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The life-cycle of *Spirometra* species from Peninsular Malaysia

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Abstract. The life-cycle of Malaysian *Spirometra* spp. was studied under experimental conditions in the laboratory. The *Cyclops* were reared as the first intermediate host, the hamster as the experimental second intermediate host and cat as the definitive host. Maturation and hatching of eggs took 6 to 12 days by incubation at temperature 30°C. The hatched coracidium measured 46 x 34 µm. The *Cyclops* used were susceptible to the coracidial infection. The procercoid older than 5 days in the *Cyclop* body cavity had minute spines at the anterior end, calcium corpuscles in the body parenchyma and the cercomer at the posterior end. Procercoids 10 to 14 days old were infective to hamster. The plerocercoids from the hamster after 30 days were long and slender and were infective to cats. The plerocercoids experimentally inoculated to cats developed to adult worms and began to produce eggs between 10 to 60 days. Based on the results that have been obtained, a complete life-cycle was successfully elucidated in the laboratory and hamster was identified to be a good laboratory model for a second intermediate host of *Spirometra* sp.

INTRODUCTION

Spirometra is a pseudophyllidean tapeworm of dogs, cats and other mammals (Mueller, 1974) with a worldwide distribution. Its plerocercoid larvae or spargana can infect humans causing sparganosis (Iwata, 1972). Although rare, sparganosis is endemic in many countries with the majority of cases reported from Southeast Asia and Eastern Africa (Schmid, 1972; Cho, 1975; Sparks, 1976; Nobrega-Lee, 2006). In Malaysia, the parasite has been reported by Mastura *et al.* (1995). Its life-cycle requires two different intermediate hosts, the fresh water *Cyclops* as the first intermediate host and amphibians, reptiles, birds and mammals as the second intermediate hosts (Iwata, 1972; Mueller, 1974; Lee, 1990). Humans act only as the accidental host in this life-cycle. There are three ways by which humans can be infected with the parasite. Firstly, by ingestion of infected raw snakes, frogs and other animals that harbour spargana. Secondly, by ingestion of infected *Cyclops* from contaminated drinking water and thirdly, by application of the flesh of infected frog (or poultices) to a wound or eye sores which may cause the spargana be transferred and then migrate to various visceral organs (Tanaka *et al.*., 1997) including subcutaneous tissues causing swellings (Garin *et al.*, 1997).

In the present study, an experimental life-cycle of a Malaysian *Spirometra* spp. using spargana infected local frogs (*Rana cancrivora* and *R. limnocharis*) was carried out and characterized, and a potential new second intermediate host was determined.

MATERIALS AND METHODS

Incubation of eggs

Plerocercoids obtained from naturally infected frogs (*Rana cancrivora* and *R. limnocharis*) (Mastura, 1995) were

introduced orally into three one-year-old domestic cats (2 females and one male) obtained from Ipoh Society for Prevention of Cruelty to Animals (I.S.P.C.A). They were treated with praziquantel at a doze of 5mg/kg body weight and their faeces checked for 10 days to confirm the absence of tapeworms in the intestine. After 10 to 60 days from the infection, eggs of *Spirometra* were collected from their faeces. Eggs were washed with tap water according to Voge (1970) and stored in bottles with tap water at 4°C and were incubated at 30°C. The process of embryonation of the eggs was observed through a microscope. The hatched coracidia, was also observed microscopically.

Experimental infection of the first intermediate host (*Cyclops*)

Cyclops obtained from canals of rice fields at Tanjung Karang by using plankton net (simple conical net 60 µm mesh). In the laboratory, an individual egg sac bearing *Cyclop* was sacked with Pasteur pipette, transferred into a petri dish with paramecium and cultured to several generations according to methods described by Adrian (1993). The fourth generation of the culture was used in this study as first intermediate host. *Cyclops* were exposed to active coracidia in petri dish, kept at laboratory temperature (21-26°C) and fed with *Paramecium*. In *Cyclops*, coracidia developed to procercoids in the body cavity and became infective to the second intermediate host.

