also observed microscopically before and after fixation in hot 10% formalin.

2. Experimental infection of the first intermediate host

Fresh water crustacean hosts had been reared in an aquarium in the laboratory. *Mesocyclops leuckarti*, *Eucyclops serrulatus* and *Moina* sp. (Chang, 1989) were exposed to active coracidia in a petri dish respectively. Each cyclops was kept in an aquarium at room temperature, and larval development was periodically observed. The larvae were used for challenge to the second intermediate host at 10, 12, 14, 17 and 20 days after infection.

3. Experimental infection of the second intermediate host

The tadpoles of *Rana nigromaculata* which had hatched from the egg in the laboratory were mixed with the copepods which harboured procercoids. After 15, 20 and 30 days from the exposure, they were dissected for the recovery of plerocercoid larvae. Also the procercoids of 15 to 21 days of age were orally introduced to mice through a gavage needle. The mice were examined for the larval infection after 30 days.

4. Experimental infection of the plerocercoids to mice, dogs and cats

Ten plerocercoids(spargana) from the tadpoles were infected to each of 3~7 mice and the mice were sacrificed 30 days after infection for the recovery. Two spargana recovered from the mice were orally infected to each of 2 dogs and 2 cats. Their feces were examined for the egg discharge.

5. Recovery and observation of adult tapeworms from dogs and cats

After 6 weeks from the infection, the dogs and cats were sacrificed and the adult worms were recovered from their intestine. One of the worms was fixed in 10% formalin under a glass plate. The proglottids at 30~35 cm, 50~55 cm, 70~75 cm and 90~95 cm from scolex were stained in Semichon's acetocarmine solution. Others were fixed in 10% formalin and a part of those proglottids of same location were transversely or sagittally sectioned after paraffin embedding. The sections were stained with hematoxylin and eosin and observed microscopically.

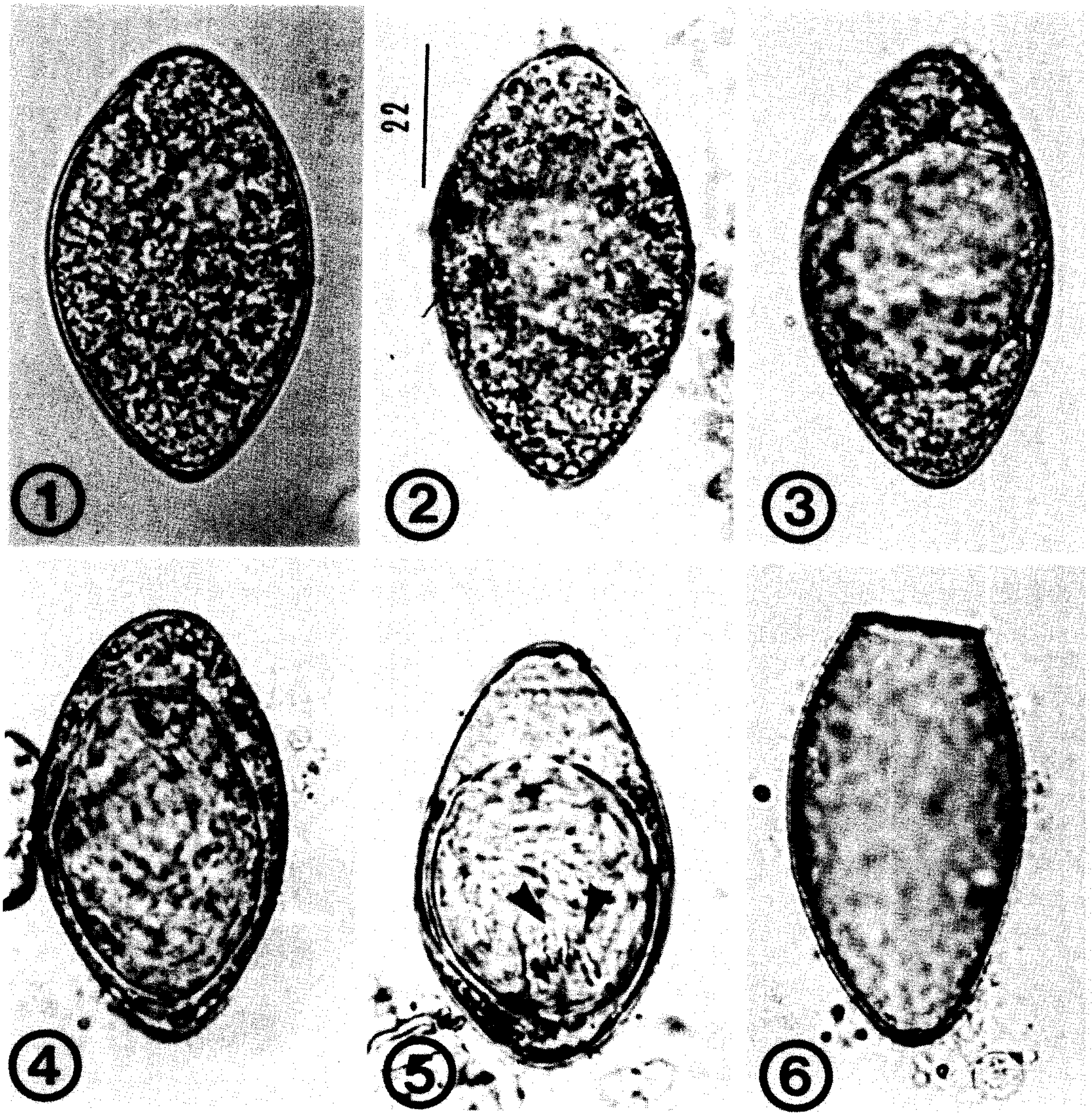
RESULTS

1. Development of coracidia within eggs

The eggs obtained from the experimentally infected dog had the operculum at a sharp pole. The eggs were slightly asymmetric and had greenish yellow shell. They included a germ cell and yolk(Fig. 1).

