

## Studies on the lungfluke, *Paragonimus iloktsuenensis*

### VII. The first intermediate host, cercaria and redia of *P. iloktsuenensis*

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#### INTRODUCTION

As a second intermediate host of *Paragonimus iloktsuenensis*, *Sesarma dehaani* has already been reported since the metacercaria had been firstly isolated in the above crab host by Yokogawa et al. (1971) in Korea. And later, *Helice tridens tridens* and *Sesarma intermedia* were also found to be served as the crab host (Seo et Kwak, 1972) at Hadan and Hadong area, South Kyong-Sang Do (=Province), Korea.

H.T. Chen (1940) was the first to describe in China that a species of brackish water snails, *Assiminea lutea* serve as the first intermediate host of *P. iloktsuenensis*. On the other hand, in Japan, the six species of snail hosts were experimentally proved to be susceptible to miracidia and enough to develop to cercaria of *P. iloktsuenensis*. These are *Assiminea parasitologica*, *A. japonica*, *A. yoshidayukioi*, *Paludinella japonica*, *Oncomelania hupensis nosophora* and *Augustassiminea nitida* (Yoshida, 1959; Yoshida et Kawashima, 1961; Miyazaki et al., 1960; Tomimura et al., 1960; Kawashima et al., 1970). Among these snails, natural infection was reco-

gnized only in *A. parasitologica* and *A. nitida*. According to Habe (1965), *A. parasitologica* and *A. yoshidayukioi* also should be assigned to the genus *Augustassiminea* in a broad sense.

In Taiwan, the snail host, *Tricula chiu* (Habe et Miyazaki, 1962) was reported as natural and experimental first intermediate host (Chiu, 1965). This species of snail was reassigned to the genus *Oncomelania* by Davis (1968) and renamed as *O. hupensis*. However, no studies on the snail host of *P. iloktsuenensis* in Korea have ever been made yet. Consequently, upto now, four kinds of snail have been found naturally infected with *P. iloktsuenensis* to serve as the first intermediate host.

In the present study, authors attempted to find the natural infection with *P. iloktsuenensis* in the brackish water snails, collected at the Sumjin River delta, where is known as enzootic areas of this fluke, and to identify the species of infected snails. The larval morphology of fluke found in the snail host was also described.

#### MATERIALS AND METHODS

As reported in the previous study, there are two enzootic areas of *P. iloktsuenensis*; Hadan and Hadong. The former is located at the Naktong River delta, western suburb of the city of Pusan and the latter at the Sumjin River

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delta. And the infection rate of metacercariae in crab hosts in the area of Hadong was found higher than in the areas of Hadan (Seo et Koo, 1971). From these results, the snail hosts were collected in the area of Hadong, in the western corner of South Kyong-Sang Do(=Province), bounded by estuaries of the Sumjin River, separating two Provinces; the South Kyong-Sang and the South Cholla Do.

The brackish water snails collected at the above area were tentatively identified and separately pooled with same species. To detect the larval flukes, especially cercariae and rediae, the shell of the snail was crushed and the viscera, particularly liver and gonads were placed between two slides and lightly pressed. Specimens from each snail were carefully examined under the microscope for cercaria and redia. All measurements of the isolated larvae from snail were made from the specimens fixed with the buffered neutral formalin under the pressure of coverglass. Some of specimens were fixed in hot formalin and stained with methyl-green pyronin, and mounted with balsam after dehydration and cleaning procedure.

## RESULTS AND DISCUSSION

### The snail host:

Brackish water snails were collected from Hadong Gun, where is known as one of enzootic areas of *P. iloktsuenensis*, located in the delta

area of the Sumjin River. Among these snails collected, some species of the family Assimineidae were tentatively identified, particularly, three or more species of the genus *Assiminea* were collected in the reedy marshy area; they were identified as *Assiminea yoshidayukioi*, *A. parasitologica* and *Assiminea* species.

*Assiminea yoshidayukioi* Kuroda, 1959; very small, alt., 3.2mm, diam., 2.2mm, ovately conical, chestnut corneous, subtransparent, rather blunt apex, very glossy, spire with slightly convex outline, some black spots on the last body whorl, narrowly opened umbilicus.

