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Occurrences of *Paragonimus westermani,* diploid type, and *P. miyazakii* in the same population of the freshwater crab, *Geothelphusa dehaani,* collected in Mikkabi-cho, Shizuoka Prefecture, Japan

HIDETO KINO

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Abstract

Prevalences of lung flukes in the freshwater crab, *Geothelphusa dehaani,* were surveyed in the eastern part of Aichi Prefecture and the western part of Shizuoka Prefecture. Metacercariae were detected in all populations in Shizuoka Prefecture and 2 populations in Shinshiro City, Aichi Prefecture. The abundance of metacercariae in the crab population was highest in Mikkabi-cho.

Adult worms were recovered from experimental hosts for 2 localities in Shizuoka Prefecture. While most worms recovered from a rat and dogs were identified as *P. miyazakii,* 2 worms from Mikkabi-cho were identified as *P. westermani* from the morphological feature of their ovaries. The seminal receptacles of the latter were filled with a number of spermatozoa, indicating that the worms were bisexual, diploid type.

The discovery of the diploid *P. westermani* would support the evidence that Nagase *et al.* (1977) detected adult worms of the diploid *P. westermani* in a fox found in Shinshiro City. Co-occurrence of *P. westermani* and *P. miyazakii* in the same crab population seemed to be important in respect to the geographical distribution of the lung flukes.

Key words: *Paragonimus westermani,* diploid, *Paragonimus miyazakii,* geographical distribution, *Geothelphusa dehaani,* Shizuoka Prefecture

Introduction

Karyotype of *Paragonimus westermani* in Japan was first shown to be triploid (3n = 33) by Sakaguchi and Tada (1976) and Terasaki (1977). Then, Miyazaki (1977) reported that the flukes found in the freshwater crab, *Geothelphusa dehaani,* in Akita Prefecture (Suzuki *et al.*, 1978) were cross fertilization type. These worms were later confirmed to have diploid chromosomes (Terasaki, 1980). The evidence that there were two types of *P. westermani* in Japan stimulated field surveys and the diploid type of *P. westermani* has successively been found in various areas in Japan.

Department of Parasitology, Hamamatsu University School of Medicine, Japan.
記野秀人（浜松医科大学寄生虫学教室）

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Nagase *et al.* (1977b) discovered diploid adult flukes in a wild fox found in the eastern part of Aichi Prefecture, though metacercariae detected in *G. dehaani* proved to be *P. miyazakii* (Nagase *et al.*, 1977a). Although the diploid worms were found in other wild mammals in this area (Nagase, via personal communication), the intermediate hosts have not been determined. If the diploid *P. westermani* in this area exploits *G. dehaani* as the second intermediate host as well as *P. miyazakii,* these two species may occur in neighboring crab populations or, possibly, in the same population. The present study was conducted to confirm the distribution of *P. westermani* in this area and to examine the pattern of the distribution.

Materials and Methods

The freshwater crab, *G. dehaani,* was collected by the pick-up method (Kino, 1990)

from 10 localities in the western part of Shizuoka Prefecture and the eastern part of Aichi Prefecture near Shinshiro City, in particular around the locality where the fox infected with *P. westermani* was found (Nagase *et al.*, 1977b). Metacercarial infection was examined according to the method described in Kino (1990).

Metacercariae obtained from all of the crabs collected were orally administered to dogs or rats. The animals were sacrificed at least several weeks after eggs were first detected in feces. Adult worms recovered from worm cysts in the lungs were pressed between two glass plates, fixed with AFA solution (alcohol, formalin, acetic acid), then stained with borax carmine solution for morphological examination.

