

Studies on the Lung Fluke, *Paragonimus westermani* -Diploid Type, in the Northern Part of Hyogo Prefecture, Japan
V. Geographical Distribution in Mikata-gun and Kinosaki-gun

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Key words: lung fluke, *Paragonimus westermani*, diploid type, geographical distribution

Introduction

It is known that the diploid type ($2n=22$) of *Paragonimus westermani* (Kerbert, 1878) is distributed in the Tohoku, Chubu, Kinki and Kyushu districts in Japan. However, recently, Yokogawa *et al.* (1984a, b) also found metacercariae of this species in freshwater crabs (*Geothelphusa dehaani*) captured in Chiba Prefecture and in mitten crabs (*Eriocheir japonicus*) captured in Okinawa Prefecture. Consequently, it became clear that the diploid type of *P. westermani* was distributed not only in those districts mentioned above, but also in the Kanto district and even in the Nansei Islands. The author has found a densely endemic area in Tajima province of Hyogo Prefecture in the Kinki district and has been investigating the geographical distribution, the morphological feature of each larval stage, development in the intermediate hosts and host-susceptibility in some kinds of mammals (Shibahara, 1981, 1982, 1983, 1984a, b, 1985). This paper describes the results of the investigation of the crabs, *G. dehaani*, collected in the mountain streams of Mikata-gun and Kinosaki-gun.

Materials and Methods

A total of 1,444 freshwater crabs (*Geothel-*

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phusa dehaani), 1,147 of which were collected at 23 localities in Mikata-gun and 297 at 9 localities in Kinosaki-gun of Hyogo Prefecture, mainly upstream in mountainous areas, during the period from July 1982 to May 1984, were examined for *Paragonimus* metacercariae (Fig. 1).

The crabs were crushed with small pincers and digested for 6 to 12 hours at 37°C in artificial gastric juice. The digested materials were filtered through mesh wire sieves. The sediments of the filtrate were washed repeatedly with water and examined for the metacercariae. They were found in crabs from three localities. The morphological features and size of the metacercariae of each locality were recorded and then they were orally administered to a dog (♀, 4.4kg) and two cats (♂, 1.9kg; ♀, 2.4kg), giving only the metacercariae from one locality to one animal.

The animals were autopsied from 93 to 100 days after infection. The worms removed from the animals were compressed and flattened to about 0.3 mm in thickness between two slide glasses, fixed in 70 % alcohol, stained with carmine, dehydrated, cleared in xylene and mounted in balsam, and were used for morphological observations and measurements.

The eggs removed from the worm cysts in the lungs were also used for morphological observations and measurements after being preserved in 10 % formalin.

