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Life history of *Echinoparyphium recurvatum* (Trematoda: Echinostomatidae) in Korea

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Abstract: The present study was performed to observe characteristics of the life history of *Echinoparyphium recurvatum* under both natural and laboratory conditions in Korea. A batch of *Radix auricularia coreana* was collected from Sunamchon, one of the stream of West Naktonggang (River), in Kangso-gu, Pusan during August and September 1992. Out of 106 snails examined by crushing, 52 (49.0%) were infected with larval *E. recurvatum*, *i.e.* rediae, cercariae and metacercariae. Cercariae naturally shed from snails encysted in the snails of same species and loaches, but not in mud-snails. Adult worms were detected from chicks and ducks experimentally infected with metacercariae, but not from rats and mice. The average recovery rate of adults from chicks was 13.1%. Rediae were sac-like, 2.437 × 0.317 mm in average size, with a muscular pharynx and a brownish cecum which reached the anterior half of the body. Cercariae consisted of a spindle-shaped body (0.262 × 0.129 mm in average) and a rod-like tail (0.528 x 0.056 mm in average). In the cercarial body, 45 collar spines were observed on the head crown, and double rows of excretory ducts with fine granules were laterally arranged between the pharynx and the ventral sucker. Metacercariae were spherical, 0.144 × 0.142 mm in average size, with thick hyaline outer and thin elastic inner walls, and many excretory granules. Adults were slender and more attenuated in the anterior end, 2.760 × 0.550 mm in average size, and had 45 collar spines including four end group spines on both ventral corners. From the above results, it was confirmed that *R. auricularia coreana* plays a pivotal role in the life cycle of *E. recurvatum* as the first and/or second intermediate hosts in Korea.

Key words: life history, *Echinoparyphium recurvatum*, *Radix auricularia coreana*, redia, cercaria, metacercaria, adult, chick, duck

INTRODUCTION

Echinoparyphium recurvatum is an intestinal fluke of wild fowls and mammals, and is an old cosmopolitan species (Skrjabin, 1947; Yamaguti, 1958). Human infections with this fluke have been reported in Taiwan, Indonesia and Egypt (Beaver *et al.*, 1984). The life history of *E. recurvatum* has been studied by many authors (Harper, 1929; Azim, 1930; Odening, 1962; Nevostrueva, 1964; Moravec *et al.*, 1974).

Odening (1964) has already suggested that *E. recurvatum* cercariae are divided into two types, *i.e.* lymnaeidarum and planorbidarum. Recently, some investigators have verified that the planorbidarum type, *Echinoparyphium* cercariae shed from planorbid snails, are those of the sibling species group (Grabda-Kazubska, 1984; Grabda-Kazubska and Kiseliene, 1989; McCarthy, 1990). New species were separated from the *E. recurvatum* group and described with studies on their life cycle (Kiseliene and Grabda-Kazubska, 1990;

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Grabda-Kazubska and Kiseliene, 1991).

In Korea, adult worms of *E. recurvatum* have been found in a house rat (Lee *et al.*, 1990). However, there are no studies on the biological characteristics of this fluke. Therefore, the present study was performed to observe the biological characteristics and morphologies of *E. recurvatum* which originated from the life cycle of this fluke in Korea.

MATERIALS AND METHODS

A batch of snails, *Radix auricularia coreana*, was collected from Sunamchon, one of the stream in West Naktonggang (River), located in Kangso-gu, Pusan during August and September 1992. Among the collected snails, 106 ones were examined respectively under a stereomicroscope by crushing to investigate the infection status of larval flukes. When echinostome larvae were found, the identity as *E. recurvatum* was confirmed by examining the number of collar spines. The detected larvae were observed alive and then measured after fixation with 10% neutral buffered formalin.