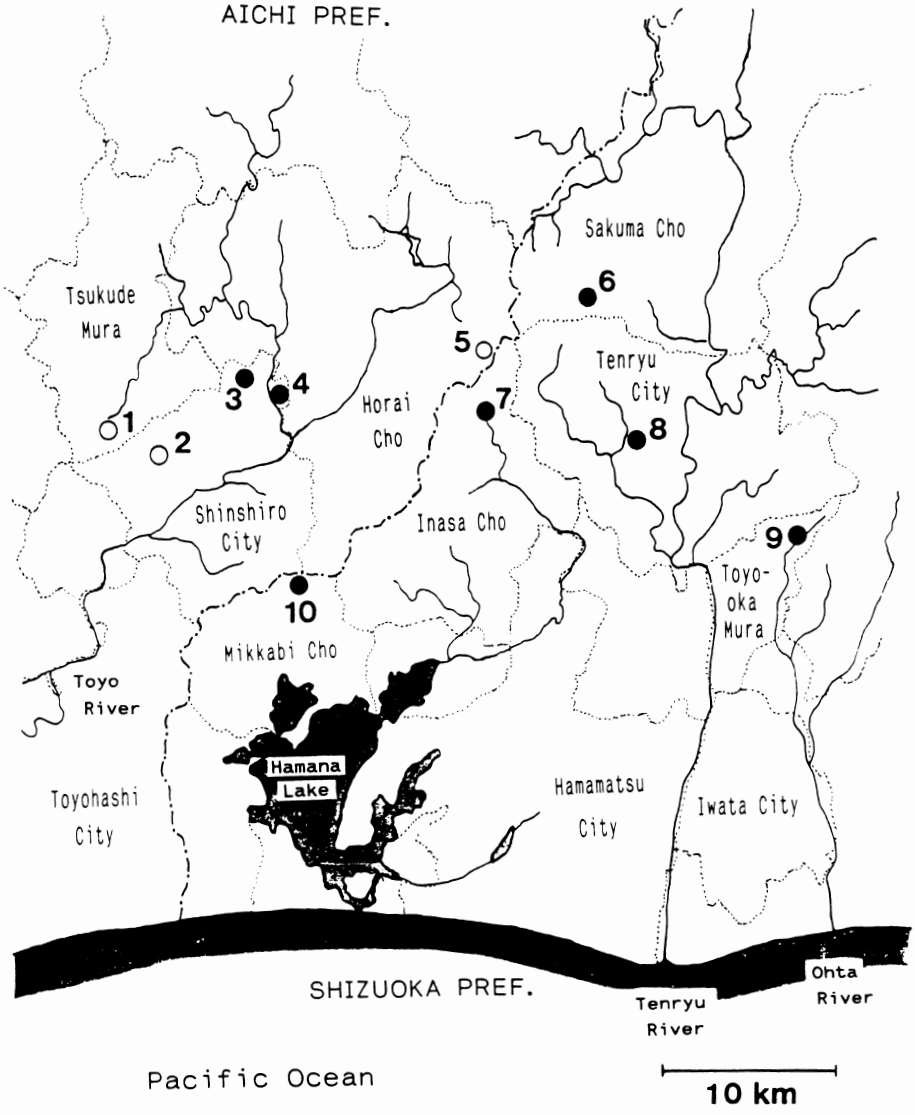

Fig. 1 Map of the western part of Shizuoka Prefecture and the eastern part of Aichi Prefecture showing the localities where crabs were collected. Numbers at the localities correspond to those shown in Table 1. The localities where metacercariae were found were shown as solid circles.

Results

Localities where *G. dehaani* was collected are shown in Fig. 1 and the prevalences of metacercariae in crabs with a carapace width of 18 mm or more are shown in Table 1. The minimum size of crabs compared was determined according to Kino (1990). Metacercariae were detected in all of the crab samples from Shizuoka Prefecture, though only one crab smaller than 18 mm was infected with a metacercaria in the case of Sakuma-cho. Samples from the eastern part of Shinshiro City were also positive, whereas those in the western part and north of the city were negative for the infection. One locality near Tadaki, Mikkabi-cho, was most endemic and the density of metacercariae was highest (Table 1).

Adult flukes were recovered from a rat for Toyooka-mura and from two dogs for Mikkabi-cho, but no worms were recovered for the other localities probably due to the small number of metacercariae obtained. From the rat, 3 worms out of 7 given metacercariae (42.8%) were recovered 113 days after infection. Five out of 40 (12.5%) from dog no. 1 and 9 out of 38 parasites (23.6%) from dog no. 2 were recovered 182 and 132 days after infection, respectively.

The adult worms derived from Toyooka-mura were identified as *P. miyazakii* with the morphological feature of the ovary having a complicated branching structure. Although most of the worms from Mikkabi-cho were also identified as *P. miyazakii* (Fig. 2a), two out of 9 worms from dog no. 2 were identified as *P. westermani* (Fig. 2b). The ovary of the worms lobulated into 6 simple branches, though the shape of the lobes was not necessarily typical of the species (Fig. 2c). The seminal receptacle was filled with a number of spermatozoa (Fig. 2d). Eggs discharged by the adult worms during mixed incubation in saline showed a morphological feature typical of *P. miyazakii* having a symmetrical shape and no thickening of the egg shell at the end.