*Assiminea parasitologica* Kuroda, 1958; shell, small, alt., 4.2mm, diam., 3.0mm, shortly ovate, dark brown corneous with obtuse apex, spire with convex outline in distinct and deeply constricted suture, a pale straw colour band zone below the suture, particularly distinct on the later portion of the last body whorl. According to Kuroda(1959), *A. parasitologica* was once erroneously identified as *Paludinella devilis* (Yokogawa et al., 1958), which is differentiated by a sharply perforated umbilicus and different radular formula of the latter.

*Assiminea* sp.; some specimens seem to be different species. Among them, there are specimens to show a close resemblance with *Assiminea parasitologica*, but the shell is slightly larger, in different colour, with somewhat glossy surface and so on.

The identification of this species was opened

Table 1. The natural infection rates of *P. iloktsuenensis* in the snail hosts reported by various authors

Snail species	Author(Year)	Locality	No. snails examined	No. snails infected(%)
<i>Assiminea lutea</i>	Chen(1940)	Canton, China	59,725	58(0.097)
<i>A. parasitologica</i>	Yoshida(1959)	Osaka, Japan	1,300	1(0.076)
<i>A. parasitologica</i>	Tomimura et al. (1960)	Osaka, Japan	14,785	132(0.89)
<i>Tricola chui</i>	Chiu(1965)	Taipei, Taiwan	4,122	3(0.073)
<i>Augustassiminea nitida</i>	Kawashima et al. (1970)	Amami-Oshima Japan	892	6(0.67)
<i>Assiminea</i> spp.	Present authors	Hadong, Korea	3,096	12(0.387)

Table 2. Measurements of the rediae of *P. iloktsuenensis* reported by various authors

Larval stage		1st generation rediae			2nd generation rediae			
Author (Year)		Chen (1940)	Chiu (1965)	Present author	Chen (1940)	Tomimura et al. (1960)	Chiu (1965)	Present author
No. of rediae measured		45	25	5	28	30	25	18
Body	Length	304—691	197—506 (419)	408.2—639.2 (497.8)	813—2,043 (1,422)	630—1,000 (796)	206—960 (638)	734.4—1,298.8 (1,020.4)
	Width	87—155	59—166 (102)	122.4—170.0 (140.1)	220—357 (257)	120—200 (160)	103—224 (159)	142.8—231.2 (186.2)
Pharynx	Length	42—122	28—63 (40)	35.7—48.1 (42.2)	(60)	—	30—51 (40)	32.6—63.6 (47.2)
	Width	45—122	28—57 (38)	34.1—45.0 (40.3)	(65)	—	24—47 (38)	38.8—60.5 (46.1)
Intestine	Length	49—233	24—90 (48)	51.2—80.6 (63.6)	98—486 (213)	45—270 (94)	33—138 (62)	65.1—141.1 (108.6)
	Width	49—158	18—83 (37)	29.5—58.9 (38.0)	(85)	30—140 (64)	20—87 (46)	31.0—77.5 (53.6)

The figures in parenthesis are averages

for further study.

As shown in Table 1, twelve of 3,780 specimens of the snail host, *Assimineea* examined were found to be infected with larval flukes of *P. iloktsuenensis*. Both cercariae and rediae were found in each six snails of 1,715 *Assimineea parasitologica* and 1,381 *Assimineea* spp. The average infection rate was 0.32 per cent. However, there were found no larvae in 684 *A. yoshidayukioi*. The infection rate of *P. iloktsuenensis* was found 0.35 per cent in *A. parasitologica*.

#### Larval morphology:

The first generation redia; The number mature redia found in a snail was 8 to 15 (average 10) The body shape was cylindrical. The digestive tract consisted of a short prepharynx, a large, stout, globose pharynx with a short esophagus, followed with a short intestinal cecum. The pharynx was muscular and distinctly characteristic, being one-eleventh of the body length in average. The cecum was sometimes brownish in colour, being about one-eighth of the body length. The birth pore was located near to

pharynx. Inside the body, there were second generation rediae with on average 6 germ balls in the posterior portion of the body cavity. The measurements from five specimens were 408.0 to 639.2 microns in length by 122.4 to 170.0 in width.

