[Jap. J. Parasit., Vol. 19, No. 2, 136-153, 1970]

Comparative studies on *Paragonimus sadoensis* Miyazaki, Kawashima, Hamajima et Otsuru, 1968 and *P. ohirai* Miyazaki, 1939. II. Susceptibility of *Oncomelania minima* (Bartsch, 1936) Davis, 1969 and *Assiminea parasitologica* Kuroda, 1958 to infection with the lung flukes

KENTARO YOSHIMURA, YOSHIMASA HISHINUMA AND MITSUKO SATO

Department of Medical Zoology, 406th Medical Laboratory
U. S. Army Medical Command, Japan
APO San Francisco 96343

(Received for publication; January 20, 1970)

Introduction

General information relative to the status of *Paragonimus sadoensis* can be found in the first report of this series (Ito *et al.*, 1969). Ito *et al.* could not differentiate the rediae and cercariae between *P. sadoensis* and *P. ohirai* since their morphological features, including the excretory systems, and size were very similar.

Most recently, Kawashima & Hamajima (1969) reported that *Oncomelania minima* (syn. *Tricula minima*) snails could be easily infected with the two lung flukes in experimental infections. Further, they noted that the intramolluscan larval developments of the two species were very similar, but a significant difference in intestinal length was found between *P. sadoensis* and *P. ohirai* rediae.

The purposes of this study are to determine whether *P. sadoensis* can be developed in *Assiminea parasitologica*, the first intermediate host for *P. ohirai*, similarly, whether *P. ohirai* can be developed in *O. minima*, the intermediate host for *P. sadoensis*, and to determine whether significant differences are found in snail infection rates, in periods necessary for the completion of larval development and in larval morphology.

Materials and Methods

Potamon dehaani crabs infected with *P. sadoensis* metacercariae were collected from small streams near the mouth of the Okura-river, Aikawamachi, Sado-gun, Niigata Prefecture, Japan, in September, 1968. At the same time, *O. minima* snails used for infection experiments were collected from the aforementioned area and from streams near Iwayaguchi, in Aikawamachi. *Sesarma dehaani* crabs infected with *P. ohirai* metacercariae and *A. parasitologica* snails used for infection experiments were collected at the mouth of the Asahi-river, Shimoda-machi, Kamo-gun, Shizuoka Prefecture, Japan, in October, 1968.

Snails were maintained as follows: 1) *O. minima*; ten snails were housed per Petri dish (87 mm wide and 20 mm deep). The bottom of the dish was covered with filter paper, upon which was placed a conical shaped mass of soil (25 mm in diameter and 10 mm in height) and a layer of water 3 mm deep, 2) *A. parasitologica*; seventy-five to eighty snails were housed per large plastic box (300 mm long, 280 mm wide and 90 mm high), the bottom of which was layered with soil 10–15 mm deep. The soil was moistened daily with water.

Snails were fed with a small amount of Heinz's rice cereal (H. J. Heinz Co., Pittsburgh, Pa.) once a month.

Adult rats and cats were experimentally infected with the metacercariae of the respective species and autopsied 50 days after infection. All adult worms harvested were incubated overnight in a Petri dish with physiological saline solution at 28°C. Eggs shed from the worms were collected, poured into several centrifuge tubes (25 mm × 120 mm in size), and then incubated at 28°C for 15–19 days in the case of *P. sadoensis* and 16 days for *P. ohirai*. Active miracidia used for snail exposure were obtained by exposing miracidia containing eggs to a light stimulus (Sylvania photoflood No. 2) for 15–20 minutes or under refrigeration (5°C for 15 minutes).

Both *O. minima* and *A. parasitologica* snails which had been previously maintained in the laboratory for 68–71 days and 72 days respectively were used for infection experiments. Snail exposure was performed *en masse* by immersing 10–20 snails in a small dish (45 mm wide and 20 mm deep) which contained miracidia in dechlorinated water. All exposed snails were returned to the aforementioned containers and maintained during the course of the experiment.