Cercariae naturally shed from *R. auricularia coreana* were challenged to 30 snails of same species, 10 mud-snails, *Cipangopaludina* sp., and 20 loaches, *Misgurnus anguillicaudatus*, to know the potentiality as the second intermediate hosts. The encysted larvae detected from *R. auricularia coreana* were fed to 5 mice, 5 rats, 5 ducklings and 5 chicks to determine suitable final hosts. The number of metacercariae fed was one hundred per host. To observe the recovery rates of worms, 25 chicks were also infected orally with 100 metacercariae each.

The adult worms recovered from chicks were fixed with 10% neutral buffered formalin under a cover-slip pressure and stained with Semichon's acetocarmine. The specimens were mounted with Canada balsam, observed and measured under a light microscope. Some adult worms were fixed with 2.5% glutaraldehyde, dehydrated in graded alcohol, dryed in critical point dryer, and coated with gold. The specimens were observed under a DS-130C SEM (ISI Korea Co.) to observe the arrangement of collar spines on the head crown.

RESULTS

Infection status with larval flukes in *R. auricularia coreana*

Out of 106 snails examined, 52 (49.1%) were infected with larval stages of *E. recurvatum*. The infection status is shown in Table 1.

Morphology of larval *E. recurvatum*

Daughter rediae are elongated and sac-like, 1.938-3.193 mm (2.437 mm in average) by 0.235-0.388 mm (0.317 mm) in size, and pale yellow in color (Fig. 1). They had a well developed pharynx and a sac-like cecum filled with dark brown contents, and contained mature cercariae.

Cercariae have a spindle-shaped body, with a well marked collar (Fig. 2). A rod-shaped tail is longer than the body, and is devoid of a finfold. Oral sucker leads to a fairly short prepharynx, followed by a muscular pharynx, a long esophagus and ceca bifurcated at the anterior portion of the ventral sucker. Ventral sucker is located post-equatorially and is

Table 1. Infection status with larval *Echinoparyphium recurvatum* in *Radix auricularia coreana*[a] from the Sunamchon (Stream) in Kangso-gu, Pusan

Larvae detected/snail	No. (%) of snails infected
rediae only	5 (4.7)
rediae + cercariae	8 (7.5)
rediae + cercariae + metacercariae	15 (14.2)
rediae + metacercariae	1 (0.9)
metacercariae only	23 (21.7)
Total	52 (49.1)

[a]A total of 106 snails was examined.

slightly larger than the oral sucker. Body is covered with a fairly thin tegument equipped with fine spines extending to the level of the anterior border of the ventral sucker. Excretory ducts between the pharynx and the ventral sucker are dilated and filled with numerous small granules. The ducts from each side unite in the posterior part of the body to form the excretory bladder. The dimensions of each organs are shown in Table 2.

Metacercariae are almost spherical, with a thick hyaline outer (0.004-0.006 mm in thickness) and a thin elastic inner walls, measuring 0.140-0.150 mm (0.144 mm in average) × 0.135-0.146 mm (0.142 mm) in diameter (Fig. 3). Head crown armed with 45 collar spines and two excretory ducts filled with numerous fine granules are characteristic features.

Table 2. Measurements[a] of *E. recurvatum* cercariae from *R. auricularia coreana*

Organs	Range	Average
Body (L)[b]	0.235-0.291	0.262
(W)[c]	0.112-0.138	0.129
Oral sucker (L)	0.036-0.048	0.041
(W)	0.039-0.046	0.043
Pharynx (L)	0.020-0.027	0.024
(W)	0.011-0.015	0.014
Prepharynx (L)	0.010-0.014	0.012
Esophagus (L)	0.033-0.046	0.041
Ventral sucker (L)	0.048-0.061	0.052
(W)	0.053-0.062	0.059
Tail (L)	0.469-0.556	0.528
(W)	0.049-0.061	0.056

[a]Unit is mm; [b]length; [c]width.

Results of the experimental infection with cercariae and metacercariae

Cercariae naturally shed from *R. auricularia coreana* encysted in snails of same species and loaches, *M. anguillicaudatus*, but not in mud-snails, *Cipangopaludina* sp. Sexually matured adults were detected from the chicks and ducks experimentally infected with metacercariae from *R. auricularia coreana*. However, they were not found in the experimentally infected rats and mice.