Discussion

Morphological differences between *P. westermani* and *P. miyazakii* are described to be present in the branching system of the ovary and, with many exceptions, the shape of the egg. Other keys used to identify *Paragonimus* species such as relative sizes of oral and ventral suckers and arrangement of spines on the worm surface are said to be the same. The 2 worms identified as *P. westermani* from Mikkabi-cho had ovaries with a morphology different from that of *P. miyazakii*. The main difference was the simplicity of branching system: whereas lobulation of the worms identified as *P. miyazakii* was complicated and formed a so-called "coral shape", the number of branches in the worms of *P. westermani* was easily counted as 6 and no cases of complicated lobulation were observed. This characteristic feature was considered to warrant the identification of the worms as *P. westermani*. The presence of spermatozoa in the seminal receptacle indicated that these worms are diploid and performed bisexual reproduction. Although the shape of their eggs did not show the characteristic feature of *P. westermani*, it can be variable even if the eggs were produced by a single individual, particularly for the diploid type (Miyazaki, 1978; Shibahara, 1983; Kanazawa, 1986).

The distribution of *P. miyazakii* has been studied in the eastern part of Aichi Prefecture (Nagase *et al.*, 1977a) and in the western part of Shizuoka Prefecture with prevalences as high as 80% at Kawane-cho (Ito and Mochizuki, 1975; Kino *et al.*, 1986). In the present study, the distribution of the species in *G. dehaani* was also confirmed in this area. In surrounding regions of the area, however, no infection in the crab host has been found. This evidence would indicate that

Fig. 2 a: Adult worm of *P. miyazakii* recovered from a dog 132 days after infection with metacercariae collected in Mikkabi-cho.
b: Adult worm of *P. westermani*, diploid type, recovered from a dog 132 days after infection with metacercariae collected in Mikkabi-cho.
c: The ovary of *P. westermani* (b) showing 6 lobes (1 lobe is hidden).
d: The seminal receptacle of *P. westermani* (b) filled with spermatozoa.