Three days after incubation, the germ cell grew with blur margin(Fig. 2). The yolk content decreased and an embryonic mass appeared after 5 days(Fig. 3). The embryo shaped a coracidium after 8 days(Fig. 4). The coracidium looked mature and began to hatch with active movements after 13 days(Figs. 5 & 6).

The hatched coracidium had numerous cilia on the surface, and swam actively. It became slender and stretched its terminal hooklets during swimming. The fixed coracidium in hot 10% formalin was oval, and the ciliated embryonic membrane enveloped a hexacanth embryo(Fig. 7). The coracidia measured 43.8 μm long and 36.9 μm wide. Cilia were 12.8 μm



Figs. 1~5. Development of the embryo.
1. The egg of *Spirometra erinacei* from an experimentally infected dog.
2. The egg after 3 days of incubation.
3. The egg after 5 days of incubation.
4. The egg after 8 days of incubation.
5. The egg after 13 days of development, contained fully mature embryo, showing hoolets(arrow heads).

Fig. 6. The egg which already hatched out. * Bar unit is μm.

long, and the embryos were 38.9 μm long and 26.4 μm wide. The hooklets were 8.6 μm long (Table 1).

2. Larval development in cyclops

The procercoids developed in 2 species of cyclops, *M. leuckarti* and *E. serrulatus.* The larvae were in the body cavity. They became elongated and still had hooklets at the body terminal by 4 days after infection(Figs. 8 & 9). On the fifth day, minute spines were found