Room temperature was recorded using an automatic recording thermometer (Ota Seisakusho). Room temperature for maintaining *P. sadoensis* infected snails varied from 16° to 28°C. Corresponding room temperatures for maintaining *P. ohirai* infected snails varied between 10°—32°C.

Exposed snails were crushed at different times after exposure to examine snail infection rates as well as the intramolluscan larval development of the respective species. Measurements of sporocyst and the first and second generation rediae were made on fresh materials under cover glass pressure. Hot

Table 1 Experimental infection of *Oncomelania minima* and *Assiminea parasitologica* snails with *Paragonimus sadoensis* miracidia

Snails	Snail exposure		Examination of exposed snails			
	No. of snails used	Average No. of Mir. exposed/ snail	Day after exposure	No. of snails examined	No. of snails infected (%)	Larval stages found
O. m	160	20	2	3	2	Spor.
			5 - 30	9	9	Spor.
			45	3	3	Spor. & 1st GR.
			71	2	2	1st & 2nd GR.
			75 - 92	3	3	2nd GR. & Cer.
			100	2	2	Spor., 1st & 2nd GR., & Cer.
			110-184	127	125	2nd GR. & Cer.
			Total	149	146 (98%)	
A. p	160	20	2 - 5	6	0	
			15 - 30	6	3	Spor.
			45	3	1	Spor. & 1st GR.
			71	1	1	1st & 2nd GR.
			75	1	1	Spor., 1st & 2nd GR.
			94	3	1	Spor., 1st & 2nd GR., & Cer.
			102-177	35	23	2nd GR. & Cer.
			Total	55	30 (55%)	

Mir. = miracidia. *O. m* = *Oncomelania minima*. *A. p* = *Assiminea parasitologica*. Spor. = sporocyst. GR. = generation redia. Cer. = cercaria.

Table 2 Experimental infection of *Oncomelania minima* and *Assiminea parasitologica* snails with *Paragonimus sadoensis* miracidia

Snails	Snail exposure		Examination of exposed snails			
	No. of snails used	Average No. of Mir. exposed/ snail	Day after exposure	No. of snails examined	No. of snails infected(%)	Larval stages found
O. m	160*	10	2	3	1	Spor.
			5 - 30	9	5	Spor.
			45	3	2	Spor. & 1st GR.
			71	1	1	1st & 2nd GR.
			75 - 80	3	3	Spor. & 1st GR.
			92	1	1	2nd GR. & Cer.
			101	2	2	Spor., 1st & 2nd GR., & Cer.
			110-184	123	121	2nd GR. & Cer.
			Total	145	136(94%)	
A. p	160**	10	2	3	1	Spor.
			5 - 30	9	0	
			45	3	1	Spor. & 1st GR.
			71	1	1	1st & 2nd GR.
			76-177	24	12	2nd GR. & Cer.
			Total	40	15(38%)	

Mir.=miracidia. *O. m*=*Oncomelania minima*. *A. p*=*Assiminea parasitologica*. Spor.=sporocyst. GR.=generation redia. Cer.=cercaria.

* A total of 155 exposed snails survived during the experiment.

** A total of 53 exposed snails survived during the experiment.

10% formalin was used to fix cercariae which were then measured under slight cover glass pressure. Morphological observations on each larval stage were made on fresh materials.

Results

1. Experimental infection of *O. minima* and *A. parasitologica* snails with *P. sadoensis* miracidia

Experiment 1 : This experiment was divided into two groups; snails were exposed to 20 miracidia per snail in group A, whereas snails in group B were exposed to 10 miracidia. The exposure period lasted for 24 hours in both groups.

The number of living miracidia remaining after completion of exposure was 25 and 11 for groups A and B of *O. minima* and 7 and 31 for *A. parasitologica* respectively.

Five snails died during the period of miracidial exposure in each group of *A. parasitologica*, however, no *O. minima* snails died.

Experimental results of groups A and B are summarized in Tables 1 and 2 respectively. Periods necessary for the completion of larval development were similar in both snail species: *P. sadoensis* cercariae were first found from *O. minima* snails 75 days after exposure; they appeared from *A. parasitologica* 76 days after exposure (Tables 1 & 2). Conversely, a distinct difference in snail infection rate was found between the two snail species; snail infection rate was markedly higher in *O. minima* (94--98%) than in *A. parasitologica* snails (38--55%; Tables 1 & 2).