The second generation rediae were found especially in the space surrounding the digestive gland and the hermaphroditic organ in the spire of the snail. Their movement was sluggish in body extension and contraction. The body was cylindrical in shape. The muscular, globose pharynx was about one twentieth of the body length. The cecum was short, slender and brownish coloured, being one-ninth of the body length. In the fully matured redia, there were 3 to 10 cercariae in various developing stages. And the germ balls in a cluster were also observed. The body length of the second generation rediae varied from 734.4 microns to 1,298.8 microns, however the average measurements from 10 specimens were 1,020.4 microns in length by 186.2 microns in width.

Cercariae were also found in the digestive gland and gonad of the snail. Their extending and contracting motility was observed only in

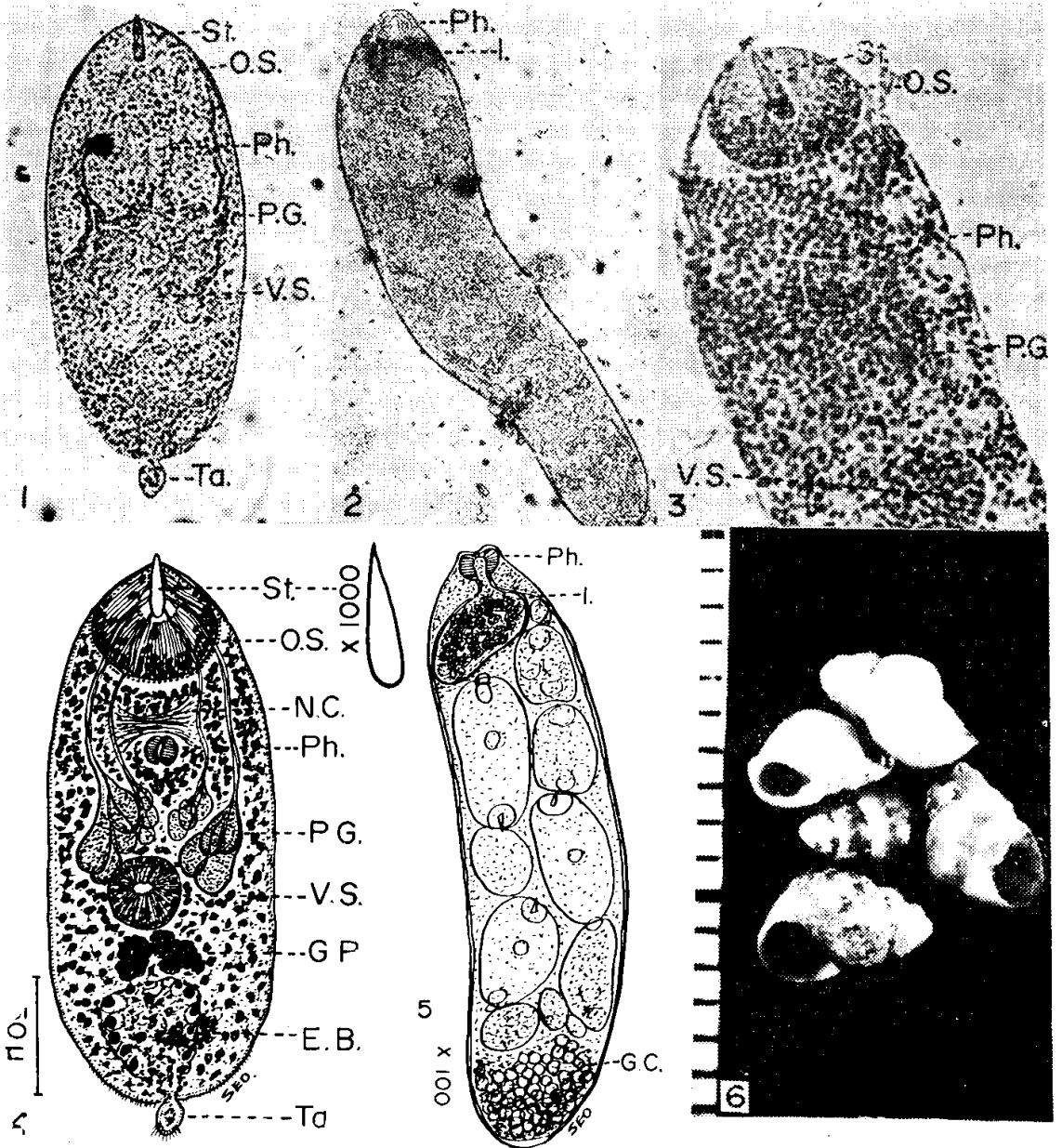


Fig. 1. Cercaria of *P. iloktsuenensis* (fixed on the buffered neutral formalin)

Fig. 2. Redia of *P. iloktsuenensis* (fixed on the buffered neutral formalin)

Fig. 3. Cercaria of *P. iloktsuenensis* (stained with methyl green pyronin)

Fig. 4. Cercaria of *P. iloktsuenensis* drawn with the aid of camera lucida (stained with methyl green pyronin)

Fig. 5. Redia of *P. iloktsuenensis* drawn with the aid of camera lucida (stained with methyl green pyronin)

Fig. 6. *A. parasitologica*, the first intermediate host of *P. iloktsuenensis*

**Table 3.** Measurements of the cercariae of *P. iloktsuenensis* reported by various authors

Author (Year)	Chen (1940)	Yoshida (1959)	Tomimura et al. (1960)	Chiu (1965)	Kawashima et al. (1970)	Present authors	
No. of cercariae measured	?	10	30	10	?	10	
Body	Length	265	165—320 (249.4)	190—275 (238)	170—328 (254)	245.0	238.0—306.0 (277.4)