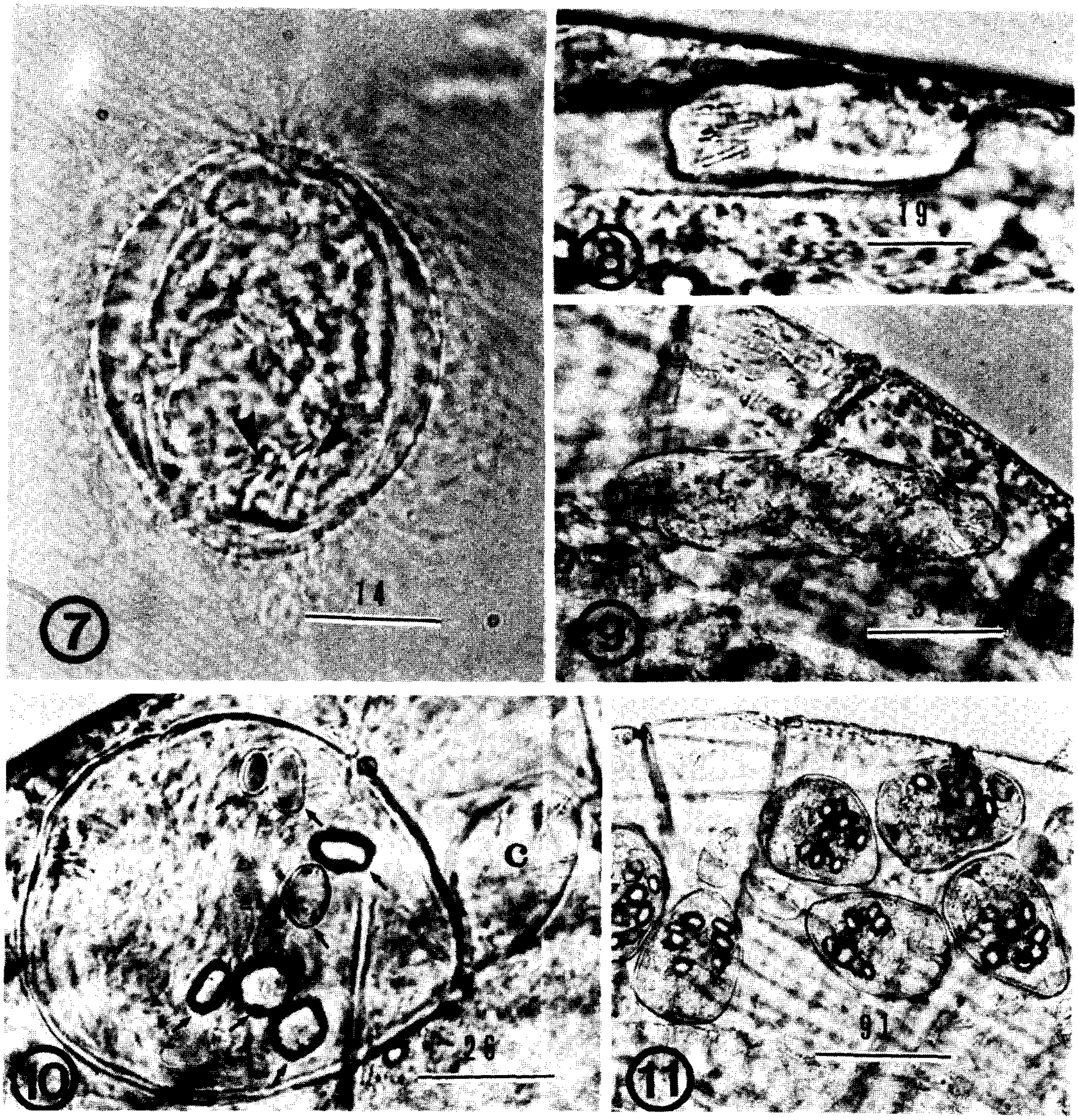
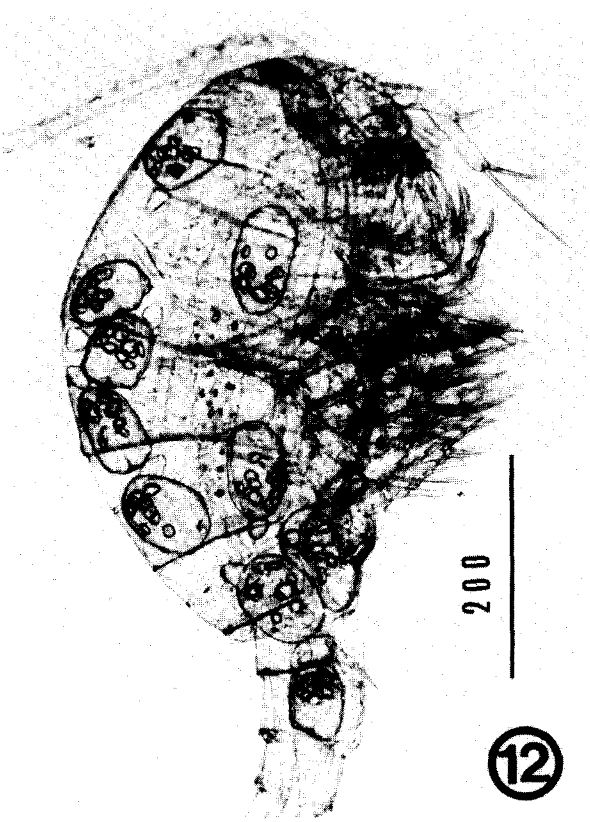
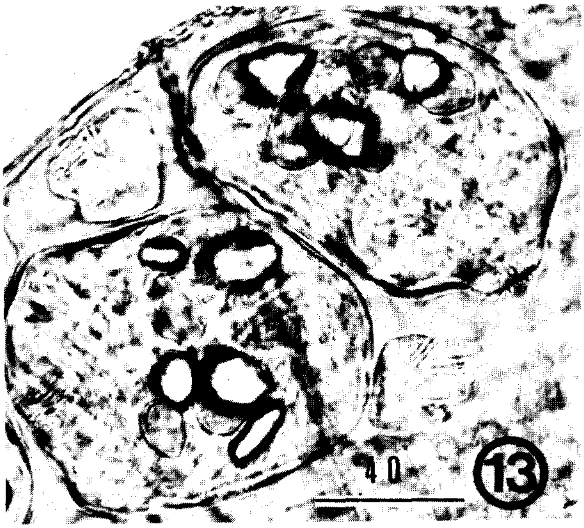


Fig. 7. Coracidium of *S. erinacei* showing numerous cilia and hooklets(arrow heads).
Figs. 8~11. Procercoids of *S. erinacei* in the body cavity of experimental cyclops.
8. A 1-day old one.
9. A 3-day old one.
10. A 5-day old one, showing minute spines at the anterior end, calcium corpuscles(arrows), and cercomer(C).
11. 11-day old ones.

* All bar unit is μm.

dense at the anterior end. Calcium corpuscles appeared in the parenchyme. The posterior body end became sharp to be a cercomer including 3 pairs of hooklets(Fig. 10). The procercoids showed no significant morphological changes after then up to 25 days(Figs. 11~14). The larvae lost their activity, number of calcium corpuscles and even some of them were calcified after 30 days(Fig. 15). The measurements of procercoids in cyclops were as variable as 56~





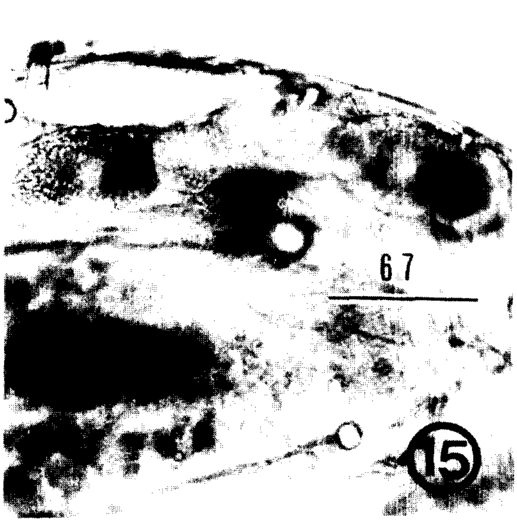


Fig. 12. 15-day old procercoids in the body cavity of experimental cyclops.
Fig. 13. Magnification of Fig. 12.
Fig. 14. A 17-day old procercoid.
Fig. 15. 30-day old procercoids, calcified in cyclops.
* All bar unit is μm.