Experimental infection of the second intermediate host (hamster)

Fifteen eight-weeks-old female hamster (*Mesocrisetus auratus*) obtained from the animal house of the Faculty of Medicine, University of Malaya were used in this study as second intermediate host. Each hamster was orally fed with 30 infected *Cylops* with procercoids. After infection, they were housed in a group of 3 per cage, fed with commercial food pellets and water. The hamsters were examined for the larval infection after 30 days.

Experimental infection of the plerocercoids to cats

Two eight- months-old female domestic cats obtained from Millenium Court area near University of Malaya were used in this study as definitive host (Ooi, 2000). They were treated with praziquantel at a doze of 5 mg/kg body weight and their faeces checked for 10 days. Two plerocercoids from hamster were orally fed to each cat. Faeces of each cat were examined daily until *Spirometra* eggs detected.

Recovery of adult worm from experimentally infected cats

After 30 days from the infection, two cats were sacrificed. Cat was pre-anaesthetized with Ketamine 20 mg/ kg IM + Xylazine 1.1 mg/kg IM, then euthanized with Dolethal 5 mg IV. A midline incision on the abdomen was made and opened in layers. The small intestine was opened and the adult worms recovered. The lengths of the worms were taken and then killed with hot 10% formol saline. One of the worms was fixed under a glass plate with AFA (85% Alcohol + 10% Formalin + 5% Glycerol) overnight. The scolex with neck and proglottids at 40-45 cm from the scolex were stained with Alum-carmine solution.

RESULTS

Development of coracidia within eggs

The morphology of eggs obtained from the experimentally infected cat observed under light microscope is ovoid, tapered at both ends and had the operculum at the sharp pole. The eggs were of mean length and width of 59.6 x 36.4 µm. Figure 1 shows stages of development of coracidia within eggs. The shell is smooth and the embryo is visible within the shell (a). Three days after incubation the germ cell grew with blur margin (b). The embryo mass appeared at day 5 of incubation (c). The egg at day 7 of incubation (d). The egg at day 9 of incubation containing definite coracidia with hooks (e). The coracidium looked mature and ready to hatch at 10 days (f). Egg already hatched with open operculum (g). The hatched coracidium

