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The Examination of the Second Intermediate Hosts of *Paragonimus westermani*, Diploid Type, in Taiwan

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Abstract

Freshwater crabs from Taipei, Hsinchu and Miaoli Counties, the previously endemic areas of paragonimiasis in Taiwan, were examined during the period from October, 1984 to December, 1985 for the metacercariae of *Paragonimus westermani.*

A total of 1777 crabs, including 1050 *Eriocheir japonicus,* 199 *Geothelphusa miyazakii,* 260 *G. candidiensis,* 264 *Candidiopotamon rathbuni* and 4 *G. chui* were examined.

None of the metacercariae of *P. westermani* was found in *E. japonicus.* On the other hand, the potamonid crabs showed quite a high prevalence and density of the metacercariae of *P. westermani* with 79.0% and 4.9/crab in *G. miyazakii,* 26.9% and 1.4/crab in *G. candidiensis,* and 17.0% and 3.7/crab in *C. rathbuni*, respectively.

All the metacercariae obtained from these potamonid crabs were identified as the diploid type of *P. westermani.*

The distribution patterns of the metacercariae in *G. miyazakii* and *C. rathbuni* were similar, with the highest density in muscles followed by gills and viscera. In *G. candidiensis*, the sequence was muscles, viscera, and gills.

Possible reasons for the negative findings of the metacercariae of *P. westermani* in *E. japonicus* and the high prevalence and density of the metacercariae of the diploid type in potamonid crabs are discussed.

Key words: *Paragonimus westermani,* diploid type, metacercariae, potamonid crab, *Eriocheir japonicus*

Introduction

The second intermediate hosts of *Paragonimus westermani* were discovered by Nakagawa (1915) at Chialo Village, Hsinchu County. He reported that *P. westermani* utilized three species of freshwater crabs as the second intermediate hosts: *Candidiopotamon* (*Potamon*) *rathbuni, Geothelphusa candidiensis* (formerly, *P. dehaani*) and *Eriocheir japonicus.*

Three other species, *Parathelphusa sinensis* (Yokogawa and Morishita, 1931), *Geothelphusa miyazakii* (Miyake and Chiu, 1965) and *G. chiui* (Chiu, 1962) were also found to be second intermediate hosts of *P. westermani.*

Among these crab hosts, *E. japonicus* is known to be the most important vector in the transmission of human paragonimiasis in the plains, while *C. rathbuni* is a more important source in the mountainous areas in Taiwan.

Recent studies on chromosomes of *P. westermani* in Japan revealed that there are two types of *P. westermani*, the triploid type and diploid type (Sakaguchi and Tada, 1976; Terasaki, 1977). In Taiwan, Miyazaki and Chiu (1980) examined the mounted worms of the so-called *Paragonimus westermani* deposited in the Departments of Parasitology at the National Taiwan University Medical College and the National Yang-Ming Medical College. They reported that the worms,

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originating from the three species of potamonid crabs, *Geothelphusa miyazakii, G. candidiensis* in Taipei County and *Candidiopotamon rathbuni* in Miaoli County, were all identified as the diploid type whereas those worms from *E. japonicus* were found to be the tripliod type of *P. westermani.*

Since then, no studies on the karyotype of the metacercariae from *E. japonicus* and potamonid crabs in Taiwan have been carried out.

One of the main objectives in this study was to examine the karyotype of the metacercariae harboured in *E. japonicus* and potamonid crabs. However, since none of the *E. japonicus* was infected with the metacercariae of *P. westermani*, this paper deals only with the present status of parasitism of the metacercariae of the diploid type *P. westermani* in potamonid crabs in the previously endemic areas in Taiwan.

Materials and Methods

Freshwater crabs, *Eriocheir japonicus* and potamonid crabs, from Taipei, Hsinchu and Miaoli Counties were examined during the period from October, 1984 to December, 1986 for the metacercariae of *P. westermani.*

First, the size of the carapace and the sex of each crab were recorded. Then, gills and viscera (including liver, gonads and heart) were removed and pressed down between large glass plates and examined under a dissecting microscope. The crabs were divided into two groups. In group A, metacercariae were found in gills and/or viscera and in group B, metacercariae were not found in either the gills or the viscera.

The two groups of crabs were processed separately and examined for the metacercariae in the muscles by the method of Huang *et al.* (1966). Briefly, the cephalothorax without carapace and the legs of the crabs were ground in a meat grinder. The minced materials were then mixed with some water and strained through coarse and fine metal meshes. The sediment was washed several times with water, and then a small amount of the sediment was poured into a 9 cm Petri dish and examined under a dissecting microscope.