Table 1. Measurements of *S. erinacei* coracidia*

Organs	Range(Mean)	
	length	width
External cellular layer (embryonic membrane)	38.3~48.5 (43.8)	31.9~45.9 (36.9)
Oncosphere	33.2~43.4 (38.9)	23.0~30.6 (26.4)
Cilia	(12.8)	
Hooklets	8.0~ 9.0 (8.6)	

* 20 coracidia were measured (unit : μm)

Table 2. Measurements of *S. erinacei* procercoids* according to the age of infection

Days after coracidial challenge	Range(Mean)	
	length	width
1	43.5~ 69.1 (56.1)	15.4~25.6 (20.1)
3	71.7~118.7 (89.6)	17.9~28.2 (24.1)
4	133.3~153.6 (142.3)	28.2~41.0 (37.4)
5	71.7~105.0 (86.2)	61.4~81.9 (70.0)
7	84.8~111.3 (98.4)	55.7~87.5 (69.8)
9	87.0~120.3 (102.6)	61.4~84.5 (72.2)
13	97.3~122.9 (107.4)	56.3~74.2 (64.6)
17	79.4~120.3 (102.6)	51.2~92.2 (63.5)
21	76.8~128.0 (92.5)	51.2~79.4 (67.4)
25	76.8~110.1 (95.0)	58.9~87.0 (67.6)

* 20 procercoids were measured (unit : μm).

142 μm long and 20~72 μm wide by the age (Table 2).

3. The infectivity of procercoids to tadpoles

All of the tadpoles exposed to the cyclops which harboured the procercoids of age 10, 12, 14, 17 and 20 days contained the plerocercoids on their mesentery after 20 days(Table 3; Fig. 16). The plerocercoids were pyriform with invaginated anterior end. They included numerous calcium corpuscles but the minute spines at the anterior end of procercoids were not found(Fig. 17). Their tail grew distinctively up to 3.38 mm after 32 days in tadpoles(Table 4; Fig. 18).

Table 3. The infectivity of *S. erinacei* procercoids to tadpoles

Age(days) of procercoid in cyclops	No. of tadpoles challenged	No. of tadpoles infected	No. of plerocer. detected range(mean)
10	7	7	1~46(18.0)
12	3	3	5~35(17.3)
14	4	4	8~42(14.5)
17	3	3	7~22(13.3)
20	5	3	14~46(22.0)

Table 4. Measurements of spargana* collected from experimental tadpoles

Age(days) of procercoids infected	Age(days) of spargana recovered	Range(Mean)	
		length	width
12	15	0.55~1.25 (0.89)	0.33~0.75 (0.44)
10	17	0.66~1.39 (0.88)	0.33~0.51 (0.42)
20	20	0.58~1.22 (0.86)	0.31~0.42 (0.37)
14	26	0.52~1.12 (0.85)	0.25~0.41 (0.32)
10	30	1.63~2.88 (1.95)	0.43~0.63 (0.48)
13	32	2.40~5.50 (3.38)	0.35~0.55 (0.46)

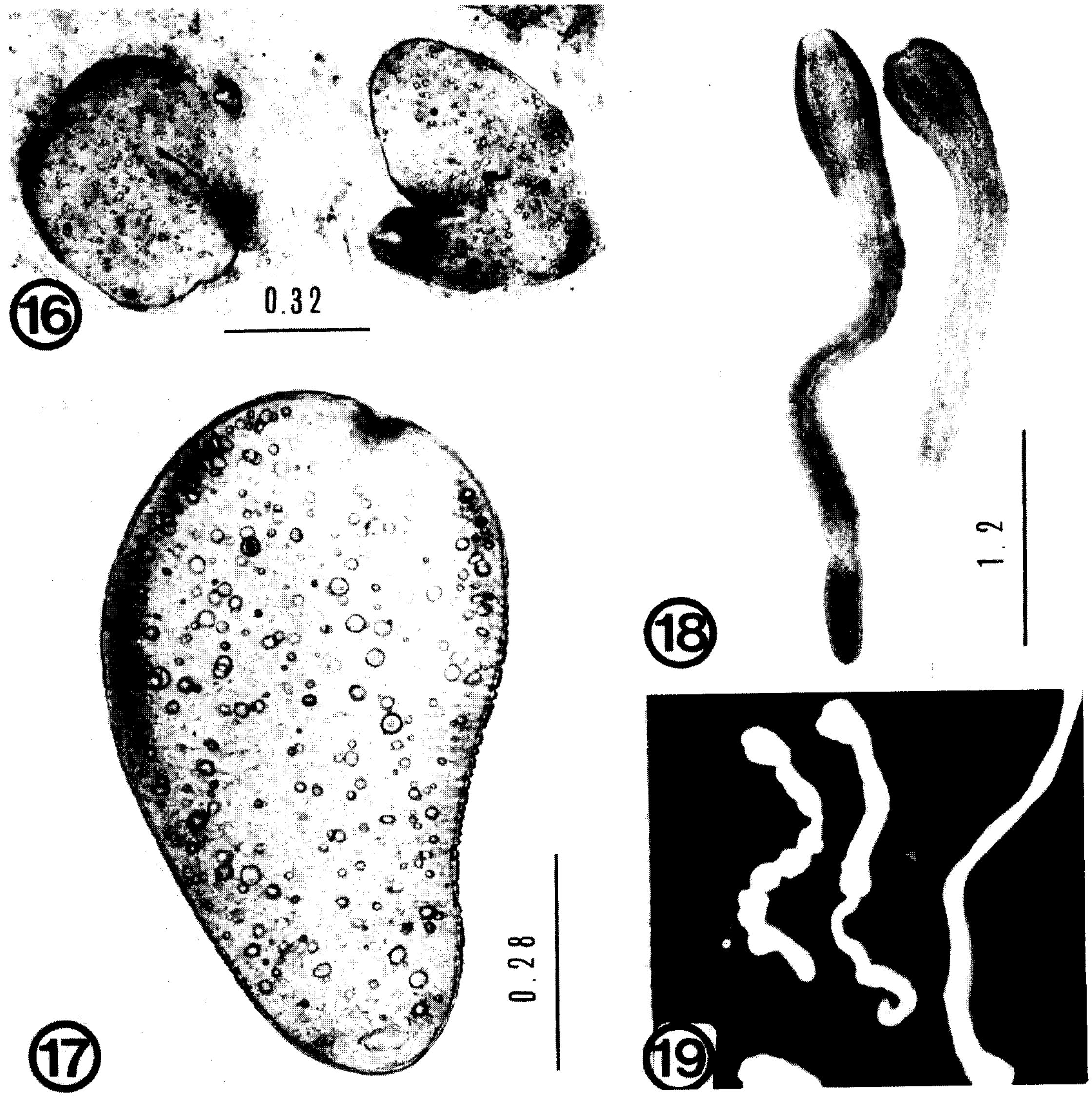
* 20 spargana were measured (unit : mm).