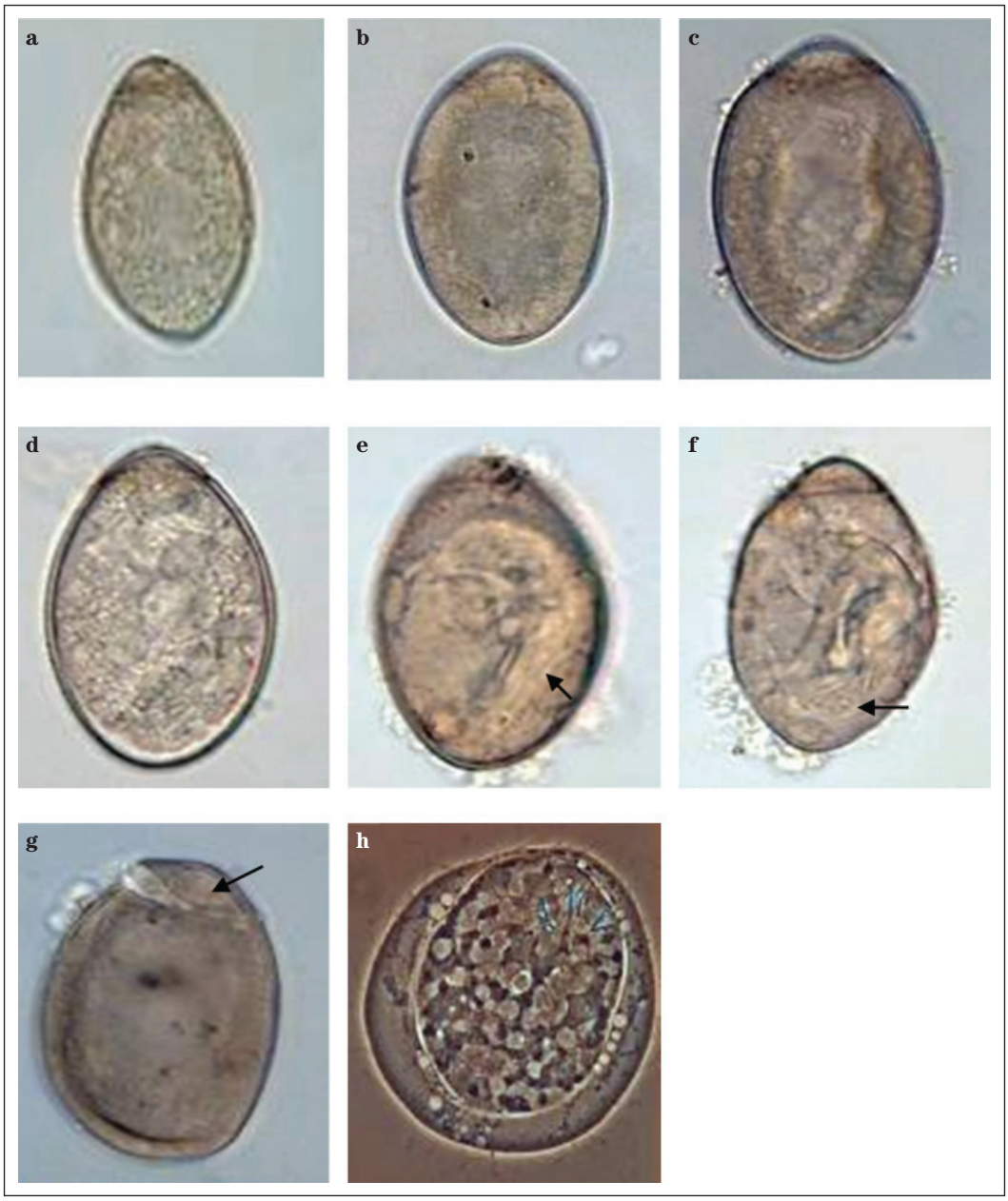

Figure 1. Development of coracidia within *Spirometra* eggs during incubation
a: Malaysian *Spirometra* egg from experimentally infected cat, appearance under light microscope
b: Appearance of egg at day three of incubation.
c: Appearance of egg at day five of incubation.
d: Appearance of egg at day 7 of incubation.
e: Appearance egg at day 9 of incubation, containing definite coracidia with hook (Arrow = coracidia).
f: Egg at 10 days of incubation, coracidia ready to hatch with hooks (Arrow = hooks).
g: Egg which already hatched with open operculum (Arrow = open operculum).
h: Hatched coracidia.