Recovery rates of adult worms from experimental chicks

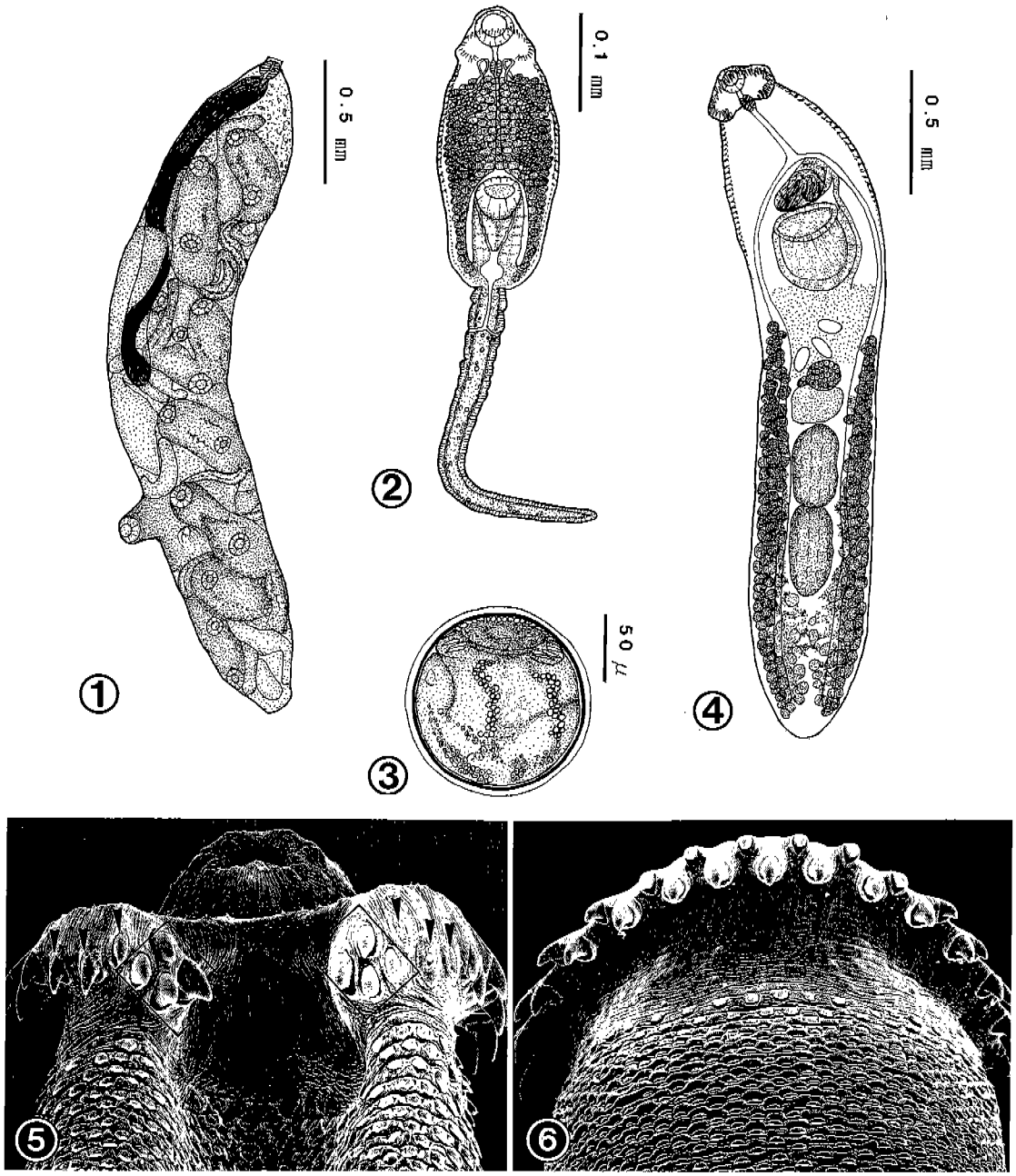
A total of 328 worms was recovered from 24 (96.0%) out of 25 chicks experimentally infected with metacercariae. The recovery rates were 7.0-22.6% (13.1% in average) depending on the age of infection (Table 3). Although 5 day-old worms had no eggs in their uterus, all worms recovered at 5-17 days after infection were sexually matured.

