## On the Lung Fluke, *Paragonimus iloktsuenensis* Chen, 1940 in Korea

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#### Introduction

Paragonimus iloktsuenensis, Chen, 1940 has been found in various areas of Japan, at the mouth of river Shinyodo, Osaka, at the mouth of the river Sendai, Kagoshima prefecture (Miyazaki, 1945), at the mouth of the river Kako, Hyogo prefecture (Miyazaki et al., 1951) and in the island Amami Oshima, Kagoshima prefecture (Sato et al., 1969). Other than the places mentioned above, Alilao village in Taipei, Taiwan (Miyazaki and Chiu, 1962) had been the only place where Paragonimus iloktsuenensis was found, except China mainland and Japan.

In September 1970 the authors found out metacercariae very similar to those of *Paragonimus iloktsuenensis* in *Sesarma dehaani* collected at the mouth of the river Nakdong in South Korea and obtained the adult worms from the lungs of rats experimentally infected with those metacercariae from *Sesarma dehaani*.

#### Meterial and Methods

The area where the authors collected crabs was Hadan district at the mouth of the river Nakdong (Fig. 1), low and damp zone where reeds were densely growing. The crabs

collected in the area were brought back to the laboratory and examined for metacercarie



Fig. 1 A map of Korea.

mainly on the livers of the crabs. The liver was placed between two slides (4×10 cm), pressed lightly and carefully examined under binocular dissecting scope. The cyst wall of the metacercariae found in the liver of crabs, *Sesarma dehaani*, was extremly week and the isolation of the metacercariae from the tissue of the liver was quite a difficult procedure.

A few metacercariae taken out complete were given orally to rats immediately after the size of the metacercariae was measured. Excysted larvae were fixed with 70 % alcohol, stained with carmine, and mounted with balsam after dehydration.

#### Results

1. Results of examination of crabs.

The species and number of crabs examined

same area. However, a great number of the metacercariae of *Microphalloides japonicum* were found in 34 (73.9 %) out of 46 *H. tridens tridens*.

The correlation of the size of *S. dehaani* and the number of metacercariae per crab was shown in Table 2. The highest infection rate (85.7 %) was noted among the crabs with the shell size from 21 to 25 mm. Though the total number of 26 *S. dehaani* might be considered not enough to deduce statistical data, a tendency is noted in the above correlation; that is, the infection rate is higher in the smaller *S. dehaani* of less than 25 mm in shell size (80.0 %) than in *S. dehaani* of more than 26 mm in shell size (50.0 %).

The number of metacercariae per crab was generally small: Only 1 to 3 metacercariae were found in 13 *S. dehaani* out of 16

Table 1	Result of	examination	of	crabs	collected	from	Hadan,	Pusan,	Korea
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Species	No. crabs	exam.	No. (%) posit. for metacercariae of <i>P. iloktsuenensis</i>	No. metacercariae recovered (No. per crab)
	M	17	10 (58.8)	30 (3.0)
Sesarma dehaani	F	9	6 (66.7)	18 (3.0)
	Total	26	16 (61.5)	48 (3.0)
	F	36	0	
Helice tridens tridens	M	10	0	
triuens	Total	46	0	
Sesarma haematocheir	F	3	0	

were shown in Table 1. In the livers of 16 (61.5%) out of 26 Sesarma dehaani, the total 48 small metacercariae very similar to those of Paragonimus iloktsuenensis were found. There were no remarkable differences in the infection rates and the average number of metacercariae between male and female crabs.

There were found no metacercariae of *Paragonimus* in 46 *Helice tridens* tridens\*, nor in 3 *S. haematocheir* collected in the

S. dehaani positive for metacercariae. The largest number of metacercariae found in one S. dehaani with the shell size of 22 mm was 11.

#### 2. Identification of metacercariae.

The metacercaiae found by the authors from S. dehaani in Korea had only one very thin membrane  $(3.0 \,\mu$  in average) and no inner wall was found as was recognized in those of P. wesrermani or P. ohirai.

The metacercaria was almost oval in shape. The larva inside the cyst was found stretching freely and it seemed to have a space to move (Photo. 1). The cyst wall was so

<sup>\*</sup> Six metacercariae of *P. iloktsuenensis* were found in 5 out of 38 *Helice tridence tridens* collected in Hadang on May, 1971.