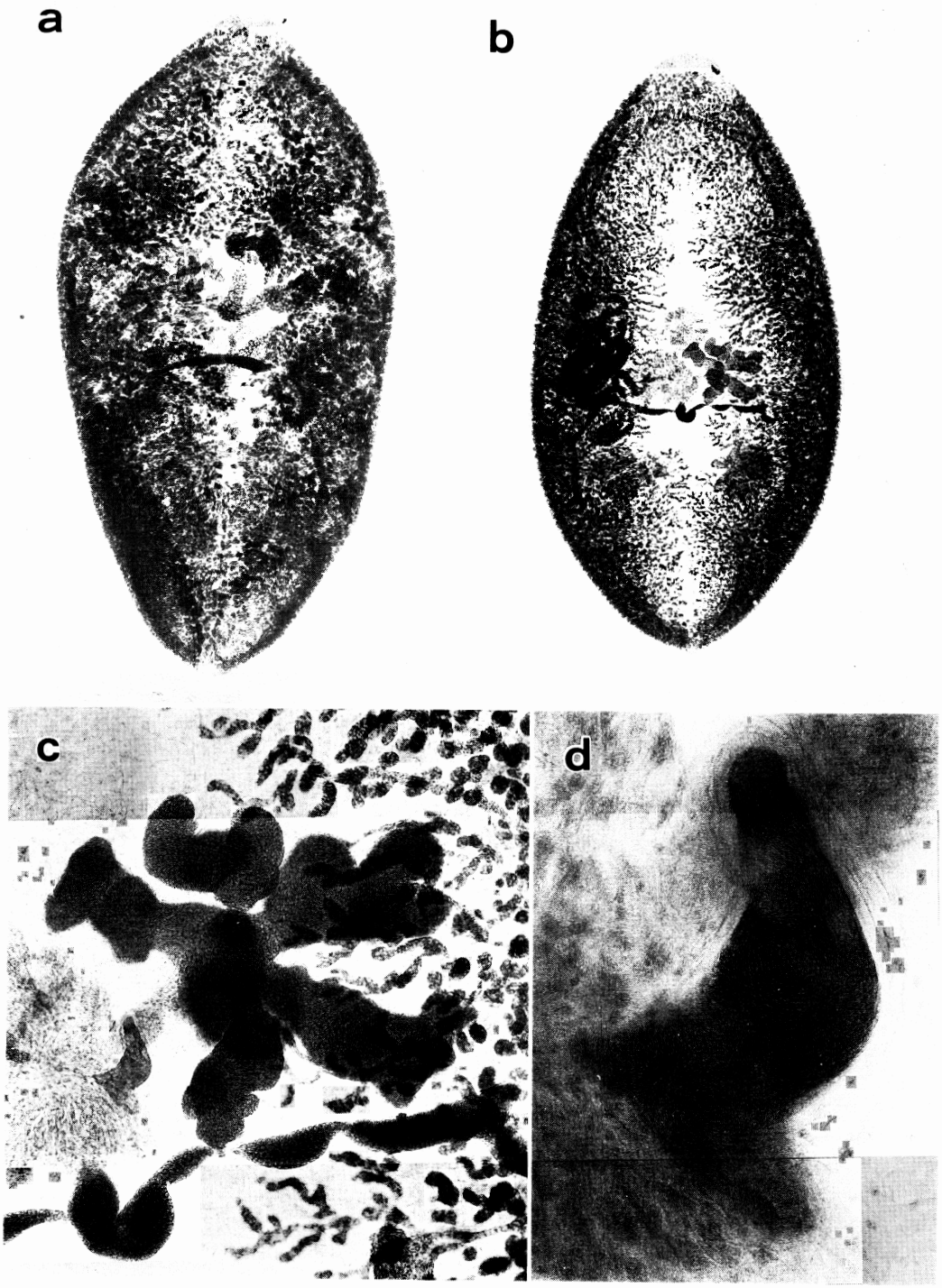
a
b
c
d

Table 1 Prevalences of metacercarial infection in *Geothelphusa dehaani* with a carapace width of 18 mm or more collected in Aichi and Shizuoka Prefectures

No.	Prefecture	Locality		No. of crabs		Infection rate (%)	Mean no. of metacercariae per crab
				examined	infected		
1	Aichi	Tsukude-mura	Yasunaga	20	0	0.0	0.0
2		Shinshiro City	Toyosaka	71	0	0.0	0.0
3			Takigawa	32	5	15.6	0.1
4			Yokokawa	41	1	2.4	0.0
5		Horai-cho	Nanasato-Isshiki	8	0	0.0	0.0
6	Shizuoka	Tenryu City	Higashi-Fujidaira	13	4	30.7	0.3
7		Sakuma-cho	Yamatokane	9	0	0.0*	0.0
8		Toyooka-mura	Mushiu	6	3	50.0	0.5
9		Inasa-cho	Shibukawa	41	3	7.0	0.1
10		Mikkabi-cho	Tadaki	94	34	36.2	1.1

*: Positive in a small crab

the distribution range forms an isolated area. This distribution pattern may have been established through geological history and hence presence of the first intermediate host snail(s) as Nishida (1988) suggested.

For *P. westermani,* in turn, while human and/or crab infections have been described throughout Japan (Radke and Davis, 1969), those with the diploid type have become apparent only in recent years. Many occurrences of the diploid have been reported from the Kinki district (Sugiyama *et al.*, 1983; 1984; Shibahara, 1982; Nishida *et al.*, 1987a, b). Outside of this area the geographical distribution appears rather sparse and has been reported from limited localities including such Prefectures as Oita (Habe and Miyazaki, 1982), Shiga (Nishida *et al.*, 1981), Ishikawa (Yoshimura *et al.*, 1983), Gifu (Shiwaku *et al.*, 1986), Chiba (Kanazawa, 1986) and Akita (Miyazaki, 1977). The discovery of the diploid type in Shizuoka Prefecture in the present study would support the first discovery of the type in Aichi Prefecture by Nagase *et al.* (1977b) and may fill the discontinuity in the geographical distribution. In a southern part of Gifu Prefecture, Ando (1915) reported both human and crab infections with *P. westermani.* The evidence that the crab species there was *G. dehaani* may raise doubt as to whether the fluke was *P. miyazakii.* However, he detected eggs in sputum of the patients and also determined the snail host to be *Semisulcospira libertina* known as the first intermediate host of *P. westermani* (Ando, 1921). These indicate that the fluke was *P. westermani* and not *P. miyazakii.* Since the karyotype of *P. westermani* that parasitized *G. dehaani* in all reported localities but Oita Prefecture was diploid, his fluke could also be the diploid type. The endemic focus shown by Shiwaku *et al.* (1986) could have derived from this locality. A similar origin of the geographical distribution of the diploid in *G. dehaani* may be considered for the population in Mikkabi-cho found in the present study, though there is a non-endemic region between the two localities at present.

The diploid *P. westermani* and *P. miyazakii* exploit the common crab species, *G. dehaani,* as the second intermediate host and the geographical distributions of the two species overlap on a large scale. However, these species have not been found to occur in the same population of the crab host except in Kyoto Prefecture (Nishida *et al.*, 1987a). This suggests some exclusive ecological interaction(s) may have operated between the two species. The crab population in Mikkabi-cho, however, harbored both the fluke species. This sympatric distribution indicates no intervention of the exclusive factor(s) between the species in selecting host population and the locality or habitat. Factors which have enabled the sympatric distribution are currently under investigation.

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