	Width	102	80—150 (114.4)	55—90 (70)	56—87 (70)	88.8	95.2—136.0 (120.4)
Oral sucker	Length	55	45—66 (49.2)	46	32—40 (37)	49.7	45.0—58.9 (51.0)
	Width	55	41—66 (49.2)	40	36—40 (38)	52.8	38.8—65.1 (49.8)
Ventral sucker	Length	37	24—52 (33.1)	34	20—28 (24)	35.7	27.9—35.7 (31.8)
	Width	37	36—55 (39.1)	35	22—32 (25)	39.8	27.9—40.3 (32.3)
Stylet	Length	—	20—30 (24.4)	—	6 (6)	26.9	23.3—31.0 (28.1)
	Width	—	—	—	—	—	4.7—6.2 (5.5)
Tail	Length	23	14—23 (20.9)	22	10—24 (15)	26.9	12.4—21.7 (17.5)
	Width	—	17—21 (18.7)	—	10—14 (11)	—	10.9—18.6 (14.6)
Excret. bladder	Length	—	42—82 (65.5)	95	47—67 (58)	—	69.8—43.3 (56.6)
	Width	—	41—33 (36.7)	—	20—28 (23)	—	—

The figures in parenthesis are averages

bottom of the containers. The cercariae were microcercus, with a long elliptical body measuring 238.0 to 306.0 microns in length by 95.2 to 136.0 microns in width. The characteristic knob-shaped tail measured 17.5 microns in length by 14.6 microns in average. The entire body was covered with minute spines. The oral sucker was larger than ventral sucker, and provided with a minute stylet measuring 28.1 microns by 5.5 microns in average. The ventral sucker located slightly behind the mid-plane of the body. The pharynx was distinctly recognized in the methyl green pyronin stained specimens, posterior to the outer margin of the oral sucker. The penetrating glands opening through the ducts at the bilateral sides of stylet consisted two types of glands; four pairs of larger lateral glands and three pairs of smaller median glands. The excretory bladder was rather cylindrical and occupied the posterior portion of the body. The genital primordia was distinctly recognized as deeply stained masses between the ventral sucker and excretory bladder only in the stained specimen.

