Research Note

## Survey for the Second Intermediate Host of *Paragonimus ohirai* Miyazaki, 1939, in Kujukuri, Chiba Prefecture

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It is known that *Paragonimus ohirai*, murine lung fluke, is distributed in the southern half of Japan (Miyazaki, 1961). In Kanto district, since Yokogawa *et al.* (1958) reported the endemic area of *P. ohirai* in Ichinomiya river, no intensive survey for *P. ohirai* has yet been conducted in Chiba Prefecture. This report deals with the survey for the second intermediate host crabs of *P. ohirai* collected from six rivers in Chiba Prefecture, which drain into the Pacific Ocean (Kujukuri beach).

The survey was conducted on the estuary areas of six rivers, the Kido, Sakuta, Maki, Nabaki, Ichinomiya and Isumi rivers during May 1981 to March 1987 (Fig. 1). Three species of brackish water crabs, Sesarma dehaani, S. intemedia and Helice tridens tridens, were collected from these rivers. All crabs were individually examined for metacercariae by Tuda's method (1959). The metacercariae were orally inoculated into Wistar rats. The adult worms recovered from the lung cyst were pressed, fixed in Carnoy's solution and stained

with carmine for morphological observations.

The brackish water crabs were found in all the rivers examined. As shown in Table 1, *P. ohirai* metacercariae were detected in *S. dehaani* and *H. t. tridens* collected from the Kido, Sakuta, Maki and Isumi rivers. The crabs collected from these four rivers showed a high level of *P. ohirai* infection. The infection rates of the metacercariae in *S. dehaani* were 81.8% in the Kido river, 74.9% in the Maki river, 60.9% in the Sakuta river and 53.3% in the Isumi river. However, crabs collected in the Ichinomiya river and the Nabaki river were not infected with *P. ohirai* metacercariae. The majority of *P. ohirai* metacercariae were de-



Fig. 1 Map of survey areas in Kujukuri beach, Chiba Prefecture.

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Table 1 Incidence of metacercarial infection of *P. ohirai* in crabs collected from various rivers in Kujukuri beach, Chiba Prefecture

			No. of crabs		Mean No.	
Locality	Date	Crabs	Examined	Infected (%)	metacercariae per infected crab	
Kido river	Nov. 1982-May 1983	Sesarma dehaani	33	27 (81.8)	27.5	
		Helice tridens tridens	16	12(75.0)	6.6	
Sakuta river	Nov. 1982-May 1983	S. dehaani	41	25(60.9)	12.4	
		H. t. tridens	6	2(33.3)	2.5	
Maki river	May 1981-Mar. 1987	S. dehaani	366	274(74.9)	19.5	
		H. t. tridens	21	11(52.4)	5.5	
Nabaki river	Sept. 1983	S. dehaani	16	0	0	
		S. intermedia	36	0	0	
Ichinomiya river	Oct. 1986	S. dehaani	41	0	0	
		S. intermedia	2	0	0	
		H. t. tridens	81	0	0 .	
Isumi river	Nov. 1982	S. dehaani	15	8(53.3)	4.9	

tected in S. dehaani. However, the metacercariae were found also in H. t. tridens although the rate was not higher than those of S. dehaani (Table 1). None of P. ohirai metacercariae was found in S. intermedia which was collected very rarely in these areas. The average numbers of metacercariae in positive S. dehaani were high in crabs collected from the Kido river (27.5 metacercariae/crab) and Maki river (19.5 metacercariae/crab). Twenty crabs of S. dehaani and twenty one of H. t. tridens collected in the Maki river were examined for the distribution of metacercariae in the body. Although some of the metacercariae were observed in the muscles and viscera, most of them were detected in the liver in both crabs (Table 2). No significant difference was observed in the infection rate between the sexes and sizes of crabs, although the average number of metacercariae per crab was higher in larger crabs than in smaller crabs (Table 3). These results are consistent with those reported by Yoshida *et al.* (1959) and Matsuo and Makiya (1985).

The metacercariae containing pinkish granules were enveloped with 2 layers of cyst walls which were ellipsoidal in shape. The average size of the inner cyst wall of the metacercaria was  $294 \ (240 - 360) \, \mu \text{m}$  in length and  $203 \ (185 - 230) \, \mu \text{m}$  in width. The measurements of adult worms recovered from the lung cyst of infected rats were 9.9 mm in length and 4.8 mm in width. The size of the ventral sucker was the same as or slightly larger than that of the oral sucker. The ovary was delicately branched and

Table 2 Number and location of the metacercariae of *P. ohirai* found in crabs collected at the Maki river

	No. of crabs	crabs No. of metacercariae found in				
	examined	Gill	Liver	Viscera	Muscles	Total
Sesarma dehaani	20	0	192(94.1)*	1(0.4)*	11(5.4)*	204
Helice tridens triden	s 21	0	60(90.9)*	0	6(9.1)*	66

by Tsuda's method (1959)

<sup>( )\*</sup> percentage

Cinou	riciocuio		
Breadth of carapace (mm)	No. o	of crabs	Mean No. of metacercariae
	Examined Infected (%) per inf		per infected crab
<20	28	20 (71.4)	12.2
21 25	112	84 (75.0)	17.4
26 30	113	86 (76.1)	17.4
31 35	59	51 (86.4)	20.6
36<	9	7 (77.8)	35. 4
Total	321	248 (77.3)	18. 2

Table 3 Relationship between incidence of metacercarial infection of *Paragonimus ohirai* and size of *Sesarma dehaani* collected at the Maki river, Chiba Prefecture

cuticular spines were arranged in groups. On the basis of the morphological observations of the adult worm, metacercaria and egg, the lung fluke from Kujukuri district was identified to be *P. ohirai*.

The present study showed the new *P. ohirai* distributions in Kujukuri beach, Chiba Prefecture. However, *P. ohirai* metacercaria was not found in crabs collected from the Ichinomiya river, in which Yokogawa *et al.* (1958) had found only one *P. ohirai* metacercaria in one out of 202 *H. t. tridens*. It seems likely that at present no *P. ohirai* can be found in the Ichinomiya river, and this may be caused by the construction of breakwaters along the river and by environmental changes of the areas.

On the other hand, Yokogawa et al. (1958) found the *P. ohirai* infection in 3 out of 168 weasels captured in environs of Mobara city which was situated up the Ichinomiya river. In the present study, we also found the *P. ohirai* infection in a weasel caught in the Maki river. Thirty six *P. ohirai* adult worms were collected from the lung cysts of the weasel. This finding suggests that the weasel is one of the final hosts of *P. ohirai* in these areas.

Ohishi and Saito (1952) reported the *P. ohirai* infection in pigs in Ibaraki Prefecture. In this prefecture, however, no *P. ohirai* metacercaria has yet been found. *P. ohirai* is considered to be distributed in surrounding areas of Kujukuri beach, probably up to southeastern parts of Ibaraki Prefecture.

It is known that the major first intermediate host of P. hirai is Assiminea parasitologica

(Yokogawa, 1965). In the present study, A. parasitologica was found in the Kido river, although P. ohirai cercariae were not found in despite of careful examinations. Since the crabs infected with P. ohirai were detected in these areas, demonstration of the infected snails could be anticipated.

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