Morphology of adult *E. recurvatum*

The morphology of adults was described with the worms recovered from the experimental chicks 10 days postinfection (Fig. 4). Body is elongated and anteriorly attenuated. Head crown is well developed, kidney shaped, armed with 45 collar spines in two alternating rows including four end group spines on each ventral corners (Figs. 5 & 6). End group spines are larger than the other spines. Their sizes are 53-59 μm (56 μm in average), 58-60 μm (59 μm), 59-63 μm (61 μm) and 65-70 μm (68 μm), respectively. The spines in the oral row are smaller than those of the aboral. Oral sucker is relatively small and located sub-

Table 3. Recovery of *E. recurvatum* from the experimentally infected chicks

Days from infection to recovery	No. of chicks challenged	Total No. of metacercariae given	No. of chicks infected	No. of worms recovered		
				Total	Range	Average
5	10	1,000	10	102	1-55	10.2
10	5	500	5	78	8-28	15.6
15	5	500	4	35	0-26	7.0
17	5	500	5	113	13-27	22.6
Total	25	2,500	24	328	0-55	13.1



Figs. 1-4. Schematic drawings of the larvae and adult of *Echinoparyphium recurvatum* from naturally infected snails, *Radix auricularia coreana*, and an experimentally infected chick. **Fig. 1.** A daughter redia has a muscular pharynx, a brownish cecum reaching the anterior half of the body, and several mature cercariae. **Fig. 2.** A cercaria consists of a spidle-shaped body and a rod-like tail. **Fig. 3.** A metacercaria has a thick hyaline and thin elastic walls, a characteristic head crown with 45 collar spines, and many excretory granules. **Fig. 4.** A adult worm is slender and more attenuated in the anterior end. It also has a well developed head crown with 45 collar spines including four end group spines on both ventral corners. **Figs. 5-6.** SEM views of collar spines on the head crown of an adult *E. recurvatum*. **Fig. 5.** Ventral view showing the four end group spines (boxed) and three lateral spines (arrow heads). **Fig. 6.** Dorsal view showing the alternately arranged collar spines.

terminally. The large ventral sucker is situated on the anterior quarter of the body. Mouth cavity in the oral sucker is followed by a short prepharynx, a muscular pharynx, a long esophagus and ceca. Esophagus bifurcates in front of the cirrus sac. Ceca extend nearly to the posterior end. Cirrus sac is well developed and contains a saccular seminal vesicle. Uterus with 1-22 (7 in average) eggs occupies the space between the ventral sucker and the ovary. Ovary is spherical or transversely oval. Mehlis' gland is situated between the ovary and the anterior testis. Testes are tandem and longitudinally oval. Vitelline glands are follicular and extend from the posterior 1/3 level of the uterus to the posterior end of the body. Eggs in the uterus are unembryonated and 0.102 × 0.068 mm in average size. The dimensions of each organs are shown in Table 4, and compared with those of previous authors.

DISCUSSION

The first description of the life cycle of *E. recurvatum* was accomplished by Mathias (1926 & 1927). *Planorbis planorbis* was used as the first intermediate host in these studies. Various kinds of snails were used as the second intermediate hosts and domestic ducks served as the definitive host. Many studies have been subsequently performed in various countries using various snail intermediate hosts (Haper, 1929; Azim, 1930; Odening, 1962; Moravec *et al.*, 1974; Diaz-Diaz, 1976). In Korea, the presence of this fluke has been confirmed by the recovery of adult worms from a house rat (Lee *et al.*, 1990). However, their biological characteristics have not been studied yet. In the present study, some kinds of biological characteristics which originated from the life cycle of *E. recurvatum* has been verified for the first time in Korea.