Measurements of the metacercariae were carried out in a suspended condition in the fluid, without use of a coverslip.

The adult worms recovered from the lungs of dogs infected separately with the metacercariae from *G. miyazakii, G. candidiensis* or *C. rathbuni* were fixed with 70% alcohol under pressure, and stained with alum-carmine for morphological observations.

The number of chromosomes in the worms was determined by the air dry technique (Terasaki, 1977; Terasaki and Nakamura, 1978).

The sign test of analysis was used to assess differences between parasite density in the gills and viscera of the infected crabs. Prevalences were compared by using the X^2-test. Differences were considered significant at $P<0.05$ (Snedecore and Cochran, 1980).

Results

A total of 1777 crabs including 1050 *E. japonicus* collected from Taipei, Miaoli, and Hsinchu Counties, 199 *G. miyazakii* and 260 *G. candidiensis* from Taipei County, 264 *C. rathbuni* from Miaoli and Hsinchu Counties and 4 *G. chiui* from Miaoli county were examined (Fig. 1).

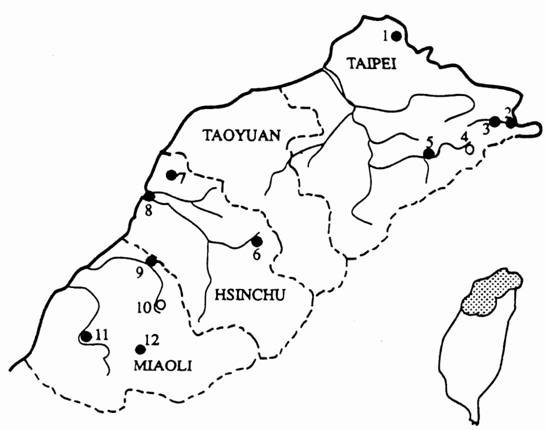

Fig. 1. Map showing the collection sites of crabs in northern Taiwan. Open circle denotes crabs positive for *Paragonimus westermani* metacercaria were found and solid circle means negative finding. Names of numbered locations are: 1 — Alilao, 2 — Gungliao, 3 — Yuhang, 4 — Taipin, 5 — Pinlin, 6 — Chialo, 7 — Boluowen, 8 — Chupei, 9 — Sanwan, 10 — Ponlai, 11 — Gonguan, 12 — Husan.

None of the *E. japonicus* was infected with the metacercariae of *P. westermani* regardless of the size, sex or locality of the crabs.

Among the potamonid crabs, the metacercariae of the diploid *P. westermani* were found in 159 (79.9%) out of the 199 *G. miyazakii* and in 70 (26.9%) of the 260 *G. candidiensis* from Taipin, Taipei County. The metacercariae were also found in 38 (17.0%) of the 223 *C. rathbuni* and in 1 of 4 *G. chui* from Ponlai, Miaoli County. *C. rathbuni* from Husan, Miaoli County and from Chialo, Hsinchiu County were all negative (Table 1).

Thirty metacercariae each from *G. miyazakii, G. candidiensis* and *C. rathbuni* measured 255 to 382 × 255 to 368, the average being 290 ± 29 × 280 ± 27 microns. No pinkish granules were found in the metacercariae.

The ovary of the adult worms originating from these 3 species of crab was branched into 6 lobes in most of the specimens, and spermatozoa were observed in the seminal receptacle and seminal vesicle. The number of chromosome was $n=11$ and $2n=22$.

On the basis of these observations, the adult worms originating from the metacercariae in *G. miyazakii, G. candidiensis* and *C. rathbuni* were all identified as being the diploid type of *P. westermani.*

The distribution patterns of the metacercariae in group A and group B are shown in Table 2. In group A, the metacercariae were found in gills and/or viscera. The distribution patterns of the metacercariae in *G. miyazakii* and *C. rathbuni* were similar, with the highest number in muscles followed by gills and viscera. In *G. candidiensis*, the sequence was muscles > viscera > gills. In viscera, the metacercariae were found more often in the liver, and occasionally in the heart or gonads.

In group B, the metacercariae were not found in either the gills or the viscera. However, they were found in muscles although in much lower numbers.

