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Geographical Distribution of the Lung Fluke, *Paragonimus miyazakii* in the South-western Part of Shimane Prefecture, Japan

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Abstract

In order to clarify the existence of localization of distribution of the lung fluke, *Paragonimus miyazakii* Kamo *et al.*, 1961 based on the hypothesis proposed by Nishida and Sakai (1978), a total of 1,328 freshwater crabs, *Geothelphusa dehaani,* were collected at 31 locations in mountainous areas of Masuda City and Mino-gun, Shimane Prefecture, between August 1988 and July 1990, and were examined for *Paragonimus* metacercariae. As a result of the survey, 644 metacercariae were obtained from 264 crabs collected at 19 locations (10 in Masuda City, 8 in Hikimi-cho and 1 in Mito-cho, Mino-gun). These metacercariae were identified as *P. miyazakii* on the basis of the morphological features of adult worms obtained by the experimental infection in a cat and rats. Although the hypothesis proposed by Nishida and Sakai (1978) is mostly reliable, some extensions of areas are known to exist (Nishida *et al.*, 1988). The new habitat of *P. miyazakii* described here was also somewhat non-applicable to the original hypothesis proposed by Nishida and Sakai (1978), but the extension from the area shown in the hypothesis almost agree with it.

Key words: lung fluke, *Paragonimus miyazakii,* geographical distribution

Introduction

It is well-known that *Paragonimus miyazakii* is widely distributed in the western Japan. Nishida and Sakai (1978) proposed a hypothesis that *P. miyazakii* is distributed in areas where there had been marine transgression since the Late Miocene. Because *Bythinella nipponica,* the first intermediate snail host of *P. miyazakii* is restrictedly distributed only in those areas and the geographical distribution of *P. miyazakii* agrees well with the areas. Thereafter, they added a lot of informations in that regard and demonstrated that most of them corresponded well with the hypothesis (Nishida *et al.*, 1988). There are, however, a few non-applicable areas to the hypothesis, for example, a few habitats are found outside the areas which had not undergone marine transgression. This study was therefore carried out to know the extent of the area in regard to the distribution of *P. miyazakii* in Shimane Prefecture beyond the area reported in the hypothesis of Nishida and Sakai (1978).

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Materials and Methods

Detection of Parasite and experimental infection in the animal hosts

A total of 1,328 freshwater crabs, *Geothelphusa dehaani,* the second intermediate host of *Paragonimus,* were collected from mountain streams at 31 locations in Masuda City and Mino-gun in the southwestern part of Shimane Prefecture between August 1988 and July 1990, as shown in Table 1 and Fig. 1. These crabs were crushed, digested for 6 to 12 hours at 37°C