Table 5. The infectivity of *S. erinacei* procercoids to mice

Age(days) of procercoids in cyclops	No. of mice		No. of procercoids	
	chal-lenged	infected (%)	chal-lenged	recover-ed(%)
15	4	0	40	0
17	6	3(50)	70	4(5.7)
19	4	1(25)	56	4(7.1)
21	4	0	40	0
Total	18	4(22.2)	206	8(3.9)

4. The infectivity of procercoids to mice

The procercoids of 17 or 19 days of age in cyclops became plerocercoids in mice but not the worms of 15 or 21 days of age(Table 5). The recovery rate was 5.7% and 7.1% respec-



Figs. **16～19.** Plerocercoids from the experimental tadpoles and mice.
16. Two plerocercoids in the mesentery of a tadpole.
17. A 20-day old one.
18. Two 45-day old ones.
19. Three from the experimental mouse.

* All bar and scale unit is mm.

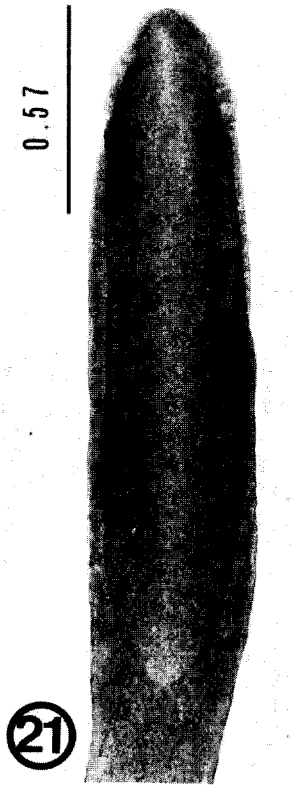
tively in 17 and 19 days group. The failure of infection with worms of 15 or 21 days looked not so significant considered the low recovery rates of 17 and 19 days group.

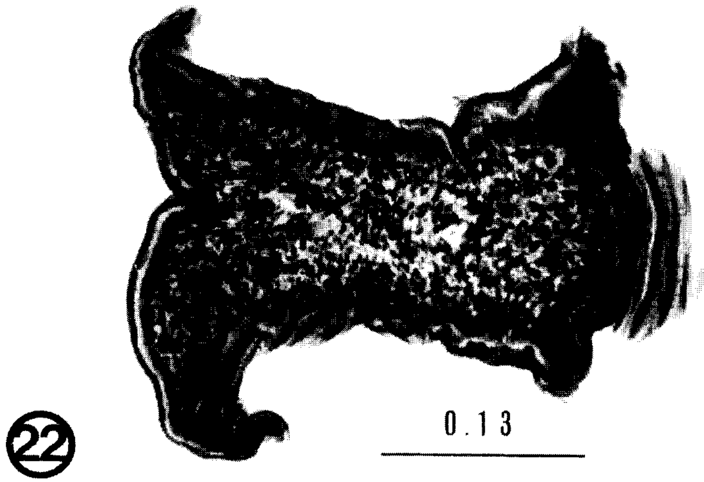
5. Growth of plerocercoids in mice

The plerocercoids of 15, 20 and 30 days of age in tadpoles showed the recovery rates of plerocercoids from mice 84.0%, 58.6% and 73.3% respectively(Table 6). Their tail was elongated to be ribbon-shaped after 20 days (Fig. 19).