Snail mortality during the course of experiments was very high in *A. parasitologica*; mortalities were 66 and 67% in groups A and B respectively. Dead *A. parasitologica* snails were usually infected with many unidentified minute nematodes. Conversely, snail mor-

tality in *O. minima* ranged only from 3 (group B) to 7% (group A).

Experiment 2: This experiment was also divided into two-groups. Both *O. minima* and *A. parasitologica* snails were exposed to 5 miracidia per snail in group A. In group B, *O. minima* snails were exposed to 5 miracidia per snail while *A. parasitologica* was exposed to 10 miracidia per snail. Snail exposure lasted for 5 hours in both groups.

At the completion of exposure, a total of 2 living miracidia remained in group A of *O. minima* snails whereas no miracidia were found from group B of the same snail. The number of corresponding miracidia recovered from *A. parasitologica* was 3 and 2 for groups A and B respectively. In this experiment, no snails of either species died during the exposure. Results of groups A and B are presented in Tables 3 and 4. *P. sadoensis* cercariae were first found from both snails 70 days after exposure (Tables 3 & 4). Snail infection rate was low in *A. parasitologica* snails (38—46%) as compared with *O. minima* (84—87%). In *A. parasitologica*, the infection rate was slightly higher in group B (exposed to 10 miracidia per snail) than in group A (5 miracidia per snail).

Snail mortality was high in *A. parasitologica* snails as noted in Experiment 1; the mortality was 85 and 84% in groups A and B respectively. Conversely, mortality varied from 6—8% in *O. minima*.

It was noted that one *O. minima* snail used had been naturally infected with an unknown species of xiphidiocercaria (Table 3).

The number of rediae infesting snails was examined using infected snails obtained from both Experiments 1 and 2. The mean number of rediae found per snail was 137 (Range=31-503; No. of snails examined=182) in *O. minima* and 137 (Range=20-300; No. of snails examined=20) in *A. parasitologica*. Both *O. minima* and *A. parasitologica* snails were found parasitized with almost the same number of rediae as well as

Table 3 Experimental infection of *Oncomelania minima* and *Assiminea parasitologica* snails with *Paragonimus sadoensis* miracidia

Snails	Snail exposure		Examination of exposed snails			
	No. of snails used	Average No. of Mir. exposed/ snail	Day after exposure	No. of snails examined	No. of snails infected (%)	Larval stages found
O. m	160*	5	2	3	1	Spor.
			5	3	1	Spor. & Unknown xiphidiocercaria
			15	3	0	
			30	3	1	Spor.
			44	3	3	Spor. & 1st GR.
			70-180	115	103	2nd GR. & Cer.
			Total	130	109(84%)	
A. p	160	5	2 - 5	6	0	
			15 - 30	6	2	Spor.
			44	3	2	Spor. & 1st GR.
			61	3	1	1st GR.
			70	3	2	1st & 2nd GR.
			75- 79	3	2	Spor. & 1st GR.
			Total	24	9(38%)	

Mir.=miracidia. *O. m=Oncomelania minima*. *A. p=Assiminea parasitologica*. Spor.=sporocyst. GR.=generation redia. Cer.=cercaria.

* A total of 150 exposed snails survived during the experiment.

Table 4 Experimental infection of *Oncomelania minima* and *Assiminea parasitologica* snails with *Paragonimus sadoensis* miracidia

Snails	Snail exposure		Examination of exposed snails			
	No. of snails used	Average No. of Mir. exposed/ snail	Day after exposure	No. of snails examined	No. of snails infected (%)	Larval stages found
O. m	160*	5	2	3	1	Spor.
			5 - 15	6	0	
			30	3	2	Spor.
			44	3	2	Spor. & 1st GR.
			70-180	112	106	2nd GR. & Cer.
			Total	127	111 (87%)	
A. p	160	10	2 - 15	9	0	
			30	3	1	Spor.
			44	3	2	Spor. & 1st GR.
			61	2	2	1st & 2nd GR.
			70	1	1	2nd GR. & Cer.
			75	1	1	1st & 2nd GR., & Cer.
			79-112	7	5	2nd GR. & Cer.
			Total	26	12 (46%)	

Mir. = miracidia. *O. m* = *Oncomelania minima*. *A. p* = *Assiminea parasitologica*. Spor. = sporocyst. GR. = generation redia. Cer. = cercaria.