From the above results obtained, it was proved that the larvae found from the snail, *A. parasitologica* collected at Hadong area were identical with those of *P. iloktsuenensis* described by

previous workers (Chen, 1940; Yoshida, 1959; Tomimura et al., 1960; Chiu, 1965; Kawashima et al., 1970). This is also verified by the following facts; in Hadong, where the snail was collected has been already known one of the enzootic areas of only one species; *P. iloktsuenensis* among the final host of house rats (Seo et Koo, 1971) and the brackish water crabs, *Sesarma dehaani* have also been found infected with metacercaria of this fluke in this area (Yokogawa et al., 1971).

Therefore, it was definitely concluded that the brackish water snail host, *A. parasitologica* is incriminated in Hadong, Korea to serve as the first intermediate host of *P. iloktsuenensis*.

## SUMMARY

A total of 3,780 brackish water snails, *Assiminea* spp. collected from Hadong, the delta area of the Sumjin River, South Kyong-Sang Do, Korea were examined for the larval fluke infection of *Paragonimus*. These included 684 *Assiminea yoshidayukioi*, 1,715 *Assiminea parasitologica* and another 1,381 *Assiminea* sp. All species of the snails examined were found infected with the larval fluke except *A. yoshida*

yukioi.

Six(0.35%) out of 1,715 individuals of the snail *A. parasitologica* and six (0.43%) out of 1,381 specimens of unidentified *Assimineae* sp. were found naturally infected with cercariae and rediae of *Paragonimus*. These larval flukes from the infected snails were verified to be identical with those of *P. iloktsuenensis* described by earlier investigators.

In addition to the above results obtained, this locality, Hadong has already been known as one of the enzootic areas of *P. iloktsuenensis* through the findings of the metacercariae and adult worms of this fluke from crabs and rodents.

Accordingly, it was definitely concluded that *Assimineae parasitologica* Kuroda, 1958 was found for the first time to be the first intermediate host for *Paragonimus iloktsuenensis* Chen, 1940 in Korea.

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#### 《國文抄錄》

怡樂村肺吸蟲(*Paragonimus iloktsuenensis* Chen, 1940)에 관한 研究

VII. 第一中間宿主 決定 및 세르카리아 및 레디아의 形態

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우리나라에 있어 怡樂村肺吸蟲(*Paragonimus iloktsuenensis*)의 第二中間宿主(말뚝개, *Sesarma dehaani*外 二種) 및 終宿主(*Rattus norvegicus*)에서 各各 被囊幼

虫 및 成虫의 自然感染狀況이 밝혀졌으나 貝類中間宿主는 究明된 바 없다.

著者等은 本肺吸蟲의 우리나라에서의 完全한 生活史를 確立할 目的으로 本蟲의 流行地域으로 밝혀진 慶南河東郡의 蟾津江 河口에서 半鹹水産貝類를 採集하고 吸蟲類 세르카리아(*Cercaria*) 및 레디아(*Redia*)의 寄生狀況을 調査하고 感染貝類를 同定하여 우리나라에서의 本蟲 第一中間宿主 決定을 試圖하였다.

總 3,780個의 貝類를 *Assimineae yoshidayukioi*, *A. parasitologica* 및 *Assimineae* sp.로 分類하고 *A. yoshidayukioi*를 除外한 2種에서 肺吸蟲幼蟲이 寄生하였음을 發見하였다. *A. parasitologica* 1,715個와 *Assimineae* 未同定種 1,381個에서 各各 6個씩의 感染貝를 檢出하였고 感染率은 各各 0.35% 및 0.43%이었다.

感染貝類에서 分離한 肺吸蟲幼虫 特히 세르카리아 및 레디아의 形態를 仔細히 檢討하였던 바 過去 여러 研究者에 依하여 記載된 *P. iloktsuenensis*의 幼虫과 同一함을 알수 있었다. 또한 同地域에서 本蟲의 被囊幼虫 및 成虫의 存在가 이미 報告된 點으로 보아 이들 肺吸蟲 幼虫은 怡樂村肺吸蟲이라 確立할 수 있었다.

따라서 우리나라에 있어 怡樂村肺吸蟲의 第一中間宿主를 *Assimineae parasitologica* Kuroda, 1958으로 報告한다. 未同定貝類에 對한 研究와 이들 貝類에 對한 本蟲의 實驗室內 實驗感染이 要求된다.