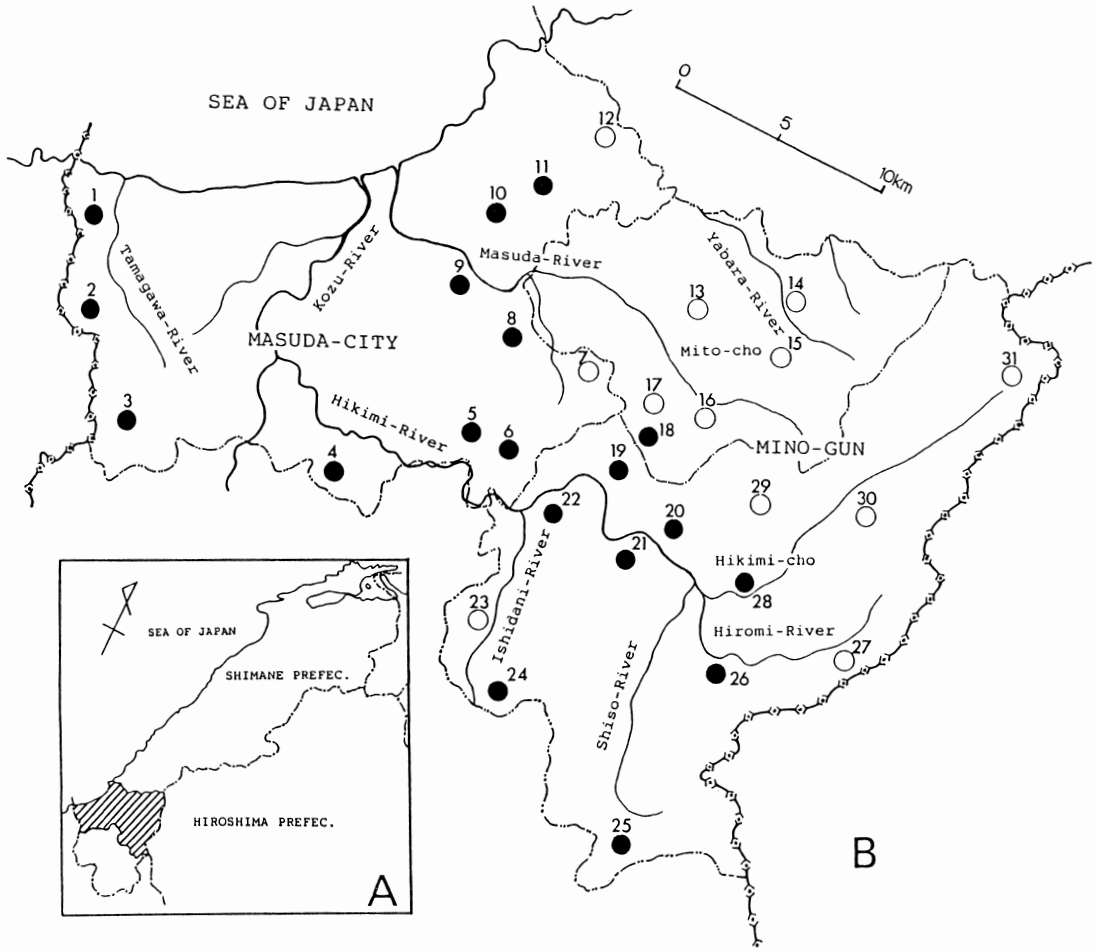


Fig. 1 A) Map showing the location where the present survey was carried out.
B) Map showing 31 localities in Masuda City and Mino-gun, Shimane Prefecture where crabs, *Geothelphusa dehaani,* were collected in the present survey.
●: positive for *Paragonimus* metacercariae. ○: negative

in artificial gastric juice and examined for the metacercariae. The metacercariae recovered were orally administered to a cat (female, 2.9kg) and 13 rats (Wistar-strain, 10 male and 3 female, 210–520g); one animal receiving the metacercariae from one locality only.

Recovery of parasite

After anesthesia was induced, the animals were bled to death and were autopsied between 71 and 165 days after administration. The investigation for worms was made in the pleural and peritoneal cavities and visceral organs by gross examination only.

Worms and eggs

The recovered worms were compressed and flattened between two slide glasses, stained with carmine and mounted in balsam, and were used for morphological observations and measurements. Eggs removed from the worm cysts in the lungs were also used for morphological observations and measurements after being preserved in 10% formalin.

Results

Prevalence of Paragonimus metacercariae in crabs

As shown in Table 1 and Fig. 1, a total of 1,328 crabs were collected from 31 different locations in Masuda City and Mino-gun, Shimane Prefecture. A total of 264 crabs, 138 of which were collected at 10 locations in Masuda City, 1 collected at one location in Mito-cho, Mino-gun, and 125 collected at 8 locations in Hikimi-cho, Mino-gun, were positive for *Paragonimus* metacercariae and a total of 644 metacercariae were obtained. The infection rate for each location varied from 1.6 to 74.5% (average 19.9%). The number of metacercariae per positive crab ranged from 1 to 32 (average 2.4).

Table 1 Prevalence of infection of crabs, *Geothelphusa dehaani*, with metacercariae (Mc) of *Paragonimus miyazakii* in Masuda City and Mino-gun of Shimane Prefecture