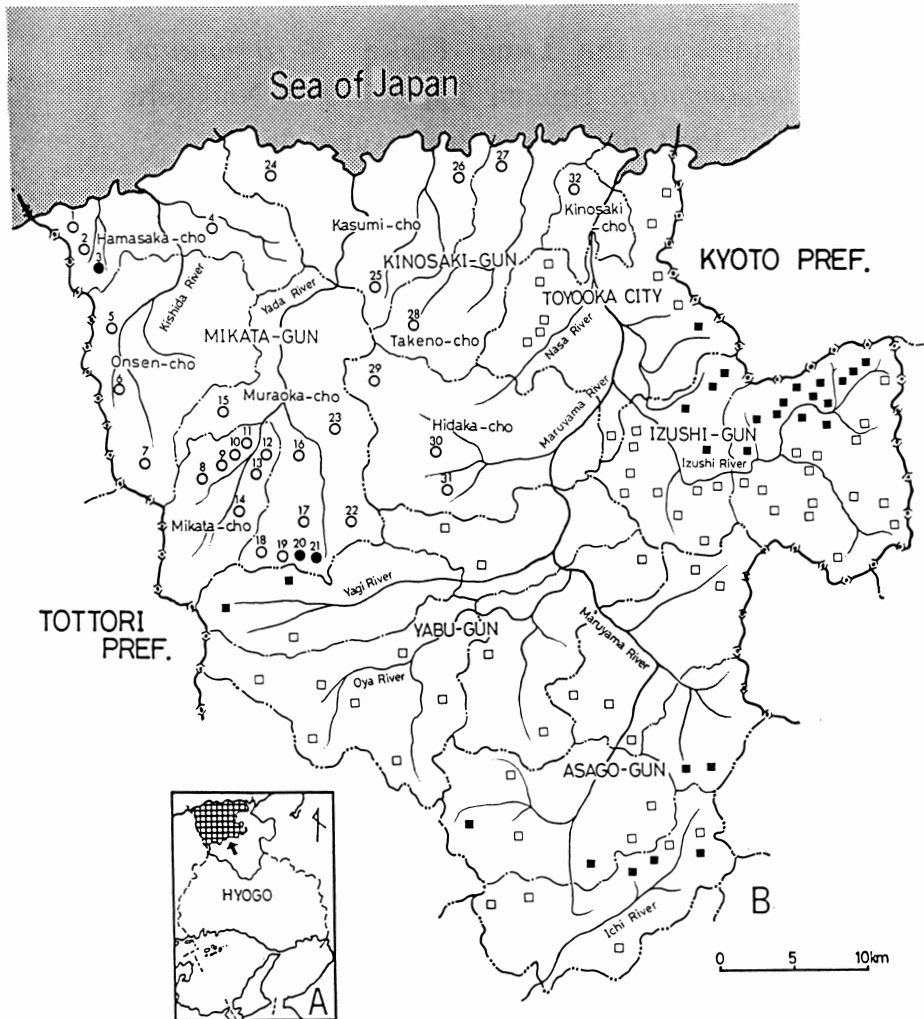


Fig. 1 A) Map showing the location where the present survey was carried out (arrows).

B) Map showing 32 localities (● : positive for metacercariae of *P. westermani*-diploid type, ○ : negative) where crabs were collected in the present survey and 89 localities (■ : positive for metacercariae of *P. westermani*-diploid type, □ : negative) in previous surveys (Shibahara, 1982, 1984b).

Results

1. Incidence of infection with metacercariae in crabs

A total of 1,444 crabs were collected for examination from 32 different localities in Mikata-gun and Kinosaki-gun of Tajima province, Hyogo Prefecture (Fig. 1, Tables 1, 2). Among them, 16 crabs, which were collected at 3 different localities in Mikata-gun (Okumoroyose, Ono and Yaidani) were

positive for *Paragonimus* metacercariae and a total of 20 metacercariae were obtained. As shown in Table 1, the infection rates for each locality were 4.1%, 7.9% and 3.5%, respectively. The average numbers of metacercariae per positive crab were also at the low level of 1.4, 1.2 and 1.0, respectively.

2. Morphology of metacercariae

The metacercariae obtained were spherical or spheroidal in shape, as shown in Fig. 2.

Table 1 Incidence of infection of crabs, *Geothelphusa dehaani*, with metacercariae (Mc) of *Paragonimus westermani* -diploid type in Mikata-gun

No.*	Locality	Date of survey	No. of crabs		Infection rate (%)	Average No. of Mc in a positive crab (Min.—Max.)
			examined	infected		
1	Igumi	Aug. 1982	38	0	0	
2	Takaragi	Apr. 1983	90	0	0	
3	Okumoroyose	July 1982 ~Apr.1983	172	7	4.1	1.4 (1 - 3)
4	Fujio	July 1982	37	0	0	
5	Chidani	July 1982	29	0	0	
6	Umigami	July 1982	29	0	0	
7	Maehata	July 1982	25	0	0	
8	Kusube	July 1982	16	0	0	
9	Kiyama	May 1984	20	0	0	
10	Kanzui	May 1984	50	0	0	
11	Ishitera	May 1984	42	0	0	
12	Kamba	May 1984	71	0	0	
13	Inodani	May 1984	81	0	0	
14	Kayano	July 1982	29	0	0	
15	Kumanami	July 1982	24	0	0	
16	Kanzaka	May 1984	50	0	0	
17	Kazuike	May 1984	22	0	0	
18	Ozasa	July 1982 ~May 1984	22	0	0	
19	Nakaotani	May 1984	61	0	0	
20	Ono	Aug.~Oct. 1982	63	5	7.9	1.2 (1 - 2)
21	Yaidani	Oct. 1982 ~May 1984	113	4	3.5	1.0 (1)
22	Sakuyama	July 1982	39	0	0	
23	Muraoka	July 1982	24	0	0	