### REFERENCES

- Chen, H.T.: *Morphological and developmental studies of Paragonimus iloktsuenensis with some remarks on other species of genus (Trematoda, Troglotremaidae)*. *Lingnan Science J.*, 19(4):429-530, 1940.
- Chiu, J.K.: *Tricula chiui Habe et Miyazaki, 1962: A snail host for Paragonimus iloktsuenensis Chen, 1940 in Taiwan*. *Jap. J. Parasit.*, 14(3):269-280, 1965.
- Davis, G.M.: *A systematic study on Oncomelania hupensis chiui (Gastropoda: Hydrobiidae)*. *Malacologia (from 6(1-2) 145-153)*, 1967), 1968.
- Habe, T. and I. Miyazaki: *Tricula chiui sp. nov., a new snail host of the lungfluke, Paragonimus iloktsuenensis Chen in Formosa*. *Kyushu J. Med. Sci.*, 13(1):47-49, 1962.
- Habe, T.: *Gastropods. New illustrated encyclopedia of the fauna of Japan*. 2, 54-55, 1965 (in Japanese).
- Kawashima, K. and I. Miyazaki: *The experimental infection of larval lung-flukes to Japanese Oncomel-*

- ania nosophora (Robson, 1915). *Jap. J. Parasit.*, 12(2):159-162, 1963.
- Kawashima, K. and F. Hamajima: A new molluscan host for *Paragonimus iloktsuenensis* Chen, 1940 in Is. Amami-Oshima, Kagoshima Prefecture, Japan. *Jap. J. Parasit.*, 19(2):199-205, 1970.
- Kuroda, T.: On the more species of *Assiminea* in Japan (A fresh water Gastropodous Genus). *Venus*, 20(1): 16-22, 1958.
- Kuroda, T.: Another new species of *Assiminea*, a trematode's intermediate host snail from Japan. *Venus*, 20(4):335-338, 1959.
- Miyazaki, I., K. Kawashima and Y. Yoshida: Studies on the snail hosts of *Paragonimus ohirai* Miyazaki, 1939 and *P. iloktsuenensis* Chen, 1940. *Kyushu J. Med. Sci.*, 11(6):261-275, 1960.
- Seo, B.S. and B.Y. Koo: Studies on the lungfluke, *Paragonimus iloktsuenensis* Chen, 1940. I. On the occurrence of *P. iloktsuenensis* in the natural final hosts at the Nakdong and Sumjin River Deltas in Korea. *Seoul J. Med.*, 12(1):31-43, 1971.
- Tomimura, T., J. Terauchi and I. Tarumoto: Studies on the first intermediate host of lungfluke, *Paragonimus iloktsuenensis* Chen, 1940 in the mouth of the River Shin-yodo, Osaka Prefecture. *Med. & Biol.*, 5(2): 45-51, 1960.
- Yokogawa et al.: On a new first intermediate host, *Paludinella devilis* (Gould, 1861) Habe, 1942, *Paragonimus ohirai* Miyazaki, 1939. *Tokyo J. Ijishinshi*, 75(2):67-72, 1958 (in Japanese).
- Yokogawa, M., K. Araki., H. Koyama, B.S. Seo, S.H. Lee and S.Y. Cho: On the lungfluke, *Paragonimus iloktsuenensis* Chen, 1940 in Korea. *Jap. J. Parasit.*, 20(3):215-221, 1971.
- Yoshida, Y.: Studies on the first intermediate host of *Paragonimus iloktsuenensis* Chen, 1940 in Japan (1) Experimental infection with the larva of *P. iloktsuenensis* to *Assiminea parasitologica* Kuroda, 1958 and *Assiminea japonica* von Martens, 1877. *Jap. J. Parasit.*, 8(5):822-828, 1959.
- Yoshida, Y. and K. Kawashima: On the distribution of the snail hosts of *Paragonimus ohirai* Miyazaki, 1939 and *P. iloktsuenensis* Chen, 1940 in Japan. *Jap. J. Parasit.*, 10(2):152-160, 1961.