It has been already suggested that *E. recurvatum* cercariae are divided into two types, *i.e.* lymnaeidarum and planorbidarum according to their snail intermediate hosts (Odening, 1964). McCarthy (1990) reported the biological differences between two sibling species in the *E. recurvatum* complex, origi-

Table 4. Measurements[a] of *E. recurvatum* adults and comparison with those of previous authors

Organs	Present study (1998)	Lee *et al.* (1990)	Bashkirova (1941)
Body	2.010-3.090 (2.760)	3.50-4.70	2.00-5.00
	× 0.460-0.610 (0.550)	× 0.50-0.65	× 0.40-0.85
Head crown	0.173-0.214 (0.188)		
	× 0.260-0.316 (0.283)	0.29-0.34	0.220-0.385
Oral sucker	0.092-0.122 (0.109)	0.12-0.15	0.099-0.130
	× 0.092-0.112 (0.099)	× 0.12-0.15	
Pharynx	0.061-0.087 (0.077)	0.11-0.13	0.077-0.170
	× 0.036-0.071 (0.046)	× 0.09-0.11	
Esophagus	0.204-0.377 (0.315)	0.16-0.19	0.282-0.605
Cirrus sac	0.209-0.337 (0.263)	0.30-0.40	0.176-0.385
	× 0.082-0.143 (0.117)	× 0.13-0.18	× 0.099-0.264
Ventral sucker	0.296-0.367 (0.333)	0.32-0.40	0.253-0.407
	× 0.306-0.383 (0.337)	× 0.32-0.39	× 0.253-0.363
Ovary	0.082-0.133 (0.112)	0.13-0.22	0.077-0.187
	× 0.092-0.153 (0.122)	× 0.13-0.20	× 0.123-0.187
Anterior testis	0.235-0.377 (0.329)	0.28-0.42	0.242-0.506
	× 0.153-0.214 (0.188)	× 0.21-0.28	× 0.154-0.275
Posterior testis	0.265-0.408 (0.355)	0.39-0.48	0.121-0.484
	× 0.163-0.204 (0.187)	× 0.18-0.29	× 0.176-0.275
Eggs	0.096-0.105 (0.102)	0.082-0.097	0.090-0.110
	× 0.064-0.071 (0.068)	× 0.054-0.059	× 0.051-0.084

[a]Length × width (average), unit is mm.

nating from *Lymnaea peregra*, and another from *Valvata piscinalis*. *Echinoparyphium pseudorecurvatum* was described as a new species on the basis of rediae and cercariae from the naturally infected *P. planorbis* and metacercariae and adults reared experimentally. This species was separated from an old collective species *E. recurvatum* by Kiseliene and Grabda-Kazubska (1990). Grabda-Kazubska and Kiseliene (1991) completed the life cycle of *E. mordwilkoi* using naturally infected *V. piscinalis* as the snail intermediate host. The aforementioned studies suggest that these three species of *Echinoparyphium* differ by the course of their life cycle, especially regarding the first intermediate hosts (*i.e.* lymnaeids in *E. recurvatum*, planorbids in *E. pseudo recurvatum* and prosobranch in *E. mordwilkoi*). Therefore, cercariae shed from naturally infected *R. auricularia coreana* in the present study should be those of *E. recurvatum* because the *Radix* snail is a member of the family Lymnaeidae.

Buscher (1978) discussed the taxonomy of the genus *Echinoparyphium* with raising *E. speotyto* as a new species. He allotted six species in the *Echinoparyphium* group bearing 45 collar spines. Among the seven species including *E. pseudorecurvatum* recently proposed, *E. recurvatum* is the most similar to *E. mordwilkoi* and *E. pseudorecurvatum*. This fluke differs from the other two species in regarding to the shape and size of the collar spines. The collar spines of *E. recurvatum* are pointed, straight and shorter, whereas those in the other two species are blunt and stumpy (Kiseliene and Grabda-Kazubska, 1990; Grabda-Kazubska and Kiseliene, 1991). The largest spine is the 4th one in *E. recurvatum*, while the 2nd and 3rd spines in *E. mordwilkoi* and in *E. pseudorecurvatum* are the largest. In the present study, the 4th collar spine was the largest. This finding corresponds closely with that of *E. recurvatum*.