Size of the shell (mm)	No. exam.	No. (%) positive	No. metacercariae found (No. per crab)
16—20	3	2 (66.7)	4 (2)
21—25	7	$ \begin{array}{ccc} 2 & (66.7) \\ 6 & (85.7) \end{array} $ 8 (80)	$\binom{4}{29}\binom{2}{(4.8)}33$ (4.1)
26—30	9	5 (55.6)	10 (2)
31—35	7	5 (55.6) 3 (42.8) 8 (50)	$ \begin{array}{ccc} 10 & (2) \\ 5 & (1.6) \end{array} $ 15 (1.9)
Total	26	16 (16.5)	40 (3.0)

Table 2 Size of the crabs, Sesarma dehaani, infected with the metacercaria of P. iloktsuenensis

thin as to change its shape when the larva inside moved and it was too easily broken even with a slight pressure. The size of the cysts was, when measured in the water without pressure using same technique as Miyazaki's (1954),  $321 \times 288 \,\mu$  in average of 5 cysts (Table 3).

The size of the excysted larvae stained was a little smaller than that of Miyazaki (1945) as shown in Table 4, but the stylet on the oral sucker was  $11 \mu$  long in average, quite the same size as measured by Miyazaki (1945) as shown in Photo. 2.

Based on the morphological features mentioned above the metacercariae found in *S. dehaani* in Korea was identified as that of *P. iloktsuenensis*.

Table 3 Measurements of the metacercaria of P. iloktsuenensis (in microns)

Authors	Range of size	Mean	
Chen (1940)	$230-302 \times 200-214$	256×216	
Miyazaki (1945)	$239495\!\times\!282396$	$338 \times 262 (30)$	
Yokogawa et al.	$315 – 323 \times 270 – 297$	$321 \times 288(5)$	

( ): showing number of metacercaria measured.

#### 3. Identification of adult worms.

Four adult worms were obtained from the two rats 40 days after the infection with the metacercariae from Sesarma dehaani in Korea. In one of the rats, a typical worm cyst was formed in the middle of the right lung and inside the worm cyst 3 actively moving adult worms were recovered. As to the other rat, a hemorrhagic lesion which seemed to be a worm cyst was noted in the right lung, but no worm was found inside. However, one adult worm was recovered which had moved freely in the pleural cavity. The number of metacercariae given to the rats is not very clear since, together with a few isolated metacercariae, the infected livers of the crabs were also given to them.

The adult worms, after being kept for one hour in physiological saline at 37°C in order to excrete the contents in intestine and eggs in the uterus, were fixed in Carnoy's solution and stained with Mayer's carmine for morphological examination. The cuticular spines and ovary of the adult worm were "arranged in group" and "profusely branched", respectively, as shown in Photos. 3 and 4. These characteristics indicated as those of

Table 4 Measurements of the excysted metacercariae of *Paragonimus iloktsuenensis* (Stained specimens in microns)

Authors	Areas collected	No. measured	Body	Oral sucker	Ventral sucker
Miyazaki (1945)	Kagoshima, Japan	7	$314-380 \times 124-165$ (333×139)	$37-46 \times 29-37$ (41 × 35)	$40-51 \times 37-51 \ (43 \times 43)$
Yokogaga et al.	Pusan, Korea	6	$239-313 \times 87-134$ $(277\pm30\times170\pm16)$	$29-37 \times 22-32$ (33±4×30±4)	$34-39 \times 32-40$ ( $36\pm 3 \times 35\pm 3$ )

Table 5 Measurements of the adult worms of *P. iloktsuenensis* recovered from the experimentally infected rats (Stained specimens)

Author	Days of autopsy after infection	No. measured	$\begin{array}{c} \operatorname{Body} \\ (\operatorname{Length} \times \operatorname{width}) \end{array}$	Oral sucker (width)	Ventral sucker	Eggs in uterus
Sato <i>et al</i> . (1969)	38	28	4.0-7.0×2.0-3.0 mm (5.7×2.6)	332–585 (469 μ)	$411-648 \times 442-648 \mu$ $(536 \times 545 \mu)$	+
Yokogawa <i>et al.</i> (1971)	40		$6.1-7.5\times3.5-4.0 \mathrm{mm} \ (7.2\pm0.7\times3.9\pm0.4)$	474-552 $(507 \pm 32 \mu)$	$\substack{697-731\times602-688\\(715\pm14\times628\pm41\mu)}$	+

( ): average.