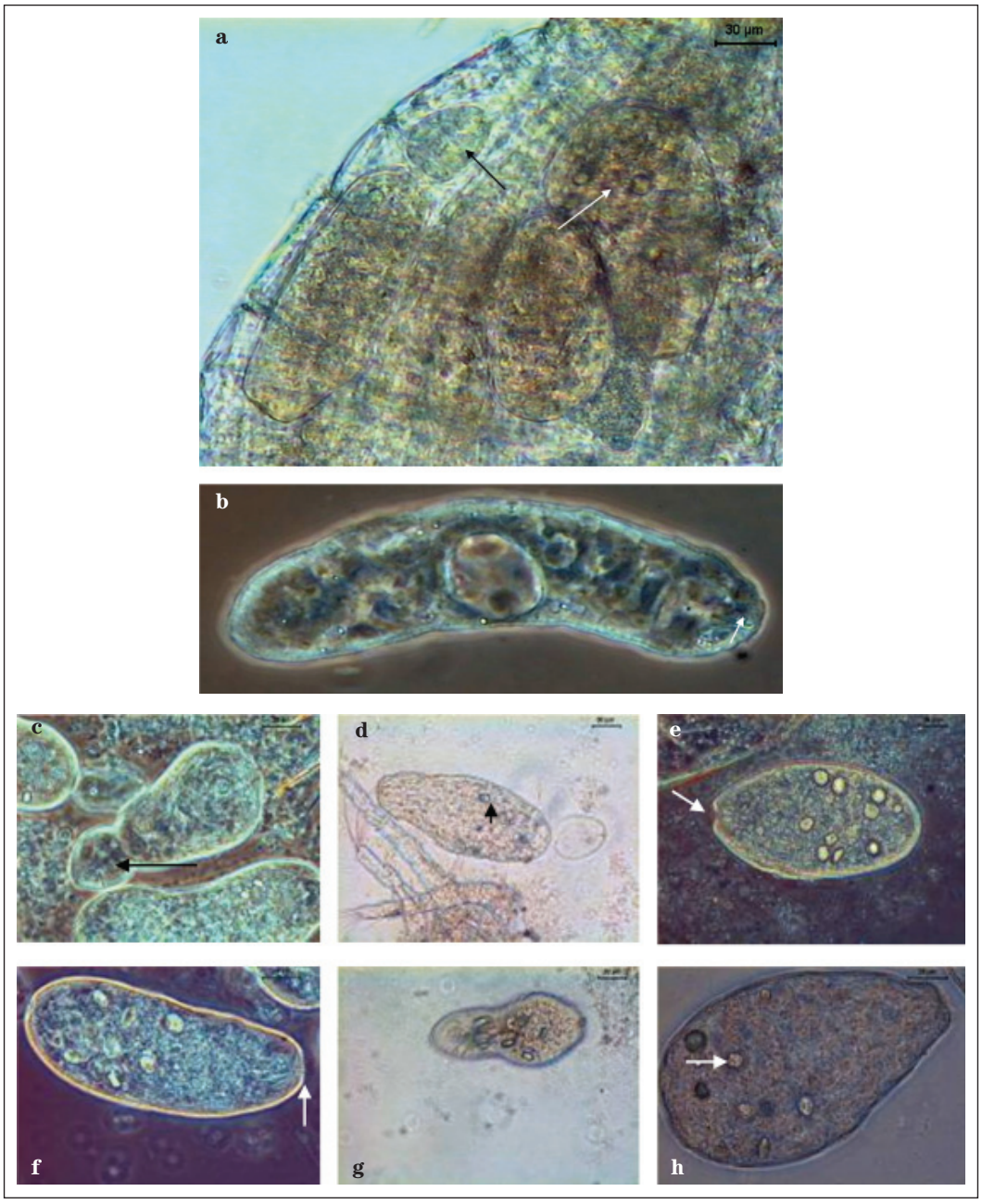

Figure 2. Development of procercoids in the body cavity of *Cyclops*

a: Part of experimentally infected *Cyclop* showing procercoids in the body (Arrow = procercoids)
b: Procercoid from *Cyclop* on the 4th day post infection, elongated and with hooks (Arrow = hooks)
c: Procercoid from *Cyclop* body cavity 5 days post infection, cercomer forming and contains hooks (Arrow = cercomer).
d: Procercoid from *Cyclop* body cavity 7 days post infection with calcareous corpuscles (Arrow = calcareous corpuscles).
e: Procercoid from *Cyclop* body cavity 9 days post infection, with indentation of anterior apical region (Arrow = indentation).
f: Procercoid from *Cyclop* body cavity 12 days post infection, showing arrangement of spines on anterior apical region (Arrow = apical spines).
g: Procercoid from *Cyclop* body cavity 22 days post infection.
h: Procercoid from *Cyclop* body cavity 30 days post infection with calcified calcareous corpuscles (Arrow = calcified calcareous corpuscles).

was oval with 3 pairs of hooks haberd-like, numerous cilia and swam actively (h). The coracidia were of mean length and width of 46 x 34 µm.

Larval development in *Cyclops*

Coracidia experimentally infected to *Cyclops* developed to procercoids. Figure 2 shows development of procercoids in the body cavity of *Cyclops*. Procercoids in the body cavity of the *Cyclop* (a). Procercoids from 4 days post infection were elongated and retained hooklets at the body terminal (b). On day 5, the posterior end became sharp and cercomer started forming with 3 pairs of hooklets (c). Calcareous corpuscles appeared in the parenchyma of procercoids on day 7 (d). The procercoid had indentation of anterior apical region on day 9 (e). On day 12, the procercoid developed spines on the anterior apical region (f). The morphology of the procercoid on day 22 (g). On day 30, procercoid had calcified calcareous corpuscles (h). The shape of the procercoids varied in relation to their age. The measurements were also variable between 40-140 µm long and 20-90ìm wide. The largest procercoid was measured on day 5.