* A total of 147 exposed snails survived during the experiment.

several hundred cercariae.

2. Morphology of *P. sadoensis* larvae

Sporocyst (Figs. 1 & 2): In the snails, sporocysts were found in the blood sinusoidal systems adjacent to the head region, stomach and intestine. Sporocyst size at different ages is presented in Table 5. Two—five day old sporocysts are light grayish white in color, and are round or ellipsoidal in shape, at times, pear shaped. A pair of flame cells was most conspicuous in the 2—5 day old sporocysts. Both the position of the birth pore and the distribution of germ balls were indistinct. One—nine sporocysts were found in the snails 2—5 days after exposure. Fifteen day old sporocysts are elongated with attenuated ends. Sporocyst parenchyme tissue surrounding the mass of germ balls usually appeared dark brown in color. Mature sporocysts which contained both first generation rediae and several germ balls were first found from *A. parasitologica* snails 30 days after exposure (Table 5). The intestine is absent and the birth pore is located at the terminal end.

First generation redia (Figs. 3 & 4): The first generation rediae were recovered from the blood sinusoidal systems surrounding the digestive tracts and liver.

1) Young form: The young form was first recovered 44 days after exposure. The body is light yellowish gray or yellowish white in color, short and truncated in form, and with an invaginated posterior end. A conspicuous collar was seen at the pharynx level and many transverse streaks were especially notable at the body surface of the cephalic region. However, both the collar and invaginated posterior end were indistinct in some individuals. The mouth is terminally located, followed by a prominent subglobular pharynx and a sac-like intestine. Intestinal contents were opalescent or brown colored. The intestine was usually not distinct in very young rediae. The birth pore was indistinct. The number of germ balls contained in young

Table 5 Size of *Paragonimus sadoensis* sporocyst obtained from *Oncomelania minima* and *Assiminea parasitologica* snails at different ages (in microns)

Days after exposure	*O. minima*					*A. parasitologica*				
	Body length		Body width		Germ balls & 1st G. R.	Body length		Body width		Germ balls & 1st G. R.
	Min.	Max.	Min.	Max.		Min.	Max.	Min.	Max.	
2 - 3	47	73	34	44	G: indistinct	52		39		G: indistinct
15	81	127	42	65	G: about 10	75	91	57	68	G: about 10
30	184	612	90	204	G: 12-14	265	536	128	180	G: several; R: 3
44-45	265	638	92	194	G: 4-12; R: 2-5	306	867	102	281	G: 6-12; R: 1-3
79-80	398	490	92	122	G: 2-3; R: 1	689	842	163	194	G: 6-13; R: 1

Min.=minimum. Max.=maximum. 1st G. R.=the 1st generation redia. G=germ ball. R=the 1st generation redia.

forms ranged from 2 to 14 (usually 4—8) which were generally together in the central part of the body.

2) Mature form: Body is light yellowish gray or yellowish white in color, elongated, cylindrical in form, with a slightly dented posterior end. Both the collar and posterior invagination were not always distinct. A subglobular pharynx and a sac-like intestine were conspicuous. Intestinal contents were white, light yellow or brown in color, usually with numerous brown colored fragments of variable size. The birth pore is located adjacent to the pharynx. Mature forms contain some second generation rediae as well as several germ balls at variable stages of development. The number of germ cells found packed near the posterior end of the body was less than 14.

Measurements of mature first generation rediae obtained from both *O. minima* and *A. parasitologica* snails are shown in Table 6. Rediae from the latter were larger than those from the former. No significant difference was observed in ratio of intestinal length to body length between the rediae from both snails.

