

## Ecological Survey on the Lung Flukes, *Paragonimus iloktsuenensis* and *Paragonimus ohirai* Metacercariae in Ibi, Nagara and Kiso Rivers in Central Japan

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(Accepted December 27, 1995)

### Abstract

A survey for detection of *Paragonimus iloktsuenensis* and *Paragonimus ohirai* metacercariae from the brackish water crab, *Sesarma dehaani*, was carried out at 14 sites about 6-10 km upstream of the estuaries of the Ibi, Nagara and Kiso rivers flowing into the Ise Bay in central Japan in 1988. *P. iloktsuenensis* metacercariae were found from crabs collected from 12 sites, no metacercaria being detected at the two sites of uppermost stream along the Ibi and Kiso rivers, respectively. The infection rates were as low as 2.6-42.5%, the average infection rate being 13.9%. The number of *P. iloktsuenensis* metacercariae per *P. iloktsuenensis* positive crab ranged from 1 to 10, the average number being 1.7. In contrast, *P. ohirai* metacercariae were found from all the sites and the infection rates varied between 22.6% and 97.5%. The number of *P. ohirai* metacercariae per *P. ohirai* positive crab ranged from 1 to 415, the average number being 20.8. There was a general trend in the survey area that *P. iloktsuenensis* metacercariae were found where *P. ohirai* infection rates were higher. This is the first record that *P. iloktsuenensis* metacercariae were found from crabs in these three rivers in Aichi and Mie Prefectures of central Japan.

**Key words:** lung fluke; *Paragonimus iloktsuenensis*; *Paragonimus ohirai*; general distribution.

### Introduction

Two species of the lung flukes, *Paragonimus iloktsuenensis* and *Paragonimus ohirai* occurring in brackish water estuaries, whose 1st and 2nd intermediate hosts are *Assiminea* snails and *Sesarma* crabs, respectively, and definitive hosts are mainly rodents. *P. iloktsuenensis* metacercaria can be differentiated from that of *P. ohirai* by the existence of an outer layer only, while *P. ohirai* has both an outer and inner layers.

*P. iloktsuenensis* has been recorded from Amami-Oshima Island and along the Sendai river in Kagoshima Prefecture, the Kako river in Hyogo Prefecture and the Shinyodo river in Osaka Prefecture, and *P. ohirai* has been found in Kagoshima,

Miyazaki, Kumamoto, Kochi, Hyogo, Kyoto, Mie, Aichi, Ishikawa, Shizuoka and Chiba Prefectures in Japan (Yoshida, 1991).

Our previous report (Matsuo and Makiya, 1985) revealed that infected crabs with *P. ohirai* metacercariae were widely distributed in the estuaries of the Ibi, Nagara, Kiso and Shin rivers which flow into the Ise Bay through the border area between Mie and Aichi Prefectures in the Tokai district, central Japan. This report, however, dealt only with *P. ohirai* metacercariae having clearly the inner layer.

The present report deals with the infection rate of *P. iloktsuenensis* metacercaria including *P. ohirai* metacercaria in the brackish water crabs, *Sesarma dehaani* collected from the estuaries of the Ibi, Nagara and Kiso rivers in Tokai district from April to October, 1988. This is the first record of *P. iloktsuenensis* in this area.

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### Survey Areas and Methods

The 2nd intermediate host crabs, *Sesarma dehaani*, were collected from 14 sites about 6–10 km upstream of the estuaries of the Ibi, Nagara and Kiso rivers flowing into the Ise Bay in the border area between Aichi and Mie Prefectures, central Japan (Fig. 1), from April to October, 1988.

After measuring the maximum carapace width of the collected crabs, their livers were removed and placed in a small amount of physiological saline in a petri dish. Metacercariae were pipetted from the dish after the livers had been torn into pieces very carefully with a fine dissecting needle under a stereomicroscope.

### Results and Discussion

#### General features of the metacercarial infection

Fig. 2 shows photographs of *P. iloktsuenensis* and *P. ohirai* metacercariae taken out of the crabs collected from the Kiso river. When the metacercariae of these two species are separated from liver, the outer layers are easily taken off. A in Fig. 2 shows *P. iloktsuenensis* metacercaria having the outer layer, and B shows *P. iloktsuenensis* active metacercaria which is freed after the outer layer was taken off. C shows *P. ohirai* resting metacercaria having the