* The Nos. are indicated in Fig. 1.

The capsules enveloping the larvae consisted of two layers, a thin outer and a thick inner cyst wall. The diameter of the inner cyst walls and the thickness of the inner or outer cyst walls varied slightly according to the location where the metacercariae were obtained. Morphologically, the metacercariae were similar to each other. The average size of the inner cyst wall was $441.7 \pm 20.1 \mu\text{m}$ in length and $426.8 \pm 16.7 \mu\text{m}$ in width. The thickness of the inner cyst wall was $15.2 \pm 3.6 \mu\text{m}$ in average and that of the outer cyst wall was $1.5 \pm 0.6 \mu\text{m}$ in average.

3. *Experimental infection of a dog and cats with metacercariae and the morphol-*

ogy of the adult worms and their eggs recovered from those animals

A dog and two cats were orally administered 5, 6 and 4 metacercariae which were obtained from the crabs collected at 3 different localities, and were autopsied on day 93 to 100 after infection. Cyst-formation was observed in the lungs of each animal. A total of 4 to 6 worms were recovered from the cysts and the recovery rates were 80 to 100 %. Most of the worms from each locality were adults and their uteri contained many eggs (Fig. 3). The adult worms obtained had singly spaced cuticular spines (Fig. 4), and ovaries divided into 6 lobes. In

Table 2 Incidence of infection of crabs, *G. dehaani*, with metacercariae (Mc) of *P. westermani*-diploid type in Kinosaki-gun

No.*	Locality	Date of survey	No. of crabs		Infection rate (%)	Average No. of Mc in a positive crab (Min.—Max.)
			examined	infected		
24	Amarube	July 1982	49	0	0	
25	Mikawa	July 1982	17	0	0	
26	Okuyasuki	Sep. 1982	21	0	0	
27	Kirihama	Aug. 1982	73	0	0	
28	Futatsuya	July 1982	23	0	0	
29	Inamba	July 1982	26	0	0	
30	Kawahata	July 1982	21	0	0	
31	Kan-nonji	July 1982	42	0	0	
32	Motoyakushi	Sep. 1982	25	0	0	

* The Nos. are indicated in Fig. 1.

both the seminal receptacle and the seminal vesicle, a large number of sperm was observed, as shown in Figs. 5 and 6. The average size of the adult worm from three localities was 7.2 ± 0.7 (6.2~8.7) mm in length and 3.9 ± 0.3 (3.4~4.5) mm in width. The average ratio of length/width was 1.9 ± 0.2 (1.6~2.1).

Observation of the eggs from the worm cyst in the lungs of a cat infected with metacercariae from Okumoro-yose revealed that most were generally inverted oval in shape although there was some variation (Fig. 7). Of the 50 eggs observed, 19 (38%) were symmetrical and the remaining 31 (62%) were asymmetrical. With regards to the location of the maximum width, 19 (38%) were located in the anterior half, 25 (50%) in the middle and 6 (12%) in the posterior half. The average size of the egg was 79.5 ± 3.0 (71.9~85.9) μm in length and 46.2 ± 2.4 (40.5~51.4) μm in width. The thickness of the eggshell was 1.2 ± 0.2 (0.8~1.5) μm in average. Thickening of the shell at the abopercular end was observed in 48 (96%), and the thickness was 3.4 ± 1.1 (1.8~6.6) μm in average. Morphologically, the eggs from the other 2 localities also exhibited a similar tendency.