6. Growth of adult worms in definitive hosts

The eggs of *Spirometra* were found from the feces of dogs at 16～18 days and from that of cats at 15 days. The eggs from dogs were 65





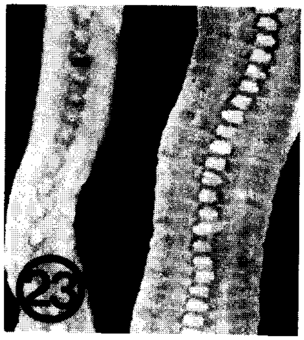
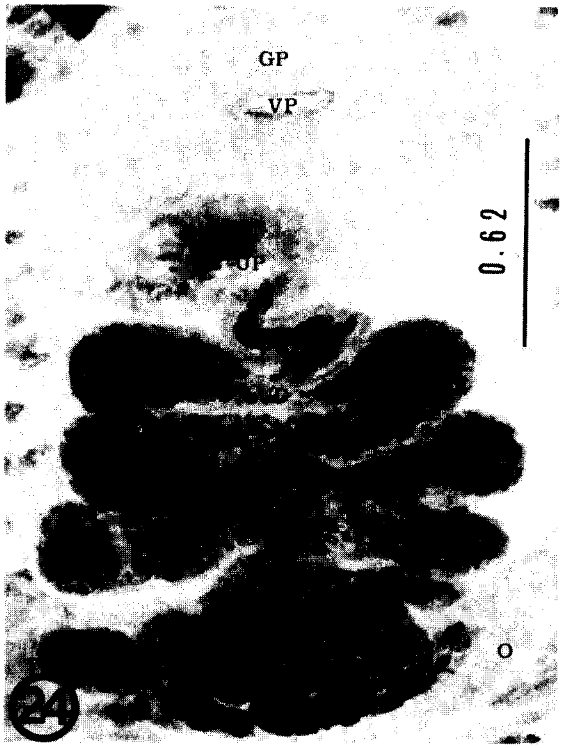
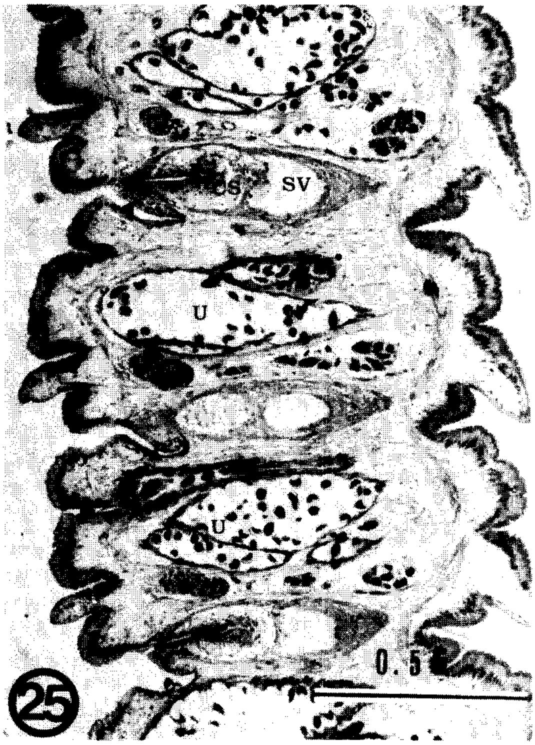


Fig. 20. Whole strobila of *S. erinacei* from an experimental dog at 6 weeks after infection.
Fig. 21. Scolex of *S. erinacei*.
Fig. 22. Cross section of the scolex.
Fig. 23. Mature proglottids of *S. erinacei*. * Bars and scale unit is mm.

μm long and 35.1 μm wide, and those from cats were 66.8 μm long and 36.1 μm wide. The tapeworm from a dog was 110 cm long and 0.8 cm wide after 6 weeks from infection(Fig. 20). The scolex measured 1.88×0.46 mm with two lateral bothria(Figs. 21 & 22). Mature proglottids measured 0.94∼2.33 mm in length and 6.03∼9.99 mm wide. The uterine tubules coiled 3 to 5 times(Table 7). Gravid proglottids contained numerous eggs(Fig. 23). Genital pore opened on the ventral midline near the anterior margin of gravid proglottids. Just distal to the genital pore, vagina opened like a slit. Uterine pore was 420 μm distal to the vaginal slit at the end of uterine coils. Mehlis' gland and dumbbell-shaped ovary was connected to the