Second generation redia (Figs. 5—10): The second generation rediae were found in the blood sinusoidal systems surrounding the stomach, intestine and liver.

The young form of the second generation rediae is short, more or less rectangular in form and light yellowish gray in color. The posterior end of the body is not invaginated but either straight or slightly convex in appearance. A subglobular pharynx is subterminally located, followed by a sac-like in-

Table 6 Size of mature first generation redia of *Paragonimus sadoensis* obtained from *Oncomelania minima* and *Assiminea parasitologica* snails (in microns)

Snail species	No. of rediae measured	Body		Pharynx		Intestine		Ratio of intestine/ body length ×100	No. of germ balls	No. of 2nd gen. rediae
		Length	Width	Length	Width	Length	Width			
		306*	112	49	52	60	36	11.3	5	3
O. m	8	1216**	192	68	81	204	133	36.6	12	5
		605.4†	149.5	55.6	60.6	119.1	89.3	21.8	9.3	3.7
		561*	112	55	55	101	52	11.1	5	1
A. p	26	1280**	255	91	109	398	173	53.8	25	8
		881.2†	188.5	68.4	71.1	160.2	94.3	19.1	13.5	2.7

O. m=*Oncomelania minima*. *A. p*=*Assiminea parasitologica*. * Minimum. ** Maximum. † Mean.

Table 7 Size of mature second generation redia of *Paragonimus sadoensis* obtained from *Oncomelania minima* and *Assiminea parasitologica* snails (in microns)

Snail species	No. of rediae measured	Body		Pharynx		Intestine		Ratio of intestine/ body length ×100	No. of germ balls	No. of cercariae
		Length	Width	Length	Width	Length	Width			
		449*	102	34	39	55	23	7.2	1	1
O. m	120	1250**	281	83	91	245	163	35.9	32	12
		750.9†	175.2	51.7	54.8	119.8	75.3	16.0	9.6	3.6
		485*	102	44	47	49	31	5.8	2	1
A. p	69	1250**	286	75	78	235	194	22.5	20	10
		783.3†	173.1	53.1	54.1	95.3	59.3	12.1	7.7	3.1

O. m=*Oncomelania minima*. *A. p*=*Assiminea parasitologica*. * Minimum. ** Maximum. † Mean.

testine which is opalescent or yellowish brown in color, containing white or brown colored refractile fragments of variable size. The young form contains 4—9 germ balls in the central part of the body as well as 2—12 small germ cells near the posterior end of the body.

Morphology of the mature second generation rediae of *P. sadoensis* was previously described in Part I of this study (Ito *et al.*, 1969). Thus, a comparison of measurements of the mature second generation rediae recovered from both *O. minima* and *A. parasitologica* snails was made (Table 7). Each of these measurements was closely related except for the ratio of intestinal length to body length. The value (16.0±5.01%) of the above ratio of redia from *O. minima* was larger than that (12.1±2.97%) of redia obtained from *A. parasitologica* : a significant difference was found between these values ($P<0.01$; t—test).

Cercaria (Figs. 11 & 12) : Morphology of the cercariae was previously reported in detail in Part I of this study (Ito *et al.*, 1969). All measurements were closely allied between the cercariae obtained from *O. minima* and *A. parasitologica* snails as shown in Table 8.

3. Experimental infection of *A. parasitologica* and *O. minima* snails with *P. ohirai* miracidia

Both *A. parasitologica* and *O. minima* snails were exposed to 20 miracidia per snail. Exposure of the snails to the miracidia lasted for 5 and 24 hours in *A. parasitologica* and *O. minima* snails respectively. At the

Table 8 Size of *Paragonimus sadoensis* cercaria obtained from *Oncomelania minima* and *Assiminea parasitologica* snails (in microns)