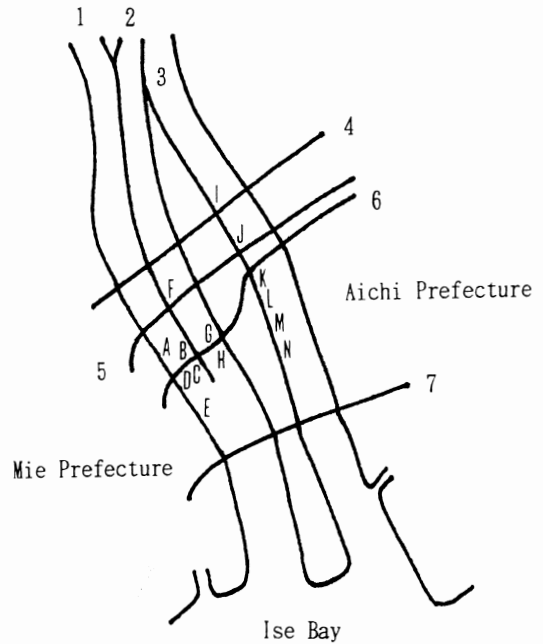


Fig. 1 Sketch map showing the collection sites of crab hosts

Collection sites:

(A)–(E) along Ibi river (1)

(F)–(H) along Nagara river (2)

(I)–(N) along Kiso river (3)

Roads: (4) Higashi Meihan Expressway, (6) Highway No. 1, (7) Highway No. 23

Railway: (5) JR Kansai Line

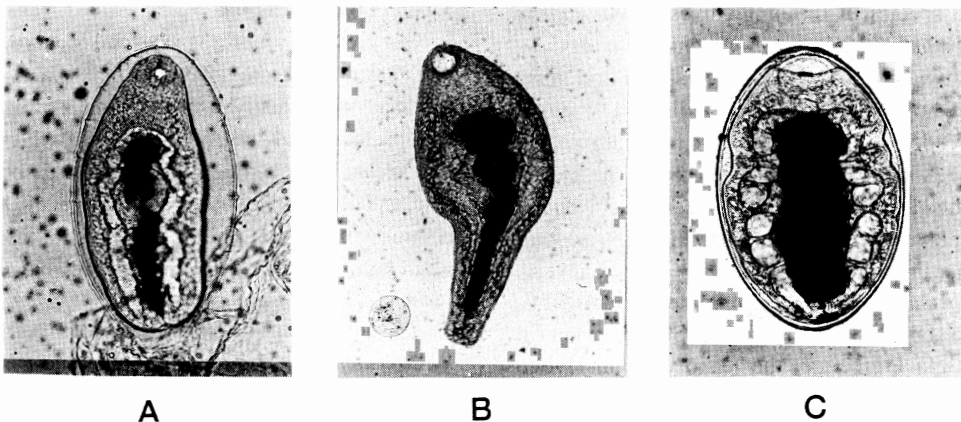


Fig. 2 Metacercariae detected from the liver of crabs collected from the Kiso river.

A: *P. iloktsuenensis* metacercaria having the outer layer

B: *P. iloktsuenensis* metacercaria not having the outer layer

C: *P. ohirai* metacercaria having the thick inner layer

thick inner layer.

A total of 793 crabs was dissected and metacercariae were recovered from 607 of them. Of these 607 crabs, 497 were infected with only *P. ohirai* metacercariae, 109 crabs concomitantly infected with both *P. ohirai* and *P. iloktsuenensis* metacercariae; while only one crab harbored *P. iloktsuenensis* metacercaria alone.

The number of metacercariae found in the crabs is summarized in Table 1 by species and collection sites (A-N). *P. iloktsuenensis* and *P. ohirai* metacercariae were found in 110 crabs and 606 crabs, respectively. The infection rates of *P. iloktsuenensis* and *P. ohirai* were thus 13.9% and 76.4%, respectively.

A total of 190 *P. iloktsuenensis* metacercariae was found. The number of *P. iloktsuenensis* metacercariae per *P. iloktsuenensis* positive crab ranged from 1 to 10 with the average number of 1.7 per positive crab. The variance/mean ratio ( $s^2/x$ ) was calculated to be 2.49 ( $=0.5966/0.2396$ ), being far larger than one. This indicated an aggregated (overdispersed) distribution of metacercariae among the crabs. As shown in Fig. 3A, the negative binomial (dispersion index  $k=0.1694$ ) was well fitted to the metacercarial distribution among the all crabs ( $X^2=3.707$ ,  $df=2$ ,  $P=0.16>0.05$ ), indicating that the metacercarial infection is highly aggregated in the crab host population.