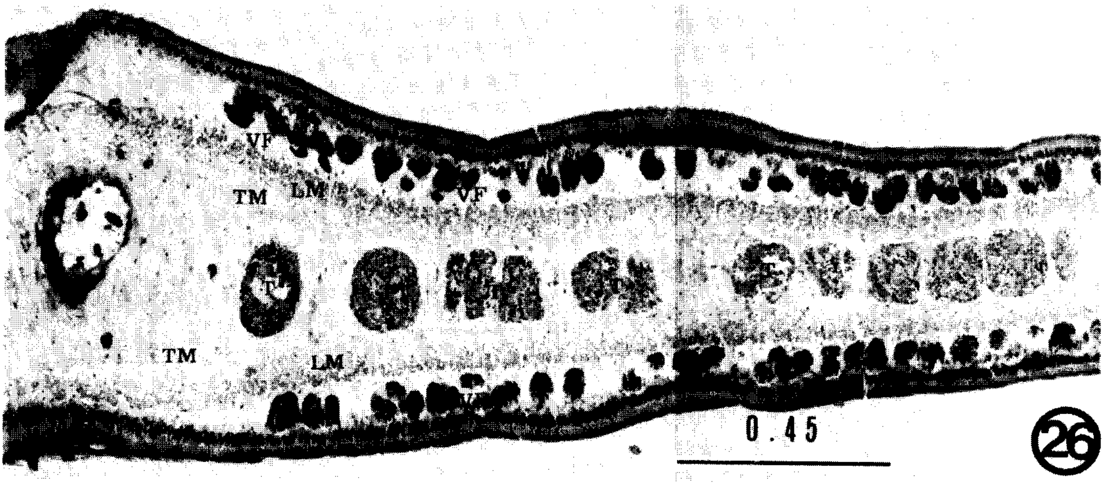


Fig. 24. Genital organs in mature proglottid, showing genital pore(GP), vaginal pore(VP), uterine pore(UP), uterine coil(UC), and ovary(O).

Fig. 25. A longitudinal section of mature proglottids, showing the relationships of genital organ: seminal vesicle(SV), cirrus sac(CS), genital pore(*), vaginal pore(▲), uterus (U), uterine pore(•) and ovary(○).

Fig. 26. A transverse section of a mature proglottid, showing longitudinal muscle(LM), transverse muscle(TM), testis(T) and vitelline follicles(VF).
* The bar unit is mm.

Table 6. The infectivity of *S. erinacei* plerocercoids to mice

Age(days) of plerocercoid in tadpole	No. of mice		No. of plerocercoids	
	used	infected	infected	recovered (%)
15	5	5	50	42(84)
20	7	7	70	41(58.6)
30	3	3	30	22(73.3)
Total	15	15	150	105(70)

Table 7. The periods for development of *S. erinacei* by stages

Stages	Periods(days)
Coracidium	8~14
Procercoid	10~20
Plerocercoid	about 10
Adult	15~18
Total	48~67

spiral uterus(Fig. 24). On the sagittal section of a gravid proglottid, genital pore opened differently from vaginal pore, and the cirrus was encircled within a sac with seminal vesicle (Fig. 25). The transverse section of a gravid proglottid showed uterine tubules at the midline, and the testes and vitellaria in the lateral fields. Small round lobular testes were at the center, but vitelline follicles were at the margin under the ventral and dorsal tegument(Fig. 26). Transverse muscles were more abundant than longitudinal ones.

Considering the observed period needed for the development of each larval stage, one cycle of the life history of *Spirometra erinacei* from egg incubation to egg discharge required 48~67 days (Table 8).

DISCUSSION

Li(1929) studied on the hatching conditions of eggs of *Spirometra.* He listed temperature, nature of the solution, light and oxygen as important factors. The embryo of *S. erinacei* was recorded to hatch out at 6 to 9 days at 35°C and at 12 to 14 days at 28°C(Kobayashi, 1931). *S. mansonoides* required 9 to 14 days for the hatching incubation(Mueller, 1938). The present result is similar to the previous data, *i.e.*, all of the incubated eggs hatched out at 8 to 14 days at 29°C.

As for the first intermediate host of *S. erinacei* or *S. decipiens*, *Cyclops affinis*, *C. phaleratus*, *C. magnus*, *C. vicinus*, *C. bicuspidatus*, *C. serrulatus*, *C. albidus* and *C. oithonoides* had been known(Li, 1929). Kobayashi(1931) reported *C. leuckarti*, *C. diaaphanus*, *C. signatus*, *C. flexopedum*, *C. phaleratus*, *C. soli*, *C. fimbriatus*, *C. viridis*, *C. serrulatus* as the first intermediate host of *S. mansoni.* As the hosts of *S. mansonoides*, *C. leuckarti*, *C. viridis* and *C. bicuspidatus* were found(Mueller, 1938). Considering the species of the crustacean hosts of those old papers and of the present results, the species of *Spirometra* may share the same crustacean hosts as the first intermediate host. The present result proves that *Mesocyclops leuckarti* (synonymous with *C. leuckarti*) and *Eucyclops serrulatus* (synonymous with *C. serrulatus*) are the experimental first host of

Table 8. Measurements* of *S. erinacei* mature proglottids from the experimentally infected dog

Items	Proglottids** placed in			
	I	II	III	IV
Length(average)	0.83~ 1.05(0.94)	1.25~ 1.50(1.38)	1.75~0.40(2.07)	2.08~2.68(2.33)
Width(average)	8.33~10.88(8.98)	9.35~10.68(9.99)	5.60~6.50(6.03)	6.38~7.40(6.81)
Length : Width	1 : 9.5	1 : 7.3	1 : 2.9	1 : 2.9
No. of uterine coils	3	3~3.5	4	4.5~5

* 10 proglottids were measured respectively (unit : mm).
** I : 30~35 cm from scolex; II : 50~55 cm; III : 70~75 cm; IV : 90~95 cm.

Spirometra erinacei in Korea. These two are common cyclops in streams or reservoirs(Chang, 1989), and are suspected as its natural first intermediate host.