The adult worms and the eggs recovered from the experimental animals were characterized by the following features; the ovary had 6 lobes, the testes had 5 to 6 lobes, the

seminal receptacle and seminal vesicle contained large amounts of spermatozoa and the presence of thickening of the eggshell at the abopercular end. From these morphological features, all the metacercariae from the freshwater crabs, *G. dehaani*, in Okumoro-yose, Hamasaka-cho and in Ono and Yaidani, Muraoka-cho, Mikata-gun were identified as those of the diploid type of *P. westermani*.

Discussion

The present author has been investigating the geographical distribution of the diploid type of *P. westermani* in Tajima province of northern part of Hyogo Prefecture since August 1979. As shown in Fig. 1, it seems that there are about 4 restricted foci of *P. westermani*-diploid type in Tajima province, taking into account the 3 positive localities in this survey and the 27 in the previous papers (Shibahara, 1982, 1984b). The four areas are as follows: 1) the mountainous area in Izushi-gun that forms a boundary between Tajima province and Tango province, Kyoto Prefecture, 2) the mountainous area in the vicinity of Ikuno in Asago-gun, 3) the mountainous area spreading from Mikata-gun to Yabu-gun and 4) the mountainous area in Hamasaka-cho, Mikata-gun that forms a boundary between Tajima province and Inaba province, Tottori Prefecture. In Tajima province, located along the Sea of Japan, an abundance of plants and wild animals still