Table 1. Measurements of *Spirometra* procercoids in *Cyclops* (µm)

Days post infection	Range (Mean)	
	Length	Width
2	40–70 (53)	20–28 (24.8)
5	90-140 (115.7)	40–90 (74.3)
8	90–120 (107.5)	50-90 (74.3)
10	100–130 (114.2)	50–90 (73.3)
12	90–110 (100.6)	60–80 (68.8)
18	100-130 (110)	50-80 (61.3)

56 coracidia were measured length and width (µm).

Infectivity of procercoids to *Cyclops*

A total of 662 *Cyclops* experimentally infected with coracidia were examined. Out of these, 100 (15.11%) were infected with procercoids, 54 (8.15%) had single procercoid, 46 (6.95%) had more than one procercoids and 5 (0.75%) had the highest number with each having 12 procercoids (Table 2).

Table 2. Experimental infection of *Cyclops* with coracidia and the recovery of procercoids per copepod after infection

No. of procercoids in each *Cyclop*s	No. of *Cyclops* infected
0	562 (84.89%)
1	54 (8.15%)
2	17 (2.56%)
3	9 (1.35%)
4	2 (0.30%)
5	2 (0.30%)
6	1 (0.15%)
7	1 (0.15%)
8	3 (0.45%)
9	1 (0.15%)
10	3 (0.45%)
11	2 (0.30%)
12	5 (0.75%)
Total number of *Cyclops*	662

The infectivity of plerocercoid to hamster

The procercoids of 10 and 14 days old in *Cyclops* became plerocercoids in hamster. The total recovery rate of larvae from all infected hamster was 1.33%. Figure 3 shows the plerocercoids recovered from hamster. The recovered plerocercoids were long with thick head and slender tail (a). The section stained with hematoxylin and eosin showed calcareous corpuscle (b).

Growth of adult worms in definitive host

The eggs of *Spirometra* were recovered from faeces of 3 cats at 12, 14 and 60 days respectively. The eggs were of mean length

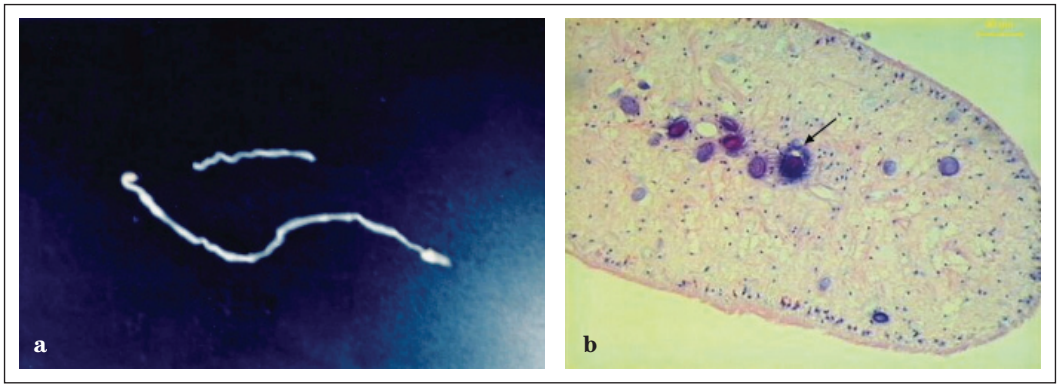

Figure 3. Plerocercoids from hamster
a: Plerocercoid recovered from experimentally infected hamster.
b: Longitudinal section of plerocercoid (hematoxylin and eosin stain) from hamster (Arrow = calcareous corpuscles).