Snail species	No. of cercariae measured	Body		Oral sucker		Acetabulum		Stylet		Tail		Excretory bladder	
		L	W	L	W	L	W	L	W	L	W	L	W
		194*	86	47	42	20	32	29	6	17	15	38	28
O. m	40	296**	129	57	53	33	38	33	8	25	19	72	48
		240.0†	107.1	51.5	45.9	26.7	35.1	30.3	6.9	20.5	16.6	52.9	37.4
		192*	98	48	43	21	33	28	6	18	16	43	30
A. p	30	286**	130	58	53	30	42	32	8	30	21	68	43
		239.0†	112.2	53.3	47.9	25.6	37.4	30.5	7.1	22.5	17.9	54.1	35.5

L=length. W=width. *O. m*=*Oncomelania minima*. *A. p*=*Assiminea parasitologica*.
* Minimum. ** Maximum. † Mean.

Table 9 Experimental infection of *Assiminea parasitologica* and *Oncomelania minima* snails with *Paragonimus ohirai* miracidia

Snails	Snail exposure		Examination of exposed snails			
	No. of snails used	Average No. of Mir. exposed/ snail	Day after exposure	No. of snails examined	No. of snails infected (%)	Larval stages found
A. p	160*	20	4	3	0	
			15 - 46	9	5	Spor.
			54 - 82	10	9	Spor. & 1st GR.
			90	1	1	1st & 2nd GR.
			95	1	1	1st GR.
			103-158	24	21	2nd GR. & Cer.
			Total	48	37 (77%)	
O. m	128**	20	4 - 35	4	4	Spor.
			47 - 54	4	4	Spor. & 1st GR.
			60 - 70	2	2	1st GR.
			74 - 82	5	3	Spor. & 1st GR.
			90	2	1	2nd GR.
			95	3	1	2nd GR. & Cer.
			103	2	2	1st & 2nd GR.
			116-144	80	50	2nd GR. & Cer.***
			Total	102	67 (66%)	

Mir. = miracidia. *A. p* = *Assiminea parasitologica*. *O. m* = *Oncomelania minima*. Spor. = sporocyst. GR. = generation redia. Cer. = cercaria.

* A total of 78 exposed snails survived during the experiment.

** A total of 112 exposed snails survived during the experiment.

*** The first generation rediae were observed with the 2nd generation rediae and cercariae only in one snail crushed 141 days after exposure.

completion of exposure, a total of 10 living miracidia remained in the dishes with *A. parasitologica* while only one miracidium was found in the dishes with *O. minima*.

Results of this experiment are summarized in Table 9. *P. ohirai* cercariae were first found from *A. parasitologica* 103 days after exposure and from *O. minima* 95 days after exposure. Snail infection rate was slightly higher in *A. parasitologica* (77%) than in *O. minima* (66%) snails.

O. minima snails were usually infested with *P. ohirai* rediae (Mean=116; Range=3-381; No. of snails examined=16) of which the number was less than one half of that (Mean=272; Range=86-702; No. of snails examined=14) found in *A. parasitologica*. First generation rediae were still recovered from one snail of *O. minima* even after 141 days of exposure (Table 9). Furthermore, *O. minima* snails were generally infested with a relatively small number of cercariae (approximately 2—200) as compared with *A. parasitologica* (approximately 100—1,000).

As seen in the aforementioned experiments, snail mortality was higher in *A. parasitologica* (51%) than in *O. minima* snail (13%).

4. Morphology of *P. ohirai* larvae

Morphological features of *P. ohirai* sporocyst, the first generation redia, the second generation redia (Figs. 13 & 14) and cercaria (Figs. 15 & 16) were almost identical with those of *P. sadoensis*. The size of sporocyst at different ages is shown in Table 10. Measurements of the mature first and second generation rediae, as well as cercaria separat-

Table 10 Size of *Paragonimus ohirai* sporocyst obtained from *Assiminea parasitologica* and *Oncomelania minima* snails at different ages (in microns)

Days after exposure	*A. parasitologica*					*O. minima*				
	Body length		Body width		Germ balls & 1st G. R.	Body length		Body width		Germ balls & 1st G. R.
	Min.	Max.	Min.	Max.		Min.	Max.	Min.	Max.	
3	81		65		G : indistinct	52	70	36	47	G : indistinct
35	245	490	112	190	G : 4-12	310	350	120		G : 8-10
46-47	347	550	166	204	G : 4-12	333	867	115	154	G : 4-7 ; R : 6
54	408	581	143	230	G : 7-11 ; R : 1-2	510		143		G : 2 ; R : 2
74	791		265		G : 12 ; R : 4	510	536	102	122	G : 3 ; R : 1

Min.=minimum. Max.=maximum. 1st G. R.=the 1st generation redia. G=germ ball. R=the 1st generation redia.