A total of 12,596 *P. ohirai* metacercariae was

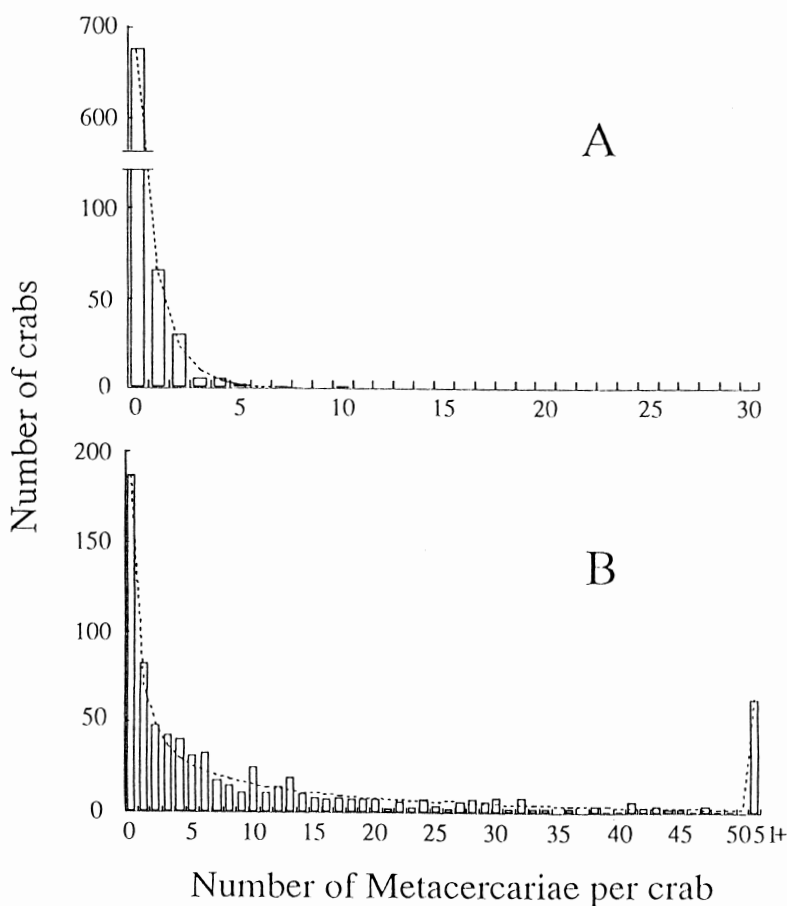


Fig. 3 Frequency distribution of *P. iloktsuenensis* (A) and *P. ohirai* (B) metacercariae in crab host population with fitted curves of the negative binomial.

found. The number of *P. ohirai* metacercariae per *P. ohirai* positive crab ranged from 1 to 415 with the average number of 20.8 per positive crab. The variance/mean ratio (1104.8/15.88=69.57) and good fit to the negative binomial (Fig. 3A;  $k=0.3862$ ,  $X^2=38.46$ ,  $df=32$ ,  $P=0.20>0.05$ ) also suggested an overdispersed distribution of metacercariae.

To summarize, the occurrence of *P. iloktsuenensis* metacercaria with an outer layer but no inner layer was found for the first time in the estuaries of the Ibi, Nagara and Kiso rivers in Aichi and Mie Prefectures, but the infection rate and the number of metacercariae per host crab were not so high.

#### Details of the infection rates at each distribution site

The followings are detailed explanations of metacercarial distribution at collection sites of the three rivers as shown in Table 1.

*Ibi river*: Crab hosts were collected from 5 sites

(A, B, C, D and E in Fig. 1). Site A is on the right bank of the uppermost stream, which is nearly midway between the bridge of the Kansai Line of the Japanese Railway (JR) and the Ise Ohashi Bridge of Highway No. 1. Sites C and D are on the both side of the Ibi river near the Ise Ohashi Bridge of Highway No. 1. Site E is on the downmost stream, being located downstream from the Ise Ohashi Bridge of Highway No. 1. Among these sites, Sites C and D are near the Sites E and F in the previous report, respectively.

No *P. iloktsuenensis* metacercaria was found in crab hosts at Site A. Both *P. iloktsuenensis* and *P. ohirai* metacercariae were found at the other four sites (B, C, D and E).

