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Comparative studies on *Paragonimus sadoensis* Miyazaki, Kawashima, Hamajima et Otsuru, 1968 and *P. ohirai* Miyazaki, 1939. III. Experimental infection of *Potamon dehaani* White and *Sesarma dehaani* H. Milne-Edwards with the cercariae of the two species

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Introduction

Basic information relative to the species status of *Paragonimus sadoensis* Miyazaki, Kawashima, Hamajima et Otsuru, 1968 can be found in the two previous reports (Ito *et al.*, 1969 ; Yoshimura *et al.*, 1970).

In part II of this series, Yoshimura *et al.* (1970) compared the susceptibility of *Oncomelania minima* and *Assiminea parasitologica* snails to infection with *P. sadoensis* and *P. ohirai*, stating that *O. minima*, the first intermediate host of *P. sadoensis*, was susceptible to *P. ohirai*, similarly, *A. parasitologica*, the intermediate host of *P. ohirai*, was susceptible to *P. sadoensis* and that, in both *P. sadoensis* and *P. ohirai*, no significant morphological differences were found between larvae developed in *O. minima* and *A. parasitologica* snails. Hence, Yoshimura *et al.* suggested that ecological dissimilarities in the first intermediate hosts were not necessarily significant differentiating characters between the two species.

The purpose of this study is to determine whether ecological dissimilarity in the second intermediate hosts can be used as a differentiating character between *P. sadoensis* and *P. ohirai*. Both *Potamon dehaani*, the second intermediate host of *P. sadoensis*, and *Sesarma dehaani*, corresponding intermediate host of *P. ohirai*, were experimentally exposed to the respective cercariae of the two lung flukes which had been developed in both *O. minima* and *A. parasitologica* snails. Special attention was given to establish (1) whether both *P. dehaani* and *S. dehaani* crabs can be infected with the respective lung flukes, (2) whether significant differences in metacercarial morphology and size are found between the two species, and (3) whether appreciable differences occur in crab infection rates or morphology of metacercariae yielded, when the crabs are exposed to the cercariae originating from the experimental first intermediate hosts.

Materials and Methods

P. dehaani crabs infected with *P. sadoensis* metacercariae were collected from small streams near the mouth of the Okura river, Aikawa-machi, Sado gun, Niigata Prefecture, Japan, in September 1968. At the same time, *O. minima* snails used for experimental infection were collected from the above described area as well as from small streams near Iwayaguchi, Aikawa-machi. Both *S. dehaani* crabs infested with *P. ohirai* metacercariae and *A. parasitologica* snails used for experimental infection were collected at the mouth of the Asahi river, Shimoda-machi, Kamo gun, Shizuoka Prefecture, Japan, in October 1968.

Adult rats and cats were experimentally exposed to *P. sadoensis* or *P. ohirai* metacercariae separated from the aforementioned crabs. The adult worms of the respective species were harvested from the above infected animals 50 days after exposure.

P. sadoensis cercariae used for experimental infection of crabs were obtained from both experimentally infected *O. minima* and *A. parasitologica* snails 140–143 days after exposure, similarly, *P. ohirai* cercariae used were harvested from both previously infected *A. parasitologica* and *O. minima* snails 130 days after exposure.

P. dehaani crabs used for experimental infection were collected from small streams in Aikawa-machi, Aiko gun, Kanagawa Prefecture, Japan, in April 1969. Uninfected *S. dehaani* crabs were collected near the mouth of the Koide river, Chigasaki city, Kanagawa Prefecture, Japan, in April 1969. Previous investigations on 169 *P. dehaani* and 170 *S. dehaani* crabs revealed that both crabs were negative for natural *Paragonimus* infection.

Five—six of either male or female *P. dehaani* crabs were housed in a large plastic box (300 mm long, 280 mm wide and 90 mm high), the bottom of which was layered with washed gravel and contained water 15 mm deep. Water in the container was changed daily. Crabs were fed daily with either boiled lettuce or commercial foods for raising tropical fish (Kinuta Dobutsu En Co., Tokyo). Fifteen—sixteen *S. dehaani* crabs were raised in a large styrol box (410 mm in length and width, and 350 mm in height) with many small holes on its lateral sides and cover, and layered with soil (50–70 mm deep). The soil was sprayed once a week to keep it moistened and changed once during the period of experiment. *S. dehaani* was fed with a small amount of apple once a week.

Crab exposure to cercariae was performed by immersing a single crab in a wide-mouthed glass bottle (53 mm in diameter and 73 mm in height) which contained 50 actively moving cercariae in dechlorinated water 5–7 mm deep. This bottle was covered with a plastic lid with a hole measuring approximately 10 mm in diameter. Crab exposure lasted for 22–24 hours in both species and was carried out at 18–28°C for *P. sadoensis* and at 16–26°C for *P. ohirai*. At the completion of exposure, the crabs were returned to the aforementioned containers. Water temperature for raising *P. dehaani* crabs varied from 20 to 25°C throughout the course of experiments while room temperature for raising *S. dehaani* varied within 20–30°C.

Exposed crabs were crushed to investigate metacercarial infection 56–76 days after exposure. Crab size was determined by measuring the maximum width of the carapace. The crab liver was removed and the remaining body parts were cut up with a pair of scissors. The liver was carefully checked under a dissecting microscope for metacercariae which were then collected. The liver and remainining body parts were crushed separately using a Waring blender. The resulting homogenates were strained through a metal sieve and sedimented in a 1-liter conical graduate. The supernate was decanted off and water was added to the sediment. This procedure was repeated until the supernate became clear. The metacercariae were then collected from the clean sediments under a dissecting microscope and placed in a watch glass with 0.6% saline solution.

Measurements of encysted metacercariae were made on fresh materials under very slight cover glass pressure. These metacercariae were used for the experimental infection of adult white, laboratory rats. Photomicrography of the metacercariae was done using a phase contrast microscope.

Excystation of the metacercariae was performed by the method described by Komiya & Tomimura (1964). The resulting excysted larvae were fixed with 10% hot formalin and measured under slight cover glass pressure. Morphological observations of the metacercariae were made on both the above described fresh and fixed materials.

Table 1 Infection rates and incidence of metacercariae in *Potamon dehaani* and *Sesarma dehaani* crabs exposed to *Paragonimus sadoensis* cercariae originating from *Oncomelania minima* snails

Crab species	Sex of crab	No. of crabs exposed	Size of crab in mm (mean)	No. of CER exposed/ crab	No. of crabs		% positive	Total No. of MC in infected crabs	No. of MC per crab	
					Examined	Infected			Maximum	Mean
*P. dehaani**	♂	16	15-22(19.0)	50	13	6	46.2	24	6	4.0
	♀	16	17-23(20.3)	50	15	5	33.3	18	11	3.6
		32†	15-23(19.7)		28	11	39.3	42	11	3.8
*S. dehaani***	♂	15	19-28(22.2)	50	13	7	53.8	24	6	3.4
	♀	15	20-26(23.2)	50	13	5	38.5	9	3	1.8
		30†	19-28(22.7)		26	12	46.2	33	6	2.8

CER=cercaria. MC=metacercaria. * Examined 61-75 days after exposure. ** Examined 56-64 days after exposure. † Total.