Table 1 Prevalence of the metacercariae of *P. westermani* in crabs in Taiwan

Locality	Grab host	No. crab examined	No. (%) crabs infected	Survey time
Taipin, Taipei	*G. miyazakii*	199	159 (79.9)	Nov. '84-Dec. '85
	G. candidiensis	260	70 (26.9)	Nov. '84-Dec. '85
Ponlai, Miaoli	*G. chui*	4	1 (25.0)	Sep. '85
	C. rathbuni	223	38 (17.0)	Apr.-Oct. '85
Husan, Miaoli	*C. rathbuni*	13	0	Apr. '85
Chialo, Hsinchu	*C. rathbuni*	28	0	Feb. '85
Pinlin, Taipei	*E. japonicus*	10	0	Oct. '84
Yunhang, Taipei	〃	120	0	Nov. '84
Alilao, Taipei	〃	26	0	Jan.-Jul. '85
Gungliao, Taipei	〃	85	0	Dec. '86
Gonguang, Miaoli	〃	180	0	Dec. '84
Sanwan, Miaoli	〃	38	0	Nov. '85
Chialo, Hsinchu	〃	2	0	Feb. '85
Chupei, Hsinchu	〃	315	0	Feb. 85-Dec. '86
Boluowen, Hsinchu	〃	230	0	Dec. '85
Unknown, from market	〃	44	0	Nov. '85

Table 2 Distribution of the metacercariae of diploid type of *P. westermani* in potamonid crabs in Taiwan

Crab host	Group	No. of crabs examined	No. of mc in				Average number of mc per crab (density)
			Gills	Viscera	Muscles	Total	
G. miyazakii	A	70	212	55	1098	1365	19.5
	B	27	0	0	59	59	2.2
G. candidiensis	A	49	31	41	91	163	3.3
	B	118	0	0	128	128	1.1
C. rathbuni	A	18	86	5	400	491	27.3
	B	87	0	0	87	87	1.0

A : metacercariae were found in gills and/or viscera
B : metacercariae were not found in either gills or viscera
mc : metacercariae

In group A, the average number of metacercariae per crab was 27.3 in *C. rathbuni*, 19.5 in *G. miyazakii* and 3.3 in *G. candidiensis*. The highest number, 27.3 in *C. rathbuni*, was considered to be due to the heavy infection of a single crab which harboured 51 and 126 metacercariae in its gills and muscles, respectively. If this crab was disregarded, the corresponding figure of 18.5 would be similar to that of *G. miyazakii*, 19.5.

Results of the examination of potamonid

Table 3 Prevalence and density of the metacercariae of *P. westermani* in gills and viscera of *G. miyazakii* from Taipin, Taipei County by size and sex

Size (mm)	Sex	No. crabs examined	No. crab positive (%) in			Average No. of mc/positive crab		
			Gills	Viscera	Total	Gills	Viscera	Total
small (11—20)	M	30	11(36.7)	7(23.3)	14(46.7)	2.3	1.1	2.4
	F	6	0	2(33.3)	2(33.3)	0	1.0	1.0
	Total	36	11(30.6)	9(25.0)	16(44.4)	2.3	1.1	2.2
medium (21—30)	M	82	62(75.6)	30(36.6)	67(81.7)	5.1	1.4	5.3
	F	66	58(87.9)	30(45.5)	61(92.4)	4.1	1.6	4.6
	Total	148	120(81.1)	60(40.5)	128(86.5)	4.6	1.5	5.0
large (31—40)	M	4	4(100)	2(50.0)	4(100)	4.8	1.5	5.5
	F	11	11(100)	2(27.3)	11(100)	6.7	1.7	7.2
	Total	15	15(100)	5(33.3)	15(100)	6.2	1.6	6.7
Total	M	116	77(66.4)	39(33.6)	85(73.3)	4.6	1.4	4.8
	F	83	69(83.1)	35(42.1)	74(89.2)	4.5	1.5	4.9
	Total	199	146(73.4)	74(37.2)	159(79.9)	4.6	1.5	4.9

mc : metacercariae

crabs, positive for the metacercariae in gills and/or viscera, are presented by species and the size of crabs as follows:

Geothelphusa miyazakii:

The prevalence of infection and density of the metacercariae of *P. westermani* in this species are summarized in Table 3. The length of the carapace ranged from 15 to 34 mm with an average of 24.7 ± 4.1 mm, and the prevalence and density of the metacercariae in gills and viscera increased with an increase in the size of the crab. The differences were most dramatic between the small and medium sized crabs.

The prevalence rates did not seem to be different between the sexes within each group, however, the X^2 test on the pooled data indicated that they were significantly higher in females than in males.