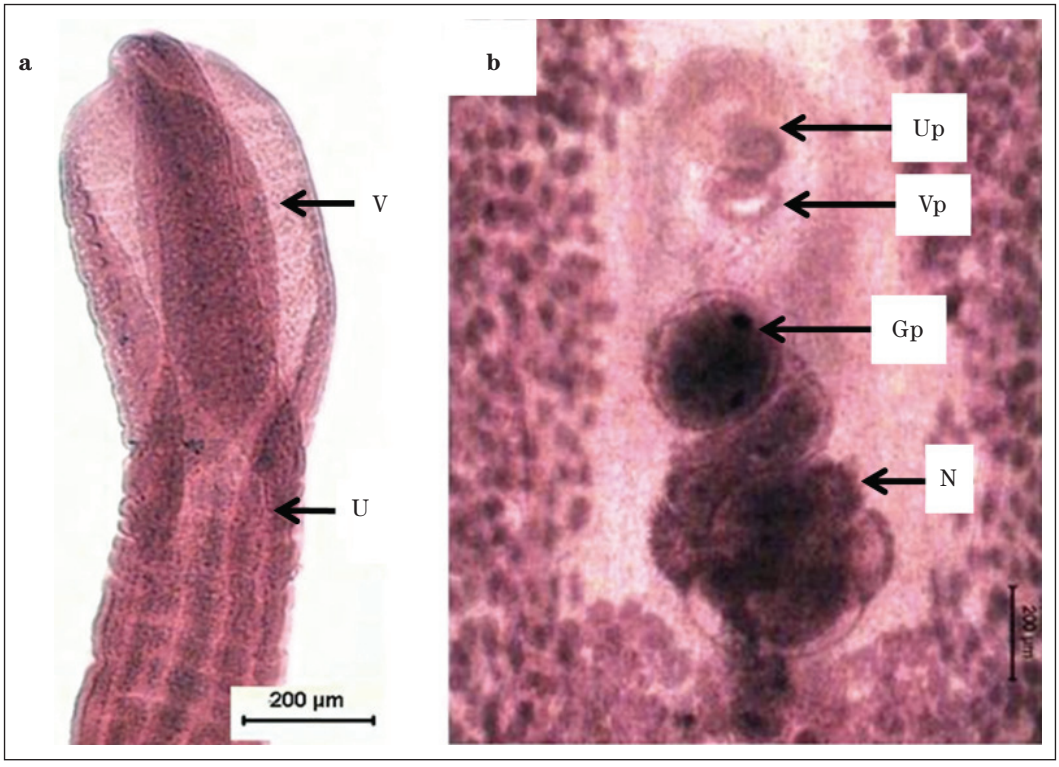

Figure 4. Scolex and proglottid of adult worm of Malaysian *Spirometra* spp. stained with hematoxylin and eosin
a: Scolex of Malaysian *Spirometra* species, bothria (Bt) and neck (N)
b: Proglottid showing genital pore (Gp), vaginal pore (Vp), uterine pore (Up) and Uterus (Ut)

and width of 59.6 x 36.4 µm. Table 3 summarises the measurements of adult worms recovered from cats. Four adult tapeworms with body length of 15.0 to 53.5 cm were recovered from the small intestines of 3 cats. Figure 4 summarises the morphology of adult worms stained with hematoxylin and eosin. The tapeworms had small head, long and thin neck. The scolex was small, spatulate and elongated. The worms were

stained with Alum carmine and studied under light microscopy. Two lateral bothria in the scolex were visible (a). The proglottid showed centrally placed uterus with 6 coils, testis, vitellaria and three openings to the surface, namely genital opening, vaginal opening and uterine opening. The genital pore was located on the upper ventral side, the vaginal pore a short distance in front of the terminal coil some distance from genital pore. Testis was not united anteriorly in the midline with the vitellaria (b). The observed time needed for the development of each larval stage, for one complete life-cycle of Malaysian *Spirometra* spp. from egg incubation to new egg discharge required 56 – 86 days (Table 4).