Table 2 Infection rates and incidence of metacercariae in *Potamon dehaani* and *Sesarma dehaani* crabs exposed to *Paragonimus sadoensis* cercariae originating from *Assiminea parasitologica* snails

Crab species	Sex of crah	No. of crabs exposed	Size of crab in mm (mean)	No. of CER exposed/ crab	No. of crabs		% positive	Total No. of MC in infected crabs	No. of MC per crab	
					Examined	Infected			Maximum	Mean
*P. dehaani**	♂	16	17-23(19.1)	50	15	8	53.3	30	8	3.8
	♀	16	17-21(19.6)	50	11	3	27.3	24	19	8.0
		32†	17-23(19.3)		26	11	42.3	54	19	4.9
*S. dehaani***	♂	14	20-26(21.6)	50	13	10	76.9	27	7	2.7
	♀	14	19-26(23.2)	50	14	5	35.7	13	4	2.6
		28†	19-26(22.4)		27	15	55.6	40	7	2.7

CER=cercaria. MC=metacercaria. * Examined 57-76 days after exposure. ** Examined 56-64 days after exposure. † Total.

Results

1. Experimental infection of *P. dehaani* and *S. dehaani* crabs with *P. sadoensis* cercariae

At the completion of crab exposure, only 1-3 dead cercariae were found in the exposing bottles of both crab species.

Results of experimental infection of crabs with cercariae originating from *O. minima* and from *A. parasitologica* are summarized in Tables 1 and 2 respectively. The results indicated that *S. dehaani* crabs were easily infested with *P. sadoensis* metacercariae. Crab infection rate was higher in *S. dehaani* (46-56%) than *P. dehaani* crabs (39-42%). However, mean metacercarial burden per crab was slightly higher in *P. dehaani* (3.8-4.9) than in *S. dehaani* crabs (2.7-2.8) (Tables 1 & 2). Male crabs of both species showed higher infection rates than females (Tables 1 & 2).

The results of metacercarial distribution within the crab body indicated that metacercariae infested not only the liver but also other body parts, including muscles and other tissues (Table 3).

2. Morphology of *P. sadoensis* metacercariae

The cyst of encysted metacercariae consists of the inner and outer cyst walls. The former is approximately 3.5 μ thick while the latter is approximately 2.0 μ thick and easily broken. The size of encysted metacercariae obtained from the two species of crabs exposed to the cercariae originating from both *O. minima* and *A. parasitologica* was similar (Table 4).

Metacercarial shape varied from round to ellipsoidal as shown in Figs. 1-8 and as indicated in the variation in ratio of inner cyst length to width (Table 4). No significant difference in ratio of inner cyst length to width was found between metacercariae obtained from *P. dehaani* and *S. dehaani* crabs both of which were exposed to cercariae originating from *O. minima* snails. However, a statistically significant difference in the above ratio was found between metacercariae obtained from the two species of crabs which were exposed to cercariae originating from *A. parasitologica* ($p<0.05$; t-test).

The body surface of excysted metacercariae is covered with a thin cuticular layer which is beset with singly spaced minute spines. The distribution of the spines is sparse in the posterior body half as compared with that in the anterior body half. The spines appear to be slightly smaller in the former

Table 3 Distribution of *Paragonimus sadoensis* and *P. ohirai* metacercariae in experimentally exposed *Potamon dehaani* and *Sesarma dehaani* crabs

Species	Crab hosts	Total No. of		In liver only		In body parts other than liver		In both liver and other body parts	
		Crabs examined	MC found	No. of crabs infested with MC (%)	No. of MC found (%)	No. of crabs infested with MC (%)	No. of MC found (%)	No. of crabs infested with MC (%)	No. of MC found (%)
P. sadoensis from OM	PD	11	42	0(0)	0(0)	6(54.5)	14(33.3)	5(45.5)	28(66.7)
	SD	12	33	4(33.3)	11(33.3)	5(41.7)	11(33.3)	3(25.0)	11(33.3)
P. sadoensis from AP	PD	11	54	3(27.3)	5(9.3)	2(18.2)	2(3.7)	6(54.5)	47(87.0)
	SD	15	40	3(20.0)	7(17.5)	5(33.3)	9(22.5)	7(46.7)	24(60.0)
P. ohirai from AP	SD	26	205	3(11.5)	10(4.9)	6(23.1)	21(10.2)	17(65.4)	174(84.9)
	PD	9	28	0(0)	0(0)	7(77.8)	21(75.0)	2(22.2)	7(25.0)
P. ohirai from OM	SD	10	24	4(40.0)	6(25.0)	4(40.0)	8(33.3)	2(20.0)	10(41.7)
	PD	2	5	0(0)	0(0)	2(100.0)	5(100.0)	0(0)	0(0)

MC=metacercaria. OM=*Oncomelania minima*. AP=*Assiminea parasitologica*.
PD=*Potamon dehaani*. SD=*Sesarma dehaani*.

Table 4 Measurements of encysted metacercariae of *Paragonimus sadoensis* obtained from experimentally exposed *Potamon dehaani* and *Sesarma dehaani* crabs (in microns)

Cercarial origins	crab hosts	No. of MC measured*	Inner cyst		Ratio of length/ width	Thickness of inner cyst wall	Thickness of outer cyst wall
			Length	Width			
O. minima	PD	28	214-298 (260.4±21.0)	173-224 (198.3±12.6)	1.01-1.72 (1.320±0.145)	1.9-4.1 (3.3±0.6)	1.5-2.6 (2.0±0.3)
	SD	25	240-293 (268.0±15.0)	180-257 (213.7±21.9)	1.03-1.59 (1.267±0.151)	2.5-4.5 (3.2±0.5)	1.5-2.6 (2.0±0.2)
A. parasitologica	PD	38	232-332 (267.8±21.1)	197-284 (223.0±21.4)	1.00-1.40 (1.206±0.091)	2.6-4.6 (3.5±0.5)	1.2-2.8 (1.9±0.4)
	SD	21	214-289 (266.6±18.3)	184-245 (211.1±14.5)	1.02-1.43 (1.268±0.115)	2.6-4.8 (3.5±0.6)	1.3-2.3 (1.8±0.3)

MC=metacercaria. PD=*Potamon dehaani*. SD=*Sesarma dehaani*. * Metacercariae obtained 56-61 days after exposure. Figures in parentheses show mean with standard deviation.

Table 5 Measurements of excysted metacercariae of *Paragonimus sadoensis* obtained from experimentally exposed *Potamon dehaani* and *Sesarma dehaani* crabs (in microns)

Cercarial origins		*O. minima*		*A. parasitologica*	
Crab hosts		*P. dehaani*	*S. dehaani*	*P. dehaani*	*S. dehaani*
No. of MC measured*		10	5	12	6
Body	Length	441-578 (503.8)	381-596 (495.0)	337-643 (480.9)	408-530 (466.5)
	Width	197-255 (228.4)	209-245 (233.8)	163-245 (218.8)	184-238 (210.8)
Thickness of cuticle		3 - 5 (3.4)	3 - 5 (4.2)	4 - 6 (4.4)	4 - 5 (4.2)
Oral sucker	Length	54 - 65 (59.3)	58 - 68 (63.0)	47 - 68 (59.0)	52 - 62 (55.5)
	Width	68 - 81 (73.8)	61 - 86 (74.8)	62 - 83 (73.3)	66 - 78 (73.3)
Stylet	Length	17.3-19.9(18.4)**	12.2-20.2(15.9)	13.3-27.2(19.5)***	11.2-17.4(13.8)
	Width	3.6 - 5.6(4.2)	3.1 - 3.6(3.3)	3.1 - 5.1(3.9)	2.6 - 4.1(3.0)
Acetabulum	Length	73 - 85 (78.7)	65 - 87 (77.6)	65 - 82 (76.3)	70 - 83 (76.0)
	Width	78 - 85 (81.9)	68 - 90 (81.6)	64 - 85 (76.5)	70 - 83 (78.5)
Pharynx	Length	28 - 39 (33.4)	26 - 36 (31.2)	29 - 37 (31.7)	29 - 35 (32.3)
	Width	30 - 39 (35.0)	29 - 38 (35.4)	30 - 39 (33.8)	27 - 36 (32.8)
Excretory bladder	Length	316-428 (359.7)	232-449 (335.2)	224-459 (338.9)	268-386 (313.0)
	Width	81-101 (94.9)	75 - 99 (88.8)	60-101 (82.7)	55 - 82 (66.2)

MC=metacercaria. * Metacercariae obtained 64-68 days after exposure. Figure in parentheses shows mean. ** Nine stylets were measured. *** Eight stylets were measured.

area than in the latter. The body is generally transparent except for the area of the excretory bladder. Red granule deposition in the larval body was almost absent in metacercariae obtained from *P. dehaani*. Conversely, the presence of the red granular deposition was usually found in metacercariae derived from *S. dehaani* crabs although the quantity of granules deposited was variable. Measurements of the excysted metacercariae are shown in Table 5.

The oral sucker is terminally located, round, and provided with a stylet in the anterio-dorsal region. The stylet length varied considerably (Table 5). The acetabulum is rounded and located at the mid-line of the body or slightly anterior to the mid-line. The acetabulum is larger than the oral sucker.

The mouth passes through the oral sucker and directly connects with the subglobular pharynx. The prepharynx is absent. The pharynx was often found slightly buried in the posterior part of the oral sucker. The esophagus is short and divides into two intestinal tracts which run along both lateral sides of the acetabulum, and, after several convolutions, terminate near the latero-posterior ends of the body. The intestines are transparent and usually appear to be vacant, rarely containing fragments.

The excretory bladder is "I" shaped and located in the median part of the body extending from just behind the intestinal bifurcation to the vicinity of the posterior end of the body. The posterior end of the bladder becomes narrow and opens as an excretory pore at the dorso-posterior end of the body. The excretory bladder is filled with large amounts of fine, refractive fragments which are dark brown or black colored in appearance.

A nerve commissure was seen between the pharynx and the intestinal bifurcation.

Measurements of the excysted metacercariae obtained from both *P. dehaani* and *S. dehaani* crabs were very similar (Table 5). The stylet length was $18.4\pm0.97\,\mu$ and $15.9\pm3.03\,\mu$ in the respective metacercariae obtained from *P. dehaani* and *S. dehaani* crabs, both of which were exposed to cercariae originating from *O. minima* snails. No significant diffe-

rence was found between the above two values. To the contrary, the stylet length was 19.5±5.99 μ and 13.8±2.04 μ in metacercariae obtained from *P. dehaani* and *S. dehaani* crabs respectively, both of which were exposed to the cercariae originating from *A. parasitologica* snails. Difference in the stylet length was statistically significant ($p<0.05$; t-test).

3. Experimental infection of *S. dehaani* and *P. dehaani* crabs with *P. ohirai* cercariae

At the completion of exposure, only 1–4 dead or surviving cercariae were found in several exposing bottles of both crab species.

Results of experimental infections of crabs with cercariae originating from *A. parasitologica* and *O. minima* snails are summarized in Tables 6 and 7 respectively. These results revealed that the crab infection rate was low in *P. dehaani* (11–26%) as compared with *S. dehaani* (67–76%) but that *P. ohirai* cercariae originating from *O. minima* snails had infectivity against both species of crabs. Infection rates in *P. dehaani* were high in males, whereas the infection rates of male crabs in *S. dehaani* were almost identical to those of females. Metacercarial burden per crab was higher in *S. dehaani* than in *P. dehaani* (Table 6).

Metacercarial distribution in crab body was analogous to those of *P. sadoensis* (Table 3). In *P. dehaani* crabs, however, the metacercariae were mainly recovered from body parts other than the liver.

Table 6 Infection rates and incidence of metacercariae in *Sesarma dehaani* and *Potamon dehaani* crabs exposed to *Paragonimus ohirai* cercariae originating from *Assiminea parasitologica* snails

Crab species	Sex of crab	No. of crabs exposed	Size of crab in mm (mean)	No. of CER exposed/ crab	No. of crabs		% positive	Total No. of MC in infected crabs	No. of MC per crab	
					Examined	Infected			Maximum	Mean
*S. dehaani**	♂	20	19–32(25.2)	50	17	13	76.5	98	19	7.5
	♀	20	19–29(23.0)	50	20	15	75.0	138	22	9.2
		40†	19–32(24.0)		37	28	75.7	236	22	8.4
*P. dehaani***	♂	20	15–22(18.9)	50	18	8	44.4	27	6	3.4
	♀	21	18–23(20.2)	50	17	1	5.9	1	1	1.0
		41†	15–23(19.5)		35	9	25.7	28	6	3.1

CER=cercaria. MC=metacercaria. * Examined 57–64 days after exposure. ** Examined 58–63 days after exposure. † Total.

Table 7 Infection rates and incidence of metacercariae in *Sesarma dehaani* and *Potamon dehaani* crabs exposed to *Paragonimus ohirai* cercariae originating from *Oncomelania minima* snails

Crab species	Sex of crab	No. of crabs exposed	Size of crab in mm (mean)	No. of CER exposed/ crab	No. of crabs		% positive	Total No. of MC in infected crabs	No. of MC per crab	
					Examined	Infected			Maximum	Mean
*S. dehaani**	♂	9	20–31(23.0)	50	8	4	50.0	7	4	1.8
	♀	10	20–23(21.9)	50	7	6	85.7	17	6	2.8
		19†	20–31(22.5)		15	10	66.7	24	6	2.4
*P. dehaani***	♂	10	15–24(17.6)	50	10	2	20.0	5	3	2.5
	♀	10	17–23(20.6)	50	8	0	0	0	0	0
		20†	15–24(18.9)		18	2	11.1	5	3	2.5

CER=cercaria. MC=metacercaria. * Examined 56–59 days after exposure. ** Examined 57 days after exposure. † Total.

Table 8 Measurements of encysted metacercariae of *Paragonimus ohirai* obtained from experimentally exposed *Sesarma dehaani* and *Potamon dehaani* crabs (in microns)

Cercarial origins	Crab hosts	No. of MC measured*	Inner cyst		Ratio of length/width	Thickness of inner cyst wall	Thickness of outer cyst wall
			Length	Width			
A. parasitologica	SD	60	221-342 (293.7±24.6)	173-226 (197.4±11.5)	1.11-1.81 (1.493±0.152)	2.6-4.1 (3.6±0.3)	1.5-2.6 (2.0±0.3)
	PD	13	243-337 (290.0±26.6)	199-278 (231.4±27.4)	1.08-1.60 (1.263±0.155)	3.1-4.4 (3.5±0.4)	1.9-2.6 (2.1±0.2)
O. minima	SD	13	250-380 (301.0±39.1)	184-286 (229.4±37.7)	1.10-1.76 (1.332±0.195)	2.0-3.6 (2.9±0.5)	1.5-2.2 (1.9±0.2)
	PD	5	217-273 (239.8±21.7)	200-219 (207.2± 7.1)	1.02-1.37 (1.162±0.138)	3.3-3.9 (3.6±0.2)	1.5-2.6 (2.1±0.5)

MC=metacercaria. SD=*Sesarma dehaani*. PD=*Potamon dehaani*. * Metacercariae obtained 56-59 days after exposure. Figures in parentheses show mean with standard deviation.

4. Morphology of *P. ohirai* metacercariae

The morphology of *P. ohirai* encysted metacercariae corresponded to that of *P. sadoensis*. Measurements of the encysted metacercariae are presented in Table 8. The sizes of metacercariae obtained from both *S. dehaani* and *P. dehaani* were similar to each other. The metacercariae were generally ellipsoidal in form but frequently rounded (Figs. 9-16, & Table 8).

A statistically significant difference in ratio of inner cyst length to width was found between metacercariae obtained from *S. dehaani* and *P. dehaani*, both of which were exposed to cercariae originating from *A. parasitologica* ($p<0.01$); the shape was more ellipsoidal in metacercariae from *S. dehaani* than in those from *P. dehaani*. A comparison of metacercarial shape was made between metacercariae obtained from *S. dehaani* and *P. dehaani*, both of which were exposed to cercariae originating from *O. minima*. No significant difference in ratio of inner cyst length to width was found between metacercariae from the two species of crabs.

The morphology of *P. ohirai* excysted metacercariae resembled that of *P. sadoensis*. As noted in *P. sadoensis*, red granule deposition in the larval body was usually found in metacercariae separated from *S. dehaani*, conversely, the red granule deposition was scarcely seen in metacercariae from *P. dehaani*.

Measurements of excysted metacercariae obtained from both *S. dehaani* and *P. dehaani* were closely related (Table 9). As noted in *P. sadoensis*, considerable variation was seen in stylet length. A statistically significant difference was found between the stylet length ($16.9\pm3.35\,\mu$) of metacercaria from *S. dehaani* and that ($18.4\pm0.74\,\mu$) from *P. dehaani* ($p<0.05$), both of which were exposed to the cercariae originating from *A. parasitologica*; the stylet length was slightly larger in metacercariae from *P. dehaani* than in those from *S. dehaani*.

5. Comparison between *P. sadoensis* and *P. ohirai* metacercariae

Special attention was given to a comparison of *P. sadoensis* and *P. ohirai* metacercariae, originating from their own natural snail and crab hosts (Tables 4, 5, 8 & 9).

A highly significant difference was found both in inner cyst length and in ratio of inner cyst length to width at the level of $p<0.001$, but not in inner cyst width between the two species; this suggests that *P. sadoensis* metacercariae are inclined to be round whereas *P. ohirai* metacercariae are ellipsoidal.

P. ohirai excysted metacercariae were slightly larger than *P. sadoensis*. The excretory bladder of *P. ohirai* metacercaria was also slightly longer than that of *P. sadoensis*;

Table 9 Measurements of excysted metacercariae of *Paragonimus ohirai* obtained from experimentally exposed *Sesarma dehaani* and *Potamon dehaani* crabs (in microns)

Cercarial origins		*A. parasitologica*		*O. minima*
Crab hosts		*S. dehaani*	*P. dehaani*	*S. dehaani*
No. of MC measured*		10	4	10
Body	Length	530-625 (585.5)	449-591 (523.0)	449-565 (518.9)
	Width	196-262 (229.9)	245-265 (254.8)	224-265 (243.0)
Thickness of cuticle		4 - 5 (4.4)	4 - 5 (4.5)	3 - 5 (3.9)
Oral sucker	Length	53 - 75 (63.9)	62 - 68 (65.3)	55 - 73 (60.9)
	Width	71 - 81 (76.4)	75 - 82 (78.0)	70 - 81 (73.3)
Stylet	Length	12.2-27.5(16.9)**	17.3-19.4(18.4)***	12.2-24.5(16.1)****
	Width	3.6 - 5.6(4.2)	4.4 - 5.6(4.9)	3.6 - 4.8(4.2)
Acetabulum	Length	81 - 86 (83.2)	78 - 82 (80.5)	81 - 88 (83.4)
	Width	81 - 86 (82.8)	83 - 88 (85.3)	75 - 88 (83.3)
Pharynx	Length	30 - 43 (34.7)	37 - 44 (39.3)	32 - 38 (34.0)
	Width	33 - 40 (36.4)	37 - 39 (38.0)	35 - 42 (38.3)
Excretory bladder	Length	388-479 (439.3)	332-452 (400.0)	316-421 (380.3)
	Width	62-112 (86.7)	109-117 (111.0)	62-125 (100.8)

MC=metacercaria. * Metacercariae obtained 59-63 days after exposure. Figure in parentheses shows mean. ** Twenty-one stylets were measured. *** Ten stylets were measured. **** Eleven stylets were measured.

Note: Five metacercariae obtained from *P. dehaani* crabs which were exposed to cercariae originating from *O. minima* snails, were not measured since they were used for the experimental infection of a rat.

the ratio of body length to bladder length was 1.4 in *P. sadoensis* and 1.3 in *P. ohirai*. The stylet length was $18.4\pm0.97\,\mu$ and $16.9\pm3.35\,\mu$ in *P. sadoensis* and *P. ohirai* respectively. No statistically significant difference was found between them.

Discussion

Miyazaki *et al.* (1968) reported that dissimilarity in the ecology of the first and second intermediate hosts was one of the differences between *P. sadoensis* and *P. ohirai*. Yoshimura *et al.* (1970) investigated the susceptibility of *O. minima* and *A. parasitologica* snails to infection with these lung flukes, reporting that the ecological differences in the first intermediate hosts could not be considered as essential differentiating characters between the two parasite species. The present investigations on the second intermediate hosts revealed that crab infection rate against *P. sadoensis* was higher in *S. dehaani* (46-56%) than in *P. dehaani* (39-42%); thus, there is no doubt that *S. dehaani* crab, the second intermediate host for *P. ohirai*, is highly susceptible to infection with *P. sadoensis*.

Yoshida (1961a, b) experimentally exposed *S. dehaani* crabs to *P. ohirai* cercariae, stating that the crab infection rates were 85.9% (6/7) in the first study and 75% (3/4) in the second. The present data (67-76%) are analogous to the results reported by Yoshida. It was also shown that *P. dehaani* crab, the second intermediate host of *P. sadoensis*, had susceptibility against *P. ohirai* infection although the crab infection rates were low in *P. dehaani* (11-26%) as compared with those of *S. dehaani*. The susceptibility of both crab species to *P. sadoensis* and *P. ohirai* implies that ecological differences between the second intermediate hosts also cannot be used as a definitive differentiating character

between the two parasite species.

When considering the results of experimental infections of both snail (Yoshimura *et al.*, 1970) and crab hosts, *P. sadoensis* has stronger affinity to the first and second intermediate hosts of *P. ohirai* than *P. ohirai* does to the corresponding hosts of *P. sadoensis*. In *P. ohirai* infection, *P. dehaani* crabs showed different results (low infection rates, less number of metacercariae parasitized and different metacercarial distribution in crab). It is of interest to note that, in *P. dehaani* crabs, *P. ohirai* metacercariae are mainly recovered from body parts other than the liver.

In the experimental infection of *P. dehaani* and *S. dehaani* crabs with *P. sadoensis* cercariae, male crabs of both species always yielded higher infection rates than females. However, since Yoshimura (1969) reported that natural infection rate of *P. dehaani* with *P. sadoensis* metacercariae was 44% (76/174) and 42% (96/231) in male and female crabs respectively (a non-significant difference), the results of the experimental infection do not necessarily coincide with those of natural infections. In the experimental infections of the crabs with *P. ohirai*, no appreciable difference in infection rates was observed between the sexes in *S. dehaani*. In *P. dehaani*, however, *P. ohirai* infection rate was higher in the male than in the female crabs as noted in *P. sadoensis* infection. Further studies are required to determine if any correlation exists between crab sex and metacercarial infections of the two *Paragonimus* species.

In both lung flukes, neither cercariae originating from *O. minima* nor those from *A. parasitologica* produced appreciably different results pertaining to both crab infection and metacercarial morphology. This indicates that both *P. sadoensis* cercariae originating from *A. parasitologica* and *P. ohirai* cercariae from *O. minima* have almost the same infectivity against the second intermediate hosts as the respective cercariae originating from their natural first intermediate hosts.

Kawashima *et al.* (1967a) and Miyazaki *et al.* (1968) reported that the inner cyst length of *P. sadoensis* encysted metacercariae was shorter, i.e., more round in shape, than those of *P. ohirai*. However, the former authors noted that metacercarial differentiation of the two species by measurements of a single specimen may be difficult. A large variation in ratio of inner cyst length to width was found in the present investigations on *P. sadoensis* encysted metacercariae, i.e., the shape varied from round to ellipsoidal. Such a variation was also notable in *P. ohirai* encysted metacercariae. Kawashima *et al.* (1967a) reported that average ratio of inner cyst length to width was 1.14 in *P. sadoensis* encysted metacercariae and 1.29 in *P. ohirai* metacercariae and that the difference between those values was statistically significant. Corresponding values seen in the present study were 1.320 in *P. sadoensis* metacercariae and 1.493 in *P. ohirai*, a difference of which was also statistically significant. Yoshida (1961b) reported that mean length of *P. ohirai* metacercaria obtained from experimentally exposed *S. dehaani* was 287.5 μ and mean width was 205.5 μ. These measurements are close to the present results (mean : 293.7 $\mu \times 197.4$ μ). When the present authors calculated the ratio of inner cyst length to width using the measurements described by Yoshida (1961b), the value was 1.399 (287.5/205.5). This value is still smaller than the present result (1.493). From the above, it is concluded that *P. sadoensis* metacercariae are inclined to be more round than *P. ohirai* metacercariae as previously pointed out by Kawashima *et al.* (1967a) and Miyazaki *et al.* (1968). Definite identification of each species by metacercarial shape is difficult even if many metacercariae are measured, since the shape and size of both *P. sadoensis* and *P. ohirai* encysted metacercariae are frequently very similar to each other.

Morphology of *P. sadoensis* and *P. ohirai* excysted metacercariae coincided with the respective descriptions previously made by Kawashima *et al.* (1967a) and Miyazaki *et al.* (1968) for the former species and by Yokogawa *et al.* (1960) for the latter. Some in-

teresting findings were seen in the red granule deposition of the larval body. In both *P. sadoensis* and *P. ohirai*, the metacercariae derived from *S. dehaani* usually contained red granules, conversely, red granule deposition was scarcely found in metacercariae from *P. dehaani* crabs. This phenomenon resembles the observations made by Kawashima *et al.* (1967b), who found the red granules in *P. westermani* metacercariae separated from *Eriocheir japonicus* but not in the metacercariae from *P. dehaani* crabs. The occurrence of red granule deposition in the larval body may be related to differences in the crab hosts, i.e., differences in various physiological conditions of crabs, although other reasons such as degree of metacercarial maturity or seasonal variation should be considered. Further evidence of the relationship between red granule deposition and crustacean host can be found in the reports of Ameel (1934) and Tang (1940). Accordingly, it seems unlikely that dissimilarity in metacercarial red granule deposition, previously suggested by Miyazaki *et al.* (1968), can be used as a differentiating character between the two species.

Kawashima *et al.* (1967a) reported that *P. ohirai* excysted metacercariae were larger than *P. sadoensis* and that the ratio of body length to excretory bladder length was slightly smaller in *P. ohirai* than in *P. sadoensis*. The present data confirmed the above observations made by Kawashima *et al.*

Mean stylet length of *P. ohirai* metacercariae observed in this study was larger than any measurements reported by Miyazaki (1939, 1947), Yokogawa *et al.* (1960), Kawashima *et al.* (1967a) and Miyazaki *et al.* (1968). However, it is well known that metacercarial stylet length is more or less related to the degree of metacercarial maturity. The present metacercariae used for measurements were obtained from crabs 59–63 days after exposure. The stylet length of *P. sadoensis* was almost identical with the data reported by Kawashima *et al.* (1967a) and Miyazaki *et al.* (1968). The stylet length of metacercariae obtained 59–68 days after exposure is slightly longer in *P. sadoensis* than in *P. ohirai*, as previously indicated by Kawashima *et al.* (1967a) and Miyazaki *et al.* (1968). However, it is doubtful whether stylet length can be used as a definitive species characteristic due to variability in its length.

In conclusion, ecological differences which exist between the second intermediate hosts do not necessarily result in essential differentiating characters between *P. sadoensis* and *P. ohirai*. Besides, differentiation of the two species by their metacercarial morphology is passably difficult. When considering the data on immunoelectrophoretic (Yokogawa *et al.*, 1968) and disc electrophoretic patterns (Yoshimura, 1969) of adult worms, morphology of the rediae and cercariae (Ito *et al.*, 1969), susceptibility of the first intermediate hosts (Yoshimura *et al.*, 1970), and the present experiments on the second intermediate hosts and metacercarial morphology, *P. sadoensis* is very closely related to *P. ohirai*.

Summary

Potamon dehaani and *Sesarma dehaani* crabs were experimentally exposed to *Paragonimus sadoensis* or *P. ohirai* cercariae, both of which were developed in *Oncomelania minima* and *Assiminea parasitologica* snails. Comparisons of susceptibility of these crabs against the respective lung flukes were conducted and, simultaneously, a comparison of metacercarial morphology was made between the two species.

Both *P. sadoensis* cercariae originating from *A. parasitologica*, and *P. ohirai* cercariae from *O. minima* snails had almost the same infectivity as the cercariae from their natural hosts.

S. dehaani was highly susceptible to infection with *P. sadoensis*, similarly, *P. dehaani* was susceptible to *P. ohirai* infection although infection rate was markedly lower. In *P. sadoensis*, infection rates of both crabs were higher in male than in female crabs. In *P. dehaani*, it was noted that *P. ohirai*

metacercariae were mainly recovered from body parts other than the liver.

In both *P. sadoensis* and *P. ohirai*, appreciable differences were found in the occurrence of red granule deposition in larval body between the metacercariae from *P. dehaani* and *S. dehaani*. The metacercariae of both species obtained from *S. dehaani* contained more or less red granules in their body, conversely, such a red granule deposition was hardly recognized in the metacercariae from *P. dehaani*.

The shape of encysted metacercariae of both species varied from round to ellipsoidal, however, *P. sadoensis* metacercariae were inclined to be round in shape whereas *P. ohirai* metacercariae were ellipsoidal. Variability in metacercarial stylet length was found in both species. The size of *P. ohirai* excysted metacercariae was slightly larger than that of *P. sadoensis*.

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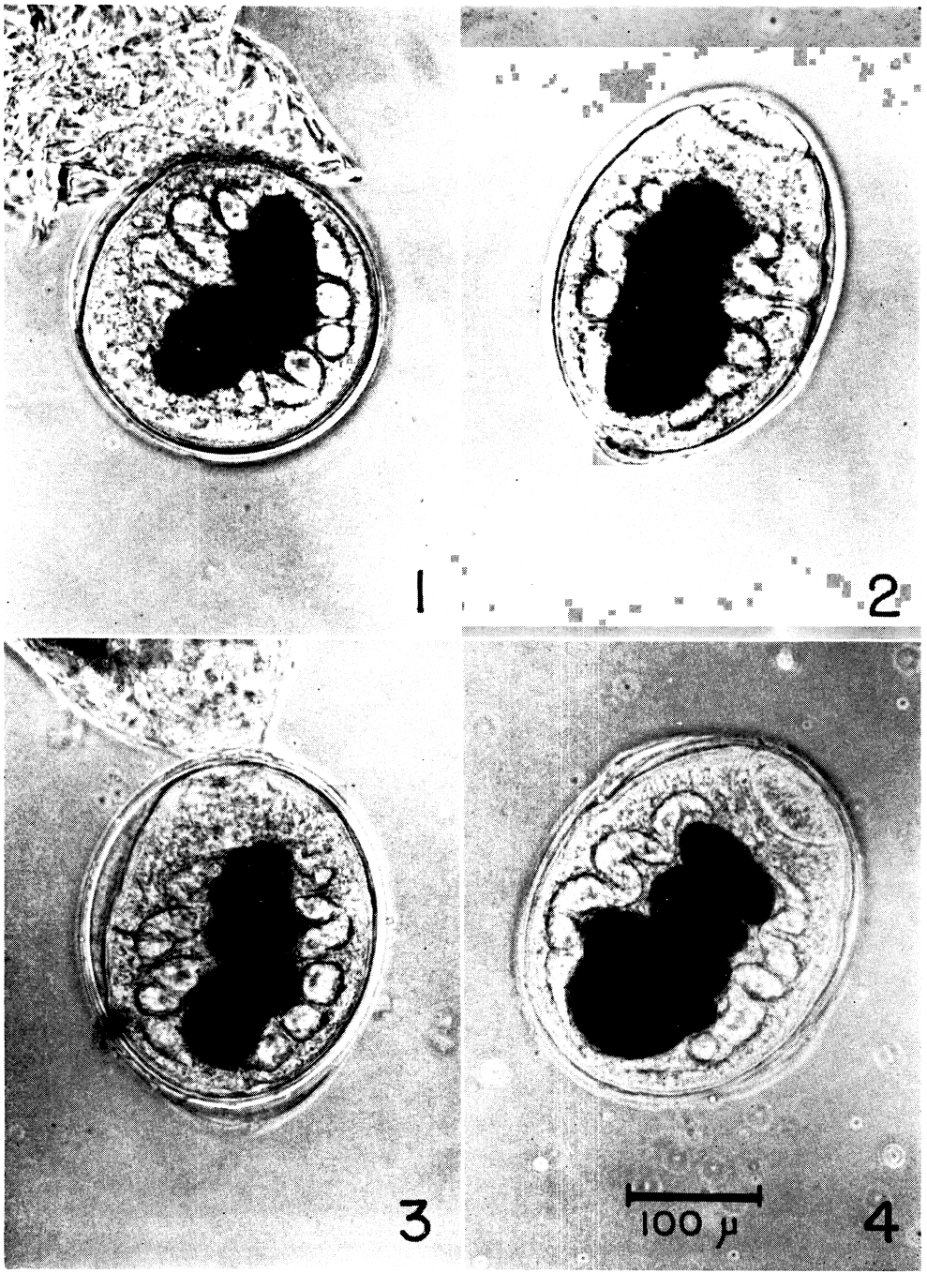
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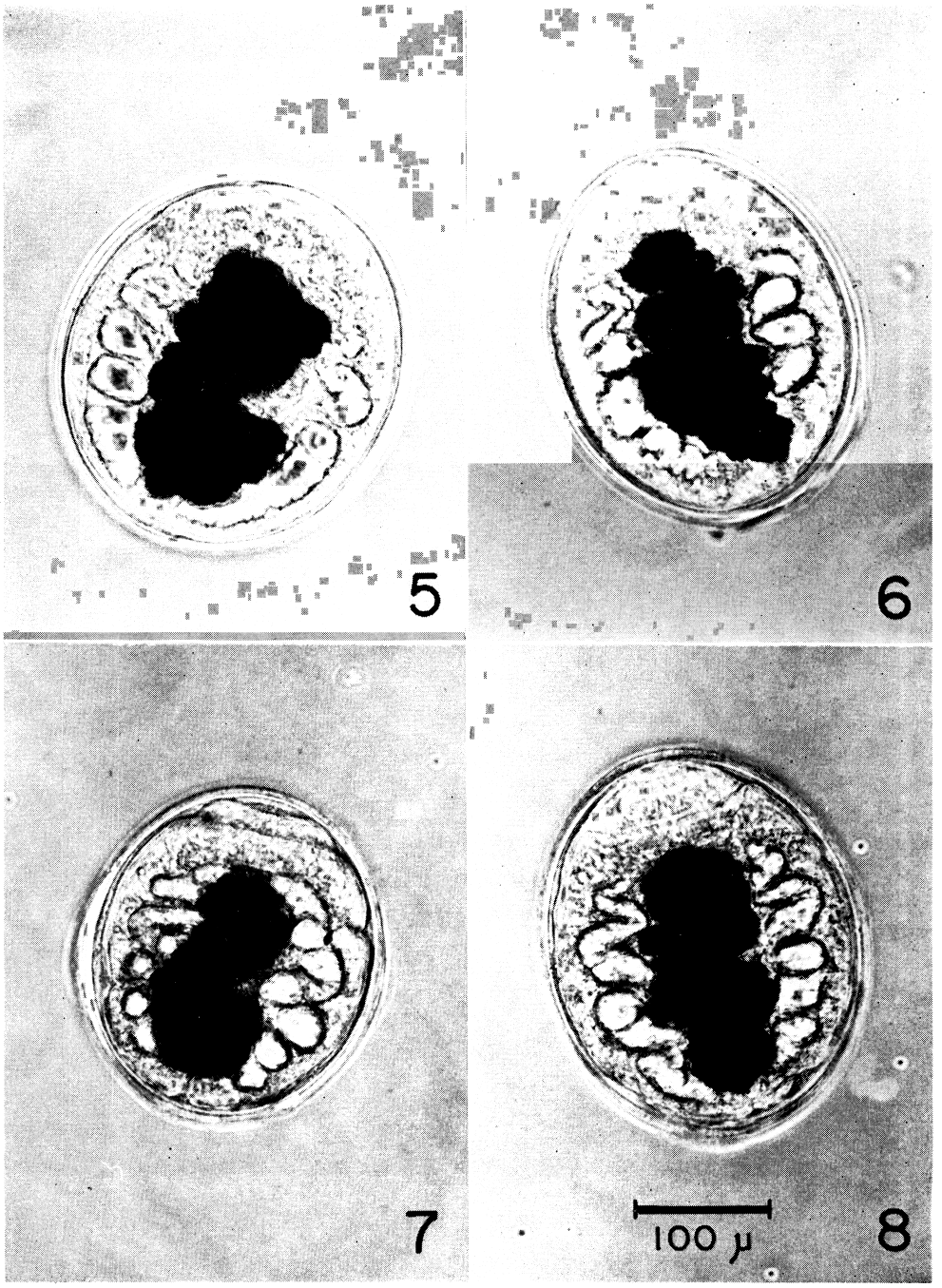
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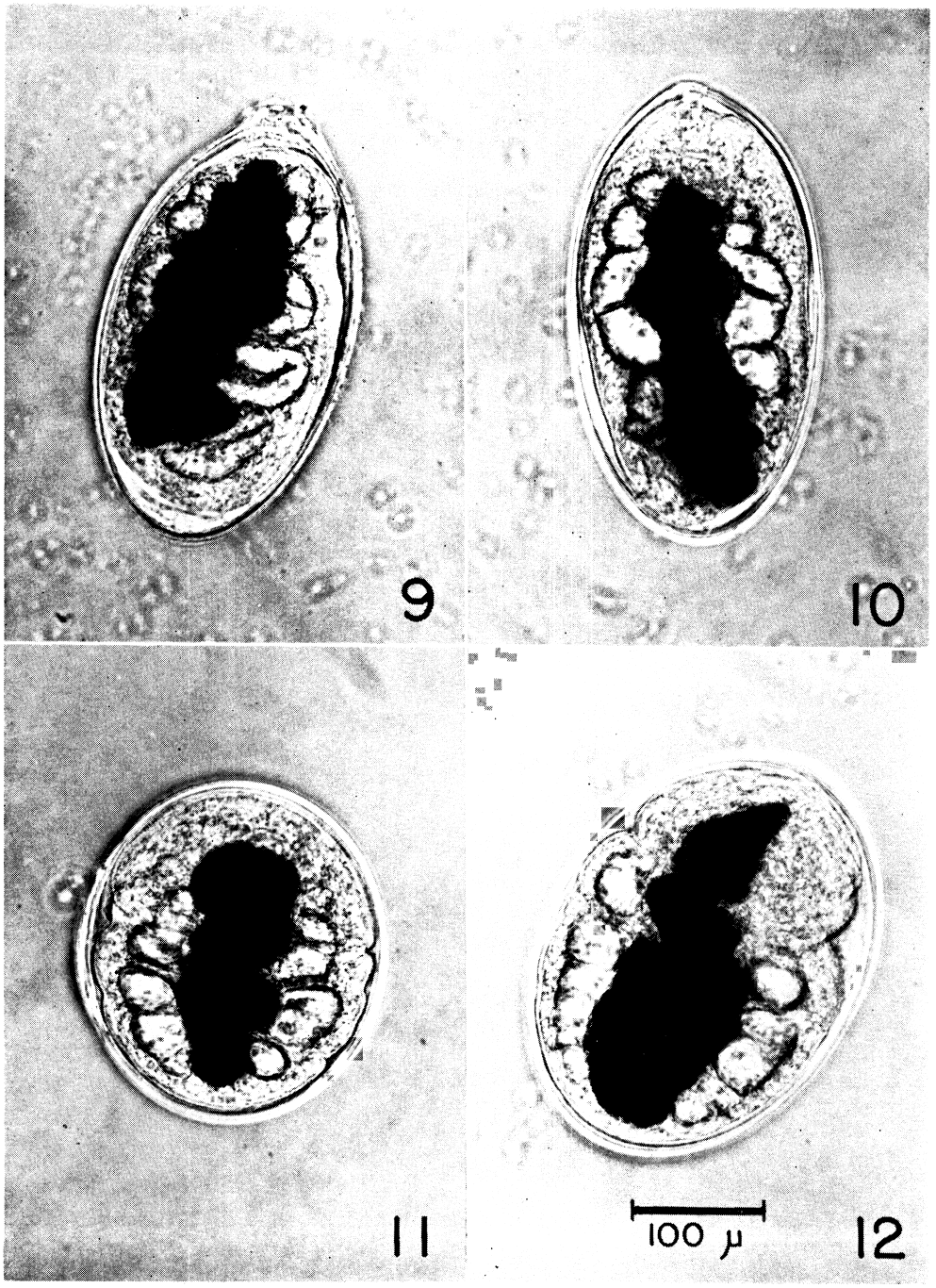
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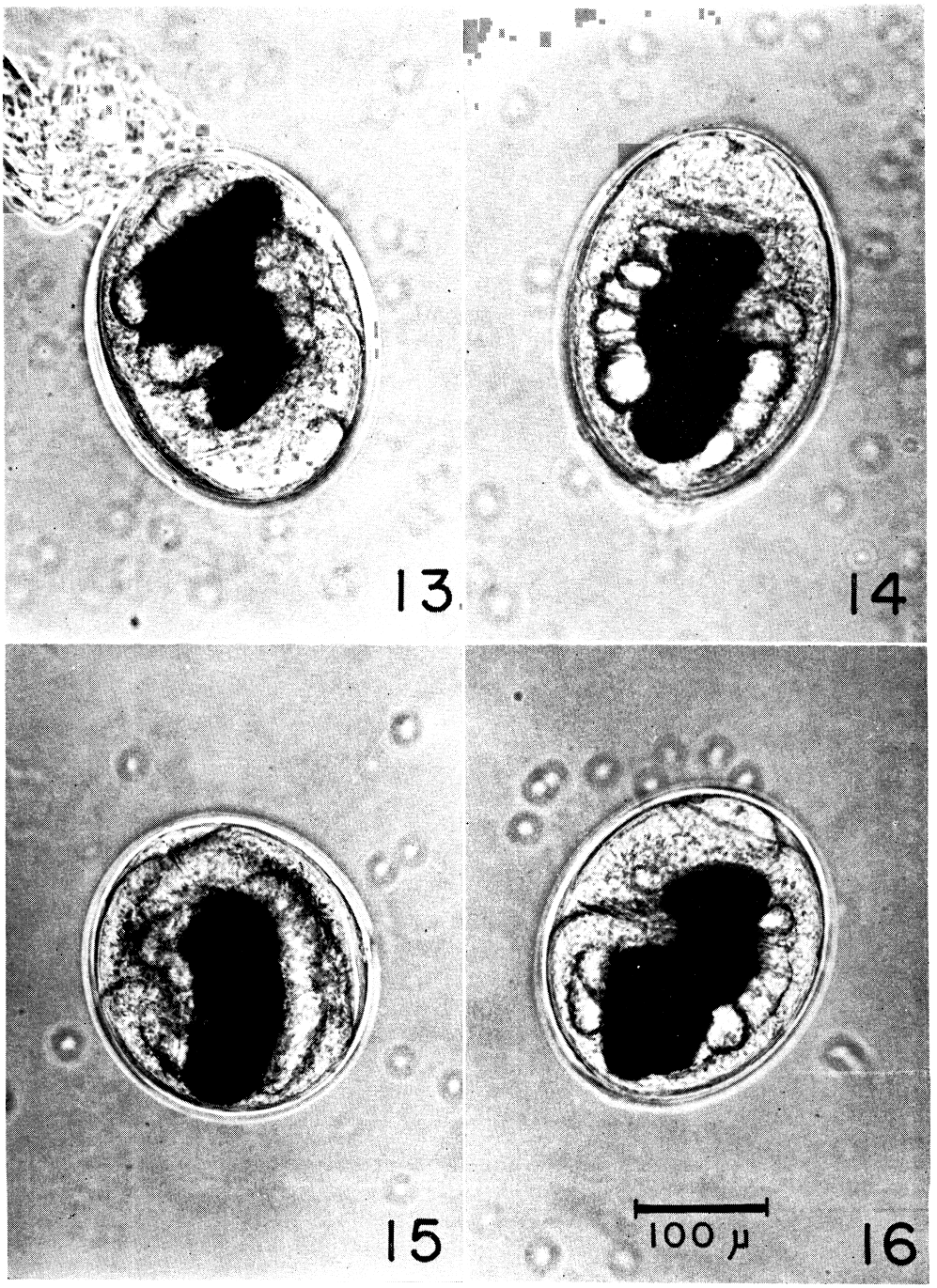
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Explanation of Figures

Figs. 1 & 2 *Paragonimus sadoensis* metacercariae separated from *Potamon dehaani* crabs which were exposed to cercariae originating from *Oncomelania minima* snails.
Figs. 3 & 4 *P. sadoensis* metacercariae separated from *Sesarma dehaani* crabs which were exposed to cercariae originating from *O. minima* snails.
Figs. 5 & 6 *P. sadoensis* metacercariae separated from *P. dehaani* crabs which were exposed to cercariae originating from *Assiminea parasitologica* snails.
Figs. 7 & 8 *P. sadoensis* metacercariae separated from *S. dehaani* crabs which were exposed to cercariae originating from *A. parasitologica* snails.
Figs. 9 & 10 *Paragonimus ohirai* metacercariae separated from *S. dehaani* crabs which were exposed to cercariae originating from *A. parasitologica* snails.
Figs. 11 & 12 *P. ohirai* metacercariae separated from *P. dehaani* crabs which were exposed to cercariae originating from *A. parasitologica* snails.
Figs. 13 & 14 *P. ohirai* metacercariae separated from *S. dehaani* crabs which were exposed to cercariae originating from *O. minima* snails.
Figs. 15 & 16 *P. ohirai* metacercariae separated from *P. dehaani* crabs which were exposed to cercariae originating from *O. minima* snails.

佐渡肺吸虫ならびに大平肺吸虫の比較に関する研究　III.　2種肺吸虫セルカリアのサワガニおよびクロベンケイガニへの感染実験

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佐渡肺吸虫と大平肺吸虫の種差を明らかにすることを目的として，第II報に引き続き，2種肺吸虫の第2中間宿主特異性に差異が見られるかどうかを検討した．すなわち佐渡肺吸虫ならびに大平肺吸虫のセルカリアをサワガニおよびクロベンケイガニに実験的に感染させ，これらのカニにおける2種肺吸虫の感染状況ならびに得られたメタセルカリアの形態を比較した．感染実験に用いたセルカリアは2種肺吸虫のミラシジアをあらかじめ実験的に感染させたナタネミズツボおよびムシヤドリカワザンショウ貝から得たものである．得られた成績は次の通りである．

1)　ムシヤドリカワザンショウで発育した佐渡肺吸虫セルカリアならびにナタネミズツボで発育した大平肺吸虫セルカリアはそれらの固有第1中間宿主内で発育したセルカリアと同様にカニに対する感染力を持つている．

2)　サワガニにおける佐渡肺吸虫の感染率は39～42%であり，クロベンケイガニのそれは46～56%であつた．

3)　クロベンケイガニにおける大平肺吸虫の感染率は67～76%であり，サワガニのそれは11～26%であつた．

4)　佐渡肺吸虫の感染率はいずれのカニの場合も雌より雄において高かつた．大平肺吸虫を感染させたサワガニでは，そのメタセルカリアは肝臓以外の体部から主として見い出された．

5)　佐渡肺吸虫ならびに大平肺吸虫のいずれにおいても，クロベンケイガニから分離されたメタセルカリアでは，多かれ少なかれ体内に紅色顆粒が認められるのに対して，サワガニから分離されたメタセルカリアでは紅色顆粒がほとんど認められなかつた．

6)　2種肺吸虫の被嚢メタセルカリアは円形～楕円形で，その形状にはかなり変異が認められる．しかし，佐渡肺吸虫メタセルカリアの方がやや円形の度が強く，大平肺吸虫のそれはやや楕円形の度が強い傾向を認めた．

7)　2種肺吸虫ともに，メタセルカリアの穿刺棘の長さにはかなりの変異が認められたが，佐渡肺吸虫の穿刺棘は大平肺吸虫のそれよりやや長い様に思われた．

8)　大平肺吸虫の脱嚢メタセルカリアは佐渡肺吸虫のそれよりやや大きい．

以上の成績から，クロベンケイガニが佐渡肺吸虫の感染に対して強い感受性を持つていること，またサワガニが大平肺吸虫感染に対して感受性を有することが解る．また2種肺吸虫メタセルカリアはその形状ならびに大きさの点において互いに類似するものが多く，従つてメタセルカリアの形態学的差異によつて2種を区別することもかなり困難であると思われる．