Table 6 Measurements of the eggs of P. iloktsuenensis (in microns)

Authors	No. measured	Length	Width	Mean
Chen (1940)		70-95	46-53	87×52
Sato et al. (1969)	35	74.3-95.7	46.2-54.5	$82.7 \times 51.1$
Yokogawa et al.	50	74-84	44-49	$78\!\pm\!2\!\times\!46\!\pm\!2$

Host: Experimentally infected rats.

"iloktsuenensis-ohirai" type. The testes of the worm seemed larger compared with those of P. ohirai as was reported by Tomimura (1959). However, precise measurement was not undertaken with the testes in the present The measurements of the worms study. were shown in Table 5. The ventral sucker was larger than the oral sucker. The eggs in the uterus and the feces were short oval in shape and egg shell was evenly thick. The mean size of 50 eggs in uterus was 78±  $2\times46\pm2\,\mu$ . This was considerably smaller than the size of the eggs of P. iloktsuenensis measured by Chen (1940) or by Sato et al. (1969).

#### Discussion

The second intermediate hosts of *P. iloktsuenensis* hitherto known are *Sesarma dehaani* and *S. sinensis* in China, *Potamon miyazakii* in Taiwan, *S. dehaani* and *Helice tridens tridens* in Japan. In Japan the metacercaria of *P. ohirai* has been found in *S. dehaani* and *H. tridens tridens*. However, diferentiation of the metacercaria of *P. ohirai* from that of *P. iloktsuenensis* was considered easy by its morphological characteristics.

Miyazaki et al. (1951) has reported that "metacercaria of P. iloktsuenesis has only one

membrane which corresponds to the outer membrane in the metacercaria of *P. ohirai*. Moreover, the cyst wall of the metacercaria of *P. iloktsuenensis* is so thin as to change its shape when the larva inside moves about. The cyst wall is easily broken even with a slight pressure, so the metacercaria is often found excysting the cyst."

The metacercariae found by the authors for the first time in Korea had all the characteristics enumerated as above by Miyazaki. Thus, the authors identified the metacercaria found in *Sesarma dehaani* in Korea as that of *Paragonimus iloktsuenensis*.

As to the adult worms, Miyazaki (1945) reported that it was difficult to differentiate P. iloktsuenensis from P. ohirai, whereas Isshiki (1962) and Tomimura (1959) considered that the adult worms of the two species of Paragonimus were discernible. The adult worms obtained by the authors in the rats experimentally infected had the cuticular spines "arranged in group" and ovarium "profusely branched", which were quite similar to those of adult worms of P. ohirai.

As regards the size of eggs, Chen (1940) reported that the average size of 100 eggs taken from the rats experimentally infected with *P. iloktsuenensis* was 70–95×46–53  $\mu$  (87

 $\times52~\mu$ ), while Miyazaki reported that, alt hough the size of eggs more or less changed according to the age of the worms, it was  $89\text{-}69\times57\text{-}44~\mu~(80\times51~\mu)$  when measured 49 days after infection and it was  $93\text{-}69\times53\text{-}44~\mu~(84\times49~\mu)$  on 60 days after the infection. Miyazaki, however, concluded that although the eggs of P.~iloktsuenensis seemed to be slightly larger than those of P.~ohirai, the size of eggs could not be considered as a diagnostic key for identifying these two species of Paragonimus.

When the authors compared the eggs of *P. iloktsuenensis* in Korea with the eggs of *P. ohirai* in Japan, the reverse result was obtained: A tendency that the eggs of *P. iloktsuenensis* were smaller than those of *P. ohirai* was observed. The authors considered, as Miyazaki did, that the size of eggs could have no significance in differentiating these two species of *Paragonimus*. However, the authors maintain that this point has yet to be studied and to be cleared in the future.