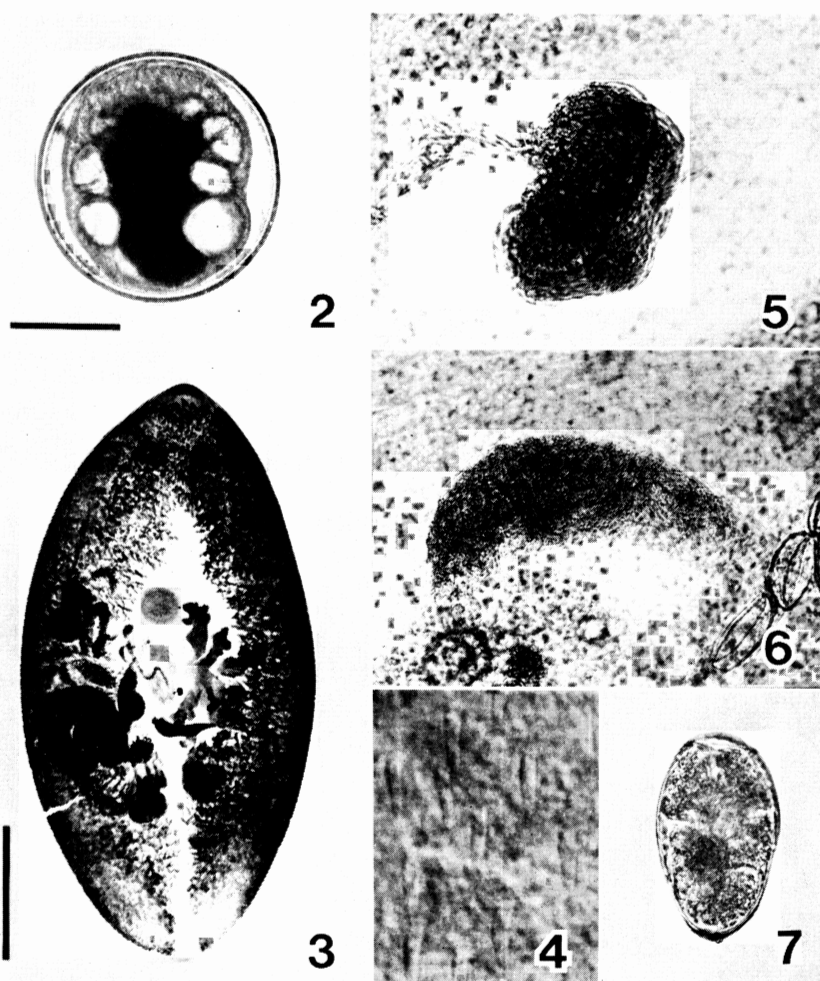


Fig. 2 Metacercaria obtained from a freshwater crab (*Geothelphusa dehaani*) collected in Okumoyose. Scale: 200 μ m.

Fig. 3 Adult worm of *P. westermani*-diploid type recovered from the cyst in the lungs of a cat 100 days after infection with metacercariae (balsam-mounted specimen, dorsal view). Scale: 2 mm.

Fig. 4 Cuticular spines on the dorso-anterior part of an adult worm, spaced singly.

Fig. 5 Seminal receptacle of an adult worm filled with spermatozoa.

Fig. 6 Seminal vesicle of an adult worm filled with spermatozoa.

Fig. 7 An egg removed from a cyst in the lungs of a cat, preserved in 10% formalin ($\times 340$)

remains. However, as clarified in this survey, the distribution of *P. westermani*-diploid type was partly restricted.

As to reports of the human paragonimiasis in the same province, as mentioned in a previous paper (Shibahara, 1982), no cases of paragonimiasis have been reported since 1956, although a few cases attributed to *P.*

westermani have been recognized in the past (Toda, 1950; Fujito and Ishihara, 1952; Yoshida *et al.*, 1955; Miyamoto, 1961). The reason for this may be that the human paragonimiasis was caused by the triploid type of *P. westermani*, which adopts to mitten crabs, *E. japonicus*, as a second intermediate host. The custom of eating these crabs

gradually disappeared, especially after it was found that they were the cause of disease. Therefore the life-cycle between the human, who was an important definitive host, and the intermediate hosts was interrupted and the triploid type of *P. westermani* gradually became extinct. However, the present survey, as well as previous surveys (Shibahara, 1982, 1984b), also revealed that there are some new habitats of the diploid type of *P. westermani* in Tajima province. Therefore, it is necessary to pay special attention to the possibility of human paragonimiasis caused by new sources.

Summary

During the period from July 1982 to May 1984, a total of 1,444 crabs, *Geothelphusa dehaani*, collected at 32 localities in Mikatagun and Kinosaki-gun of Tajima province of Hyogo Prefecture were examined for the lung fluke. Among them, 16 (4.6%) of 348 crabs collected at three localities were positive for *Paragonimus metacercariae*.

Twenty metacercariae were obtained from the crabs. Twelve adult worms were recovered from two cats and a dog 93 to 100 days after infection with the metacercariae. On the basis of the morphological features of the adult worms recovered from the animals, all flukes were identified as the diploid type of *Paragonimus westermani*.

Based on the present results, Mikatagun was proved to be a new habitat of *P. westermani* -diploid type.

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兵庫県北部におけるウエステルマン肺吸虫（二倍体型）に関する研究
 V. 美方郡および城崎郡における地理的分布

柴原壽行

(愛媛大学医学部附属動物実験施設)

1982年7月から1984年5月にかけて、兵庫県但馬地方の美方郡および城崎郡の32地点において合計1,444匹のサワガニを採集して、これらを対象に肺吸虫の寄生状況に関する調査を行った。その結果、美方郡浜坂町および村岡町の3ヶ所で採集したサワガニ16匹から肺吸虫メタセルカリアを検出した。これらのメタセルカリアをイス

あるいはネコに感染させて得た成虫および虫卵を精査し、その形態的観察結果に基づいてこれらをウエステルマン肺吸虫（二倍体型）と同定した。よって、兵庫県美方郡浜坂町および村岡町をウエステルマン肺吸虫（二倍体型）の分布地として新たに追加する。