The infection rate of *P. iloktsuenensis* metacercaria was very low (2.6–20.3%), the maximum infection rate being at Site C. The number of *P. iloktsuenensis* metacercariae per *P. iloktsuenensis* positive crab ranged from 1 to 4 with the maximum

Table 1 Infection rate of crab hosts, *Sesarma dehaani*, with *Paragonimus iloktsuenensis* and *Paragonimus ohirai* metacercariae collected along Ibi, Nagara and Kiso rivers in 1988

Site*	Date of survey	No. of crabs examined	<i>P. iloktsuenensis</i> metacercaria			<i>P. ohirai</i> metacercaria		
			No. of positive crabs (%)	Total	Average no. per positive crab (range)	No. of positive crabs (%)	Total	Average no. per positive crab (range)
A	Oct. 26	80	0			33 (41.3)	109	3.3 (1–13)
B	Aug. 3	50	7 (14.0)	9	1.3 (1–2)	36 (72.0)	680	18.9 (1–110)
C	Jul. 6	59	12 (20.3)	19	1.6 (1–4)	50 (84.7)	1165	23.3 (1–136)
D	Oct. 26	38	1 (2.6)	2	2.0 (2)	30 (78.9)	195	6.5 (1–28)
E	Oct. 5	56	9 (16.1)	11	1.2 (1–3)	52 (92.9)	713	13.5 (1–59)
F	Aug. 24	65	3 (4.6)	3	1.0 (1)	46 (70.8)	357	7.8 (1–52)
G	Sep. 8	50	7 (14.0)	17	2.1 (1–4)	41 (82.0)	482	11.8 (1–44)
H	Sep. 8	92	33 (35.9)	68	3.4 (1–10)	78 (84.8)	876	11.2 (1–109)
I	Aug. 24	35	0			8 (22.9)	43	5.4 (1–16)
J	Jul. 6	59	6 (10.2)	11	1.8 (1–3)	50 (84.7)	1450	29.0 (1–159)
K	Aug. 24	44	5 (11.4)	5	1.0 (1)	36 (81.8)	1493	41.5 (1–415)
L	Jul. 6	40	17 (42.5)	36	2.1 (1–7)	39 (97.5)	2872	73.6 (3–272)
M	Apr. 6	30	2 (6.7)	2	1.0 (1)	24 (80.0)	153	6.4 (1–27)
N	Apr. 6	35	1 (2.9)	1	1.0 (1)	31 (88.6)	701	22.6 (1–105)
N	Aug. 3	60	6 (10.0)	6	1.0 (1)	52 (86.7)	1307	25.1 (1–138)
Total		793	110 (13.9)	190	1.7 (1–10)	606 (76.4)	12596	20.8 (1–415)

\*collection sites A–N are indicated on the sketch map in Fig. 1  
A–E: Ibi river, F–H: Nagara river, I–N: Kiso river

at Site C. The average number per positive crab ranged from 1.2 to 2.0 with the maximum average number at Site D.

In contrast, the infection rate of *P. ohirai* metacercaria varied between 41.3% and 92.9%, the maximum infection rate being at Site E. The average number of *P. ohirai* metacercariae per *P. ohirai* positive crab ranged from 3.3 to 23.3 with the maximum average number at Site C. The number of *P. ohirai* metacercariae per positive crab ranged from 1 to 136, the maximum number being at Site C, where the infection rate of *P. iloktsuenensis* metacercaria was highest among all the sites along the Ibi river. The lowest infection rate of *P. ohirai* metacercaria was 41.3% at Site A, where no *P. iloktsuenensis* metacercaria was found as stated above. In the previous report, the infection rates of *P. ohirai* metacercaria at nearby Sites C and D were 56.4% and 90.0% in *Sesama dehaani*, and 88.9% and 100% in *S.intermedia*, respectively.

*Nagara river*: Crab hosts were collected from 3 sites (F, G and H in Fig. 1) along this river. Site F is on the right bank near the bridge of the JR Kansai Line, and Sites G and H are on the left bank of up and downstream from the Ise Ohashi Bridge of Highway No. 1, respectively. The Site G is near to the Site H in the previous report.

Both of *P. iloktsuenensis* and *P. ohirai* metacercariae were found from crabs at all the sites along this river.

The infection rates of *P. iloktsuenensis* metacercaria were 4.6%, 14.0% and 35.9% at Sites F, G and H, respectively, the maximum infection rate being at Site H. The number of *P. iloktsuenensis* metacercariae per *P. iloktsuenensis* positive crab ranged from 1 to 10 with the average number of 1.0–3.4 per positive crab. The maximum number and the maximum average number per positive crab were also highest at Site H. One crab at Site H harboured only *P. iloktsuenensis* metacercaria.