The prevalence of infection and density of the metacercariae in gills and viscera were similar in small crabs, but in medium and large sized crabs, those in the gills were significantly higher than those in the viscera.

Geothelphusa candidiensis:

This species is relatively small in size. The length of the carapace ranged from 11 to 23 mm with an average of 16.7 ± 2.0 mm. Among the 260 crabs examined, only 10 of them exceeded 20 mm in length of carapace and these were all females (Table 4).

The prevalence of infection and density of the metacercariae were 14.9 to 35.3% (av. 26.9%) and 1.0 to 2.3/crab (av. 1.4/crab), respectively. They did not differ with the size or sex of the crabs. Those in the gills were similar to those of the viscera in different sized crabs.

Table 4 Prevalence and density of the metacercariae of *P. westermani* in gills and viscera of *G. candidiensis* from Taipin, Taipei County by size and sex

Size (mm)	Sex	No. crabs examined	No. positive (%) in			Average No. of mc/positive crab		
			Gills	Viscera	Total	Gills	Viscera	Total
small (11—15)	M	47	3(6.4)	5(10.6)	7(14.9)	1.0	1.4	1.4
	F	17	3(17.6)	3(17.6)	6(35.3)	1.0	1.0	1.0
	Total	64	6(9.4)	8(12.5)	13(20.3)	1.0	1.2	1.3
medium (16—20)	M	125	21(16.8)	25(20.0)	41(32.8)	1.1	1.4	1.4
	F	61	6(9.8)	9(14.8)	13(21.3)	1.3	1.2	1.5
	Total	186	27(14.5)	34(18.3)	54(29.0)	1.1	1.4	1.4
large (21—25)	M	0						
	F	10	1(10.0)	2(20.0)	3(30.0)	4.0	1.5	2.3
	Total	10	1(10.0)	2(20.0)	3(30.0)	4.0	1.5	2.3
Total	M	172	24(14.0)	30(17.4)	46(27.9)	1.1	1.4	1.4
	F	88	10(11.4)	14(15.9)	22(25.0)	1.5	1.2	1.5
	Total	260	34(13.1)	44(16.9)	70(26.9)	1.2	1.3	1.4

mc : metacercariae

in 1962 — 1963 were recorded as 20.0% and 51.6%, respectively (Huang *et al.*, 1964). The corresponding figures in the present study were 79.9% and 26.9% respectively in the same areas. Although the figures for both crabs were inverted, there is no doubt that the prevalence of the metacercariae in these crabs still remained high.

With regard to *C. rathbuni*, Chiu (1965) stated that the prevalence and density of the metacercariae in *C. rathbuni* in Miaoli County were 31.8% (76/239) and 3.0/crab respectively, and these figures were much lower than those of Nakagawa (1917) and Kinugasa (1943). In our survey, they were 17.0% (38/223) and 3.7/crab, respectively. The prevalence rate was lower than that of Chiu (1965), yet the density of the metacercariae was almost the same, 3.7 vs 3.0.

It is noteworthy that the prevalence rate and density of the metacercariae of the diploid type in potamonid crabs, *G. miyazakii, G. candidiensis* and *C. rathbuni*, still remained high in the past endemic areas. These areas are located in remote mountainous regions where no paragonimiasis patient was found in recent years except a few infected dogs and cats (Lee, 1986; Liu, 1986).

In Japan, it is commonly known that *G. dahaani* is usually found in the streams in remote mountainous areas where population density is low, and therefore, the life cycle of this fluke may be primarily maintained among wild animals.

Further studies on the role of reservoir hosts such as dogs, cats and other animals in maintaining the life cycle of the diploid type of *P. westermani* in these areas in Taiwan are needed.

A comparison of the distribution of the metacercariae in the potamonid crabs revealed that in both *G. miyazakii* and *C. rathbuni*, the metacercariae were found mainly in muscles, followed by gills and viscera whereas in *G. candidiensis*, the sequence was muscles, viscera (mainly liver) and gills.

This pattern in *C. rathbuni* was similar to previous investigations (Nakagawa, 1917; Chiu, 1965) in Taiwan. The distribution pattern in *G. candidiensis* which was classified as *Potamon dehaani* by early workers was similar to that of *G. dehaani* in Japan, its sequence was muscles, liver and gills.

In general, the prevalence of the metacercariae in potamonid crabs did not differ with the sex. However, the prevalence and density of the metacercariae in *G. miyazakii* increased with an increase in size, but they were similar in *G. candidiensis* irrespective of size.

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