Table 11 Size of mature first generation redia of *Paragonimus ohirai* obtained from *Assiminea parasitologica* and *Oncomelania minima* snails (in microns)

Snail species	No. of rediae measured	Body		Pharynx		Intestine		Ratio of intestine/ body length ×100	No. of germ balls	No. of 2nd gen. rediae
		Length	Width	Length	Width	Length	Width			
		439*	122	44	47	60	34	8.7	4	1
A. p	26	836**	204	68	78	194	143	26.6	16	2
		662.9†	161.2	57.6	60.8	114.1	81.3	17.4	10.5	1.5
		316*	102	36	39	55	26	10.5	1	1
O. m	11	995**	153	55	57	107	78	22.7	10	4
		547.3†	127.9	46.8	48.4	83.5	56.4	16.4	5.5	1.9

A. p=*Assiminea parasitologica*. *O. m*=*Oncomelania minima*. * Minimum. ** Maximum. † Mean.

Table 12 Size of mature second generation redia of *Paragonimus ohirai* obtained from *Assiminea parasitologica* and *Oncomelania minima* snails (in microns)

Snail species	No. of rediae measured	Body		Pharynx		Intestine		Ratio of intestine/ body length ×100	No. of germ balls	No. of cercariae
		Length	Width	Length	Width	Length	Width			
		490*	143	42	44	62	34	7.0	2	1
A. p	42	1479**	265	62	65	163	114	24.5	22	8
		882.4†	208.6	51.8	53.4	106.7	64.2	12.6	10.3	4.0
		428*	102	38	40	49	26	8.0	1	1
O. m	43	995**	224	57	65	143	113	19.9	14	5
		672.2†	164.9	47.8	50.3	87.2	54.3	13.1	6.2	2.0

A. p=*Assiminea parasitologica*. *O. m*=*Oncomelania minima*. * Minimum. ** Maximum. † Mean.

ed from *A. parasitologica* and *O. minima* snails are presented in Tables 11, 12 and 13 respectively.

Measurements of each of *P. ohirai* larval stages from both snails were analogous to each other. However, mean body size was slightly smaller in the rediae from *O. minima* than those from *A. parasitologica*

Table 13 Size of *Paragonimus ohirai* cercaria obtained from *Assiminea parasitologica* and *Oncomelania minima* snails (in microns)

Snail species	No. of cercariae measured	Body		Oral sucker		Acetabulum		Stylet		Tail		Excretory bladder	
		L	W	L	W	L	W	L	W	L	W	L	W
		192*	92	50	45	21	33	27	6	16	14	47	31
A. p	40	298**	130	62	57	32	41	32	8	25	21	78	46
		233.6†	113.7	54.7	49.5	26.3	36.7	29.9	7.1	20.7	18.2	58.2	36.7
		173*	76	40	41	25	31	27	6	17	14	39	20
O. m	30	286**	127	56	58	41	41	31	8	32	22	67	43
		226.4†	103.3	50.1	48.3	29.8	36.7	28.7	6.9	22.0	19.0	50.8	33.0

L=length. W=width. *A. p=Assiminea parasitologica*. *O. m=Oncomelania minima*.
* Minimum. ** Maximum. † Mean.

and the former generally contained less germ balls than the latter. No significant differences in ratio of intestinal length to body length were found between rediae obtained from the two snail species.

The value (17.4±4.97%) of the above ratio of *P. ohirai* first generation redia obtained from *A. parasitologica* was slightly smaller than that (21.8±8.09%) of *P. sadoensis* redia from *O. minima* snail, however, no statistically significant difference was found between them.

Discussion

The contaminations of natural *Paragonimus* infection in both *O. minima* and *A. parasitologica* snails used for this study can not be excluded because of collecting these snails from respective endemic areas for paragonimiasis sadoensis and paragonimiasis ohirai. According to the investigations made by Hamajima *et al.* in 1966, the natural *P. sadoensis* infection rate of *O. minima* snails collected from small streams near the mouth of the Okura river was 0.15% (Hamajima *et al.*, 1968), similarly, the examinations made by the present authors in October 1967 (unpublished data) revealed that the infection rate of the snails collected from the same area was 0.82% (2/245). Therefore, the contamination of natural *P. sadoensis* infection in *O. minima* snails used in the present experiments is disregarded when considering both the strikingly high infection rates and uniform larval developments as obtained in this study. Yokogawa *et al.* (1958) reported that the natural *P. ohirai* infection rate of *A. parasitologica* snails collected at the mouth of the Asahi river was 0.06%. The investigations made in this laboratory in 1966 (unpublished data) indicated that the natural infection rate of the snail collected from the same station was 0.2% (2/1,000). Thus, the contamination of natural *P. ohirai* infection in *A. parasitologica* snails can also be disregarded by the same reasons as described for *O. minima*.

Experimental infections of *O. minima* with *P. sadoensis* have been made by Hamajima *et al.* (1968), Hashiguchi *et al.* (1968) and Kawashima & Hamajima (1969). The snail infection rates reported by the above investigators were 52.3% (11/21) (Hamajima *et al.*, 1968), 100% (10/10) (Hashiguchi *et al.*, 1968) and 100% (Kawashima & Hamajima, 1969). The present results of *O. minima* snails ranged from 84 (109/130) to 98% (146/149).

As far as the authors know, experimental infection of *A. parasitologica* with *P. sadoensis* has never been conducted. The infection rates of *A. parasitologica* against *P. sadoensis* ranged from 38 (15/40 and 9/24) to 55% (30/55). These infection rates are low as compared with those from *O. minima*

snails but resemble the infection rate (52.5± 9.6%) of *Bythinella nipponica akiyoshiensis* experimentally exposed to *P. sadoensis* (Hashiguchi *et al.*, 1968). Relatively high snail infection rates were obtained in *A. parasitologica* when the snails were exposed to a large number of the miracidia as compared with exposure to a small number of miracidia (Tables 1—4).

Infection rates of several snail species against *P. ohirai* have been reported ; 33.3% (Yokogawa *et al.*, 1958), 60.8% (Yoshida & Miyamoto, 1959), and 28.5% (Kawashima, 1961) in *A. parasitologica* snails, 2.0% (Yoshida & Miyamoto, 1959) in *Assiminea japonica*, 57.6% (Yoshida & Miyamoto, 1960) in *Assiminea yoshidayukioi*, 30.0% (Yoshida, 1960) in *Paludinella japonica*, 100% (Kawashima & Miyazaki, 1963a) in *Oncomelania hupensis nosophora*, 87.5±16.1% and 77.6±12.8% (Hashiguchi *et al.*, 1968) in *B. n. akiyoshiensis* and 50% (Hashiguchi *et al.*, 1968) and 100% (Kawashima & Hamajima, 1969) in *O. minima*. Thus, the present infection rates of *A. parasitologica* snails against *P. sadoensis* are not necessarily low in comparison with the above data previously reported for *P. ohirai*.

P. sadoensis cercariae were found from both snails 70 days after exposure. The number of rediae found was very similar between the two snail species. The morphology of *P. sadoensis* larvae from the two snails, and their measurements were also analogous to each other. Thus, it can be concluded that *A. parasitologica* is susceptible to *P. sadoensis* infection, although the snail infection rates were always lower than those of *O. minima* snails.

According to Yoshida & Kawashima (1961), *A. parasitologica* is not distributed on Sado Island. If one considers the habitat of the second intermediate host for *P. sadoensis*, *Potamon dehaani* (fresh water crab), it is unlikely that *A. parasitologica* (brackish water snail) could