Table 3. Measurements of adult worm's length

Worm	Length (cm)
1	15.0
2	47.5
3	53.5
4	16.0
Mean	33.0
Range	15.0–53.5

Table 4. The periods for development of Malaysian *Spirometra* by stages

Stage	Period (days)
Coracidium (from eggs)	6–12
Procercoid	10–14
Spargana	30
Adult	10–60
Total	56-86

DISCUSSION

The results of the present study indicated that the incubated eggs needed 6 to 12 days to hatch at 30ºC. This is in line with results of previous studies by Li (1929) which indicated that *S. erinacei* needed 6 to 9 days to hatch at 35ºC by Lee (1990), 8 to 14 days at 29ºC by Kobayashi (1931), 12 to 14 days at 28ºC and by Mueller (1959) 9 to 14 days for *S. mansonoides*.

Li (1929) found that the first intermediate host for *S. erinacei* were numerous including *Cyclops affinis*, *C. phaleratus*, *C. magnus*, *C. biscudatus*, *C. serrulatus*, *C.albidus* and *C. oithonoides*. Kobayashi (1931) reported *C. leuckarti*, *C. diaphanous*, *C. signatus*, *C.* flexopedum, *C. phaleratus*, *C. soli*, *C. fimbriatus*, *C. viridis*, *C. serrulatus*, were first intermediate hosts for *S. mansoni*. Although the species of *Cyclops* used in the present study was not known but the identified *Spirometra* may also share the same type of the first intermediate host. Further study on the species identification of the *Cyclops* used is a worthwhile effort.

The second intermediate host is as important as the first intermediate host for the survival of the species. The second intermediate hosts in the case of *Spirometra* infection in the natural habitat are the reptiles, amphibians, birds and mammals (Mueller, 1959; Opuni & Muller, 1974; and Lee, 1990). These researchers looked at the infection dynamics of procercoids and found that procercoids in *Cyclops* become infective to animals at different days post infection. Some of them, such as procercoids of *S. mansonoides* become infective on 7, 8, 9, 13 and 14 days post infection (Mueller, 1959), *S. erninacei* at 21 days (Lee, 1990); and *S. theilleri* at 7 days (Opuni & Muller, 1974). In the present study, it was observed that the procercoids of Malaysian *Spirometra* to be infective from 10 to 14 days post infection. Based on these observations, it can be concluded that the infection of the second intermediate hosts depends on the infectivity of the procercoids in the infected *Cyclops*, the longer they remain in the *Cyclops* the less infective they become. The literature review shows the life of the procercoid in the *Cyclops* is about 30 days, after which they die (Arme *et al.*., 1983). In the present study, the life span of procercoids in the body cavity of *Cyclops* appeared relatively short. The procercoids were infective to the second experimental intermediate host hamster after 10 days in the *Cyclops*.

Having established the role of the first and second intermediate host, the study elucidates the role of the domestic cat as a definitive host and laboratory model for life-cycle studies of Malaysian *Spirometra* spp. In the present study, 3 cats were orally fed with plerocercoids from naturally infected frogs and 2 cats from experimental hamster. The plerocercoids developed to adult worms and started discharging eggs. The development period to adult stage was 10 to 60 days. Early studies by McIntosh (1937) and Mueller (1938) showed the presence of *Spirometra* infection in cats, bobcats, dogs and other carnivores. The adult worm developed in the small intestine of cat after oral feeding of plerocercoids (McIntosh, 1937; Mueller, 1938; and Lee, 1990).

The life-cycle of two *Spirometra* species, *S. mansonoides* (Muller, 1938) and *S. erinacei* (Lee, 1990; Miyazaki, 1991) has been studied and the life-cycle elucidated. Members of the genus *Spirometra* have three parasitic stages in the life cycle (the procercoid, spargana and adult) and one non-parasitic stage (the coracidium). They utilize a wide range of different hosts, usually the definitive host is canidae and felidae, the first intermediate host is the *Cyclop* and the second intermediate and paratenic hosts are the amphibians, reptiles, birds and mammals. The flow of development in one complete life-cycle is from egg incubation to egg discharge. In the present study, it is the first time that the life-cycle of the Malaysian *Spirometra* species was completely elucidated starting with the larva obtained from *R. cancrivora* and fed to cats. The present study also identified the hamster to be a good laboratory model for a second intermediate host as it is infective to procercoid infection which then developed into plerocercoid and infective to cat.

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