Locality		No.*	Date of survey	No. of crabs examined	No. of crabs infected	Infection rate (%)	Average No. of Mc in a positive crab (Min.–Max.)
Masuda City	Futami	[1]	August 1989	37	1	2.9	6.0
	Kouchi	[2]	August 1989	50	19	38.0	2.2 (1– 6)
	Wanagatabara	[3]	August 1989	50	18	36.0	1.3 (1– 3)
	Hachigase	[4]	August 1989	36	24	66.7	1.5 (1– 4)
	Kakihara	[5]	August 1989	26	10	38.5	1.4 (1– 3)
	Kaminagasawa	[6]	August 1989	47	32	68.1	2.3 (1– 8)
	Hara	[7]	August 1989	63	0		
	Ooyakata	[8]	August 1989	44	5	11.4	2.4 (1– 6)
	Yokono	[9]	August 1989	37	21	56.8	5.1 (1–32)
	Otoko-nakagumi	[10]	July 1990	55	6	10.9	2.2 (1– 6)
	Yamaori	[11]	July 1990	60	2	3.3	1.5 (1– 2)
	Noji	[12]	July 1990	61	0		
Mino-gun							
Mito-cho	Kubara-nakagou	[13]	July 1990	53	0		
	Nagahashi	[14]	July 1990	16	0		
	Tabara	[15]	July 1990	37	0		
	Sagadani	[16]	August 1989	46	0		
	Fukaori	[17]	August 1988 ~July 1990	15	0		
	Kanedani	[18]	July 1990	63	1	1.6	1.0
Mino-gun							
Hikimi-cho	Noto	[19]	August 1989	31	13	41.9	1.3 (1– 3)
	Wamata	[20]	August 1988	51	27	52.9	3.4 (1–12)
	Takenohara	[21]	August 1988	55	41	74.5	2.0 (1– 5)
	Taniguchi	[22]	August 1988	33	14	42.4	3.1 (1–11)
	Ishihara	[23]	August 1989	28	0		
	Kamiutsudani	[24]	August 1989	35	1	2.9	1.0
	Mikazura	[25]	August 1988	37	19	51.4	2.9 (1–31)
	Yamane-kami	[26]	August 1988	41	2	4.9	1.0
	Hiromi	[27]	August 1988	42	0		
	Hagihara	[28]	August 1988	43	8	18.6	2.5 (1– 8)
	Yao	[29]	August 1988	51	0		
	Shimomichikawa	[30]	August 1988	49	0		
	Usukidani	[31]	August 1988	36	0		
Total				1,328	264	19.9	2.4 (1–32)

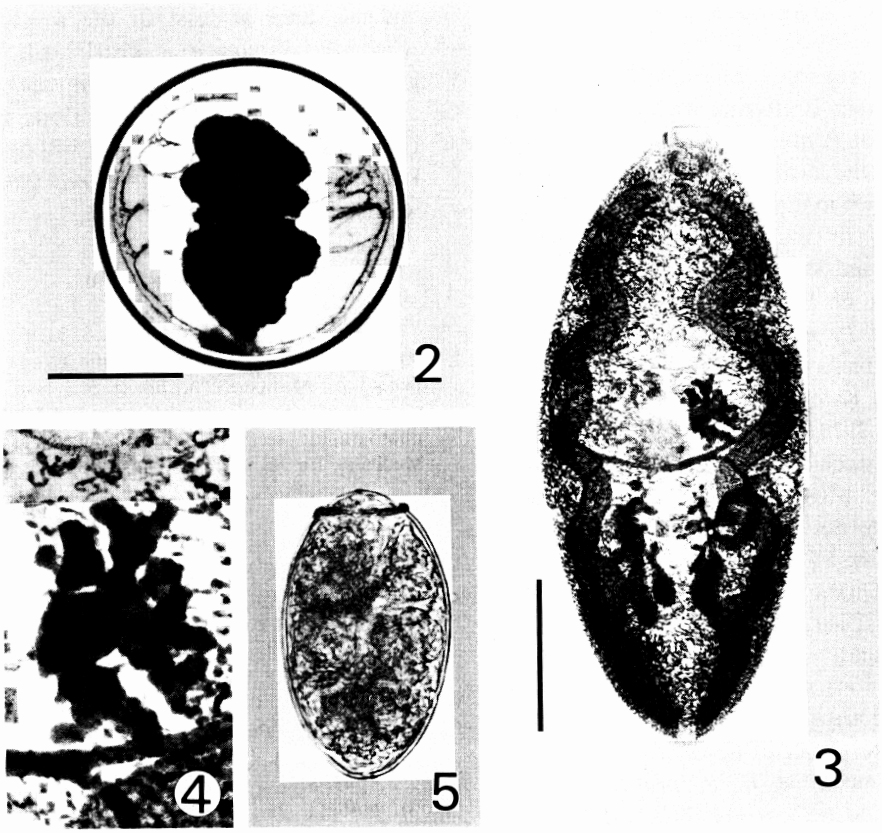
* The Nos. are indicated in Fig. 1.