Buscher (1978) distinguished species by the distribution of vitellaria in *E. recurvatum* and *E. mordwilkoi*. The vitellaria of *E. mordwilkoi* extend anteriorly to posterior edge of the ventral sucker, while those of *E. recurvatum* do not extend. In the present study, vitellaria were distributed from the posterior 1/3 level of the uterus to the posterior end of the body. This finding also corresponds to that of *E. recurvatum*.

Echinoparyphium recurvatum found in wild birds in Korea was reported as the name of *E. koidzumii* (Chu *et al.*, 1973). Although *E. koidzumii* was described as a new species in Taiwan by Tsuchimochi (1924), it was later regarded as a synonym of *E. recurvatum* by many authors (Yamaguti, 1933; Skrjabin, 1947; Yamashita, 1964; Rim, 1982). Adult *Echinoparyphium* specimens recovered from naturally infected hosts like that in Chu *et al.* (1973) must be carefully examined for correct species identification since their biological information is not available except adult morphology. The specimen found from a house rat by Lee *et al.* (1990) should also be reexamined on the species level, because of the aforementioned reason and the difference of the definitive host.

In conclusion, it has been confirmed by the present study that the life cycle of *E. recurvatum* is well maintained in the laboratory and *R. auricularia coreana* plays a pivotal role as the first and/or second intermediate host of this fluke in Korea.

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=초록=

한국에서의 오리극구흡충 (*Echinoparyphium recurvatum*)의 생활사

손운목

경상대학교 의과대학 기생충학교실

이 연구는 자연계 및 실험실 내 조건 하에서 오리극구흡충의 생활사를 관찰하기 위하여 수행하였다. 1992년 8월과 9월에 부산시 강서구에 소재하는 선암천에서 다량의 물달팽이 (*Radix auricularia coreana*)를 채집하였다. 채집한 물달팽이 중 106개를 파쇄법으로 조사하였던 바, 52개 (49.0%)에서 오리극구흡충의 유충 (레디아, 유미유충, 피낭유충)이 한 종류 이상씩 검출되었다. 물달팽이로부터 자연 유출되는 유미유충을 접촉감염시킨 물달팽이, 논우렁 및 미꾸리 중 물달팽이와 미꾸리에서 피낭유충이 검출되었다. 물달팽이로부터 검출한 피낭유충을 경구감염시킨 병아리, 오리, 흰쥐, 마우스 중 병아리와 오리에서 성충이 검출되었다. 물달팽이로부터 검출한 피낭유충을 경구감염시킨 병아리에서의 충체 회수율은 평균 13.1%이었다. 레디아는 주머니 모양으로 평균 2.437 × 0.317 mm 크기이었으며 근육성의 인두와 체장의 약 1/2 부위까지 내려와 있는 맹장을 하나씩 갖고 있었다. 유미유충의 체부위는 방추형으로 평균 0.262 × 0.129 mm 크기이었고, 꼬리부위는 긴 막대모양으로 평균 0.528 × 0.056 mm 크기이었다. 유미유충의 체부위에서는 45개의 두극이 배열되어 있는 두관과 미세한 과립을 다수 보유하고 있는 배설관이 특징적으로 관찰되었다. 피낭유충은 구형으로 평균 0.144 × 0.142 mm 크기이었으며 2중의 낭벽을 가지고 있었다. 성충은 비교적 길쭉하였고 평균 2.760 × 0.550 mm 크기이었으며 두관에는 특징적인 45개의 두극이 배열되어 있었다. 이상의 결과로 우리 나라에서 물달팽이가 오리극구흡충의 제1 및 제2중간숙주로서 생활사 유지에 중요한 역할을 수행하고 있음을 확인하였다.

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