The life span of procercoids in the body cavity of cyclops appears relatively short. The larvae were infective to the second intermediate host after 10 days in the cyclops. Thirty day old larvae were already degenerated and some of them were calcified. However, any effect of heavy burden of the worms in a cyclops should be considered. All of the present cyclops were simultaneously infected with more than 10 worms individually.

Tadpoles may be the natural second intermediate host by engulfing infected cyclops in the water. Most of the plerocercoid larvae were on the omentum of tadpoles. The larvae in the age of 10 days or more were found infective to mice. They looked to migrate into the soft tissue of the thighs after molting of tadpoles into frogs(Kim, 1983). The tail of plerocercoids grows according to the host and also to the age of infection in a host. The length of spargana measured up to about 4 mm in tadpoles at 45 days after infection. Spargana have years long life span to wait for the chance of invasion into definitive hosts. During this period in the second intermediate host or the transport host, the larvae grow slowly but continuously in both length and width(Hong *et al.*, 1989). The spargana from humans usually measure about 20 cm or longer and 3 mm thick(Cho *et al.*, 1975).

The procercoids from cyclops can infect the mouse through oral ingestion, and grow into plerocercoids. However, the recovery rate was lower than that of infection with plerocercoids. The successful infection may confirm the mode of human infection by swallowing infected cyclops, though this mode may not be so common as the infection by eating live plerocercoids.

The present data suggest that the life cycle of *Spirometra* from egg to egg requires about two months. Being considered the natural activity of intermediate hosts and the temperature in Korea, the maturation and transmission of the larvae may be concentrated in May and July. This tapeworm is considered thriving in almost all over the country.

The present morphological or biological characteristics of the coracidia, procercoids and plerocercoids were compatible with previous records of *Spirometra* sp. (Mueller, 1938; Iwata, 1972). Gross or sectional morphology of the experimentally obtained adult worms revealed that the species of the sparganum in Korea is *Spirometra erinacei*(Iwata, 1972).

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=국문초록=

우리 나라에 분포하는 스파르가눔의 실험실 내 생활사

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우리 나라에서 인체감염을 일으키는 스파르가눔의 중간숙주를 파악하고, 각 단계의 유충을 관찰하기 위하여 이 연구를 수행하였다. 실험실에서 키운 물벼룩(*Mesocyclops leuckarti; Eucyclops serrulatus; Moina* sp.)과 참개구리의 올챙이를 각 단계의 유충에 접촉 감염시키고, 다음 단계의 유충을 얻어 다른 숙주에 감염시켰다. 각 시기에 있는 유충의 발육과 형태를 관찰한 바 다음의 결과를 얻었다.

1. 실험적으로 스파르가눔을 개와 고양이에 감염시켜 얻은 *Spirometra*의 충란을 증류수에서 29°C로 배양한 결과 10~14일에 유충(coracidium)이 탈각 부화하였다. 이 때 광선이 탈각을 촉진하였다. Coracidium은 평균 43.8×36.9 μm의 크기이었고, 길이 12.8 μm의 섬모로 덮혀 있었다.

2. Coracidium에 노출된 물벼룩 중에서 *M. leuckarti*와 *E. serrulatus*가 procercoid 유충을 체강에 가지고 있었다. 감염 5일에 형태가 갖추어지고, 10일에 제 2 중간숙주에 감염력을 가지며, 한 달 이후에는 석회화되었다. 이 유충의 전단에는 미세한 소극이 밀생하여 있었고, 체내에 칼슘소구(calcium corpuscles)가 관찰되고, 후단에 cercomer가 형성되어 있었다.

3. 감염된 물벼룩과 접촉시킨 모든 올챙이의 복강에서 스파르가눔(plerocercoid 유충)이 회수되었다. 올챙이에서 감염 후 20일에 길이 1 mm 이내의 서양배 형의 작은 충체가 관찰되었고, 45일에는 길이가 약 4 mm 정도의 유충이 회수되었다.

4. 마우스에 경구감염시킨 procercoid 유충은 감염 후 17일 및 19일에 스파르가눔으로 성장하였고 각각 5.7% 및 7.1%의 충체가 회수되었다.

5. 올챙이에서 얻은 스파르가눔을 개와 고양이에 감염시킨 결과 15~17일에 대변에서 *Spirometra*의 충란이 검출되었다. 감염 후 6주에 개에서 회수한 충체는 길이가 110 cm, 폭이 0.8 cm이었다. 성숙 편절의 자궁이 4~5회의 나선형이고 한 편절의 길이와 폭의 비율이 1 : 2.9이었다. 횡단 절편된 편절에서 생식공과 질구가 간격을 두고 개구하고, 음경낭과 저정낭이 하나의 낭을 형성하여 *Spirometra erinacei*의 기술과 일치하였다.

이 연구의 결과로 우리 나라의 논, 저수지, 수로, 개천 등에 흔히 분포하는 담수 갑각류 중에서 *M. leuckarti*와 *E. serrulatus*가 스파르가눔의 중간숙주가 될 수 있음을 확인하였다. 충란의 배양에서부터 종숙주의 충란 배출까지 약 2개월 정도의 기간이 소요되었고, 우리 나라 자연환경에서는 5월에서 7월에 주로 이 충체의 유충이 발육되고 전파되는 것으로 추측되었다.

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