Morphology of metacercariae

The metacercariae obtained were spherical in shape, as shown in Fig. 2. The capsules enveloping the larvae consisted of two layers, a thin outer and a comparatively thick inner cyst wall. The larvae contracted in the cyst and had an I-shaped excretory bladder filled with blackish granules. The metacercariae obtained from each location were morphologically similar to each other. The metacercariae from some locations were almost of the same sizes. The mean size was $458.7 \pm 31.4\,\mu$m in length and $448.4 \pm 31.9\,\mu$m in width. The mean thickness of inner cyst wall was $26.6 \pm 5.5\,\mu$m.

Morphology of the adult worms and their eggs

As the results of experimental infection, cyst-formation was observed in the lungs of each animal. In a cat, a total of 39 worms (recovery rate: 78.0%) were recovered on day 80 after administration and 29 of which were from the cysts in lungs. Most of the worms from the lungs were adults with eggs in their uteri. On the other hand, in rats, a total of 63 worms (recovery rate: 48.1%) were recovered between 71 and 165 days after administration and 49 of which were from the cysts in lungs. Most of these flukes were in sexual maturity and had eggs in their uteri. All adult flukes recovered were slender in shape (Fig. 3) and had singly spaced cuticular spines



Figs. 2–5 *Paragonimus miyazakii* – 2: A metacercaria obtained from freshwater crab, *Geothelphusa dehaani,* collected in Hikimi-cho, Mino-gun. Scale: 200 μm. 3: A mounted adult specimen from the lung cyst of the rat 75 days after administration with metacercariae. Scale: 2mm. 4: The ovary of the adult worm with a coral structure. 5: An egg from the lung cyst of the rat.

on the surface of the body. They also had coral-shaped ovary (Fig. 4). Oral sucker was slightly smaller than the ventral sucker. The testes were larger than the ovary and divided moderately.

Eggs from the cysts in lungs of the cat were oval in shape (Fig. 5). The maximum widths of eggs were at the middle point. No thickenings of the egg-shell at non-operculated and were observed. The mean size of 50 eggs was 71.8 ± 2.9 μm in length and 41.4 ± 1.8 μm in width.

The morphological features of the adult flukes, eggs and metacercariae mentioned above were identical to the description of *P. miyazakii* by Kamo *et al.* (1961). Therefore, all the flukes originating from the metacercariae we collected were finally identified as *P. miyazakii.*

Discussion

In the present study, Masuda City and Mino-gun in Shimane Prefecture were found to be a new habitat of *P. miyazakii.* However, it became evident that the locations are somewhat outside the area shown in the hypothesis of Nishida and Sakai (1978). In Chugoku District, Yamaguchi, Hiroshima, and Shimane Prefectures are known as a habitat of *P. miyazakii* but till now no existence of *P. miyazakii* had been reported from Tottori and Okayama Prefectures (Hatsushika, 1967; Kamo *et al.*, 1961; Nishida *et al.* 1978, 1985, 1988; Torii *et al.*, 1988). For that disparity, Nishida *et al.* (1988) gave the following explanation: Most of eastern two-third of Chugoku District had sunk under sea in the Middle to Late Miocine and was called the first Setonaikai. On the other hand, western one-third of Chugoku District had formed northern part of the old land.

The locations surveyed were the places adjacent to the areas where Torii *et al.* (1988) had found the prevalence of *P. miyazakii,* therefore it was assumed that *P. miyazakii* would be present around here before the present survey. As both places were outside the area shown in the hypothesis of Nishida *et al.* (1978) related to paleogeographical features, the continuity of prevalence is of absorbing interest, In the present survey, as shown in Fig. 1, it became clear that a line connecting Yamaori (No. 11) at northern part of Masuda City to Hagihara (No. 28) in Hikimi-cho via Kanedani (No. 18) at the southwestern part of Mito-cho almost restrict the distribution of *P. miyazakii.* However, the area is not so isolated from the area shown in the hypothesis by Nishida and Sakai (1978). The reason why such an extension of prevalence exists is as follows: This area is situated at the downstream of the river following through the endemic area and snails like *B. nipponica,* the first intermediate host of this fluke, might have moved and dispersed during the long period, and has resulted in some extension of endemic area.

Some scientists still claim the truth of the hypothesis of Nishida and Sakai (1978). However, although there are some extensions of endemic area at least in the western Japan where *B. nipponica* is distributed, the geographical distribution of *P. miyazakii* mostly agrees to the hypothesis. Therefore, the hypothesis is worthy of believing. It will be useful to know the distribution of *P. miyazakii* in the western Japan.

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