In contrast, the infection rates of *P. ohirai* metacercaria were 70.8%, 82.0% and 84.8% at Sites F, G and H, respectively. The number of *P. ohirai* metacercariae per *P. ohirai* positive crab ranged from 1 to 109, the maximum number per positive crab being at Site H. The average number of *P. ohirai* metacercariae per positive crab ranged from 7.8 to 11.8. In the previous report, the infection rate

of *P. ohirai* metacercaria near Site G was also high; 70.8% in *Sesarma dehaani* and 57.1% in *S. intermedia*, respectively.

*Kiso river*: Crab hosts were collected from 6 sites along the river including a further upstream area than along the two other rivers. The uppermost Site I is on the right bank at some 10 km up from the estuary and close to the Kisogawa Bridge of the Higashi Meihan Motorway. The downmost Site N is nearly midway between the Kisogawa Bridge of Highway No. 23 and the Owari Bridge of Highway No. 1 (some 6 km upstream of the estuary). Among these six sites, the Sites I, J, M and N are near to Sites L, N, P and R, respectively, in the previous report.

No *P. iloktsuenensis* metacercaria was found in crab hosts at Site I. Both *P. iloktsuenensis* and *P. ohirai* metacercariae were found from the other five sites (J, K, L, M and N).

The infection rates of *P. iloktsuenensis* metacercaria varied between 2.9% and 42.5%. The maximum infection rate at Site L was 42.5%, being the highest in the present report. The number of *P. iloktsuenensis* metacercariae per *P. iloktsuenensis* positive crab ranged from 1 to 7 with the maximum number at Site L. The average number per positive crab ranged from 1.0 to 2.1, the maximum average number being also at Site L. The infection rates and the number of metacercariae per *P. iloktsuenensis* positive crab were as low as in the Ibi and Nagara rivers.

In contrast, the infection rates of crab with *P. ohirai* metacercaria varied between 22.9% and 97.5%. The maximum infection rate was 97.5% at Site L, being the highest among all the sites in this report, where the *P. iloktsuenensis* infection rate was also highest (42.5%). The minimum infection rate of *P. ohirai* metacercaria was 22.9% at Site I, being the lowest in the present survey, where no *P. iloktsuenensis* metacercaria was found as stated above. In the previous report, the infection rate of *P. ohirai* metacercaria near the present Site I was also low; 33.3% in *Sesarma dehaani* and 9.1% in *S. intermedia*, respectively. The number of *P. ohirai* metacercariae per *P. ohirai* positive crab ranged from 1 to 415 with the maximum number at Site K. The average number of *P. ohirai* metacercariae per positive crab ranged from 5.4 to 73.6. The maximum average number per positive crab was 73.6 at Site L.

being highest in the present report.

To summarize the results in Table 1, *P. iloktsuenensis* metacercariae were found at 12 sites along the three rivers, but no metacercaria was found from the uppermost Sites A and I along the Ibi and Kiso rivers, respectively. The infection rates of *P. iloktsuenensis* metacercaria were less than 42.5%, the average infection rate being 13.9%. The number of *P. iloktsuenensis* metacercariae per *P. iloktsuenensis* positive crab ranged from 1 to 7 with the average number of 1.7 per positive crab. In contrast, *P. ohirai* metacercariae were found at all the 14 sites and the infection rates varied between 22.6% and 97.5% with the average infection rate of 76.4%. The infection rates of *P. ohirai* metacercaria at the two uppermost sites were lowest. The number of *P. ohirai* metacercariae per *P. ohirai* positive crab ranged from 1 to 415 with the average number of

20.8 per positive crab. There was a general trend that the *P. iloktsuenensis* infection rate was higher where the *P. ohirai* infection rate was higher.

#### References

- 1) Matsuo, K. and Makiya, K. (1985): Ecological studies on the lung fluke, *Paragonimus ohirai* Miyazaki, 1939. 1. Infection rate with *P. ohirai* metacercariae of brackish water crabs collected from the six rivers in Tokai district, central Japan. Jpn. J. Trop. Med. Hyg., 13, 307–313.
- 2) Miyazaki, I. (1944): On the distribution of lung fluke, *Paragonimus ohirai* (1). Medicine and Biology, 6, 23–26 (in Japanese).
- 3) Yoshida, Y., Matsuo, K. and Nakanishi, Y. (1958): On the distribution of *Paragonimus ohirai* in Mie and Aichi Prefectures. Medicine and Biology, 49, 1–4 (in Japanese).
- 4) Yoshida, Y. (1991): Illustrated Human Parasitology. Nanzando, Tokyo, 152–153 (in Japanese).