As the first intermediate hosts of *P. iloktsuenensis*, *Assiminea lutea* in China, *A. parasitologica* and *A. yoshidayukioi* in Japan and *Trichuris chui* (*Oncomelania chui*) in Taiwan are known. The discovery of the first intermediate host in Korea will be the problem of study for future.

As to the definitive host of *P. iloktsuenensis* in Korea, Dr. Seo (1971) one of the present authors, found out adult worms of *P. iloktsuenensis* in the lungs of wild rats caught in Hadan, the field of the present study, and in Hadong, the western corner of Kyongsang-nam Do and gave evidence that they precisely accord with the adult worms of *P. iloktsuenensis* experimentally obtained by the authors.

As mentioned above, the discovery of *P. iloktsuenensis* in *Sesarma dehaani* and in wild rats for the first time in Korea is considered to be of great interest.

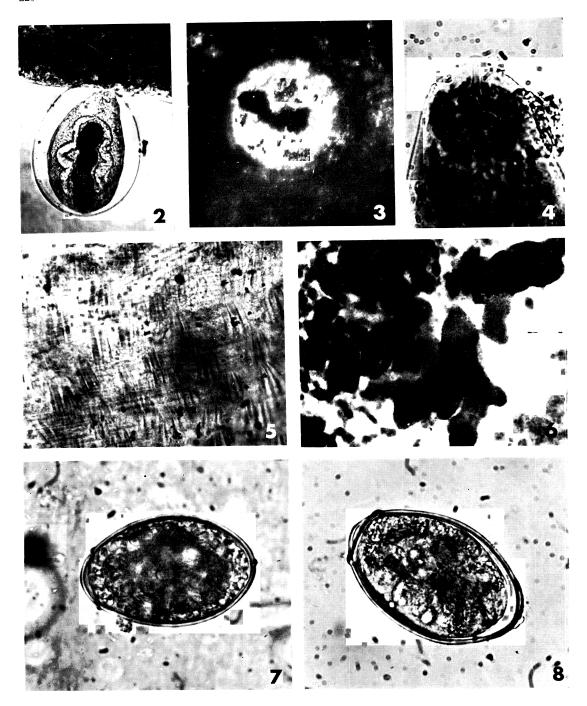
#### Summary

In September 1970, the authors found for the first time in Korea small metacercariae of *Paragonimus* in *Sesarma dehaani*, other than *P. westermani*, at the mouth of the river Nakdong, Pusan, Kyongsangnam Do. By the morphological study, this metacercaria was identified as those of *P. iloktsuenensis*. In May 1971, the authors also found the metacercariae of *P. iloktsuenensis* in *Helice tridens tridens* collected in the same area.

From the observations on the adult worms obtained from the rats experimentally infected with the metacercariae, it was proved difficult to differentiate the adult worm of *P. iloktsuenensis* from that of *P. ohirai*.

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Figs. 2-8 Paragonimus iloktsuenensis found in Korea.

- 2. Metacercaria, 3. Metacercaria in liver of crab, 4. Stylet of Metacercaria,
- 5. Cuticular spines "arranged in group",6. Ovarium "confusely branched",7. Egg in feces of rat (40 days after infection),
- 8. Egg of P. ohirai in feces of rat (40 days after infection).

# 韓国においてはじめて見出された小形大平肺吸虫(Paragonimus iloktsuenensis)について

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著者らは1970年9月,韓国慶尚南道釜山近郊の洛東口河口の Hadan (下端地区) のクロベンケイ Sesarma dehaani, アシハラガニ Helice tridence tridence およびアカテガニ Sesarma haematocheir を採集し, その肝臓を調べたところ,26匹のクロベンケイ中16匹(61.5%) の肝臓に小形の肺吸虫メタセルカリアが見出された. その形態および大きさから,これを小形大平肺吸虫のメタセルカリアと同定した.また,このメタセルカリ

アを2匹のラットに餌食させ、40日後に4虫の成虫を得た.その形態的特徴として皮棘の群生および卵巣の複雑な分岐がみられ、いわゆる小形-大平肺吸虫型を示していた.虫卵の形状は大平肺吸虫卵のそれと極めて類似しており、大きさは大平肺吸虫卵のそれよりやや小さかつた.

なお、その後同地域で採集したアシハラガニよりも本 種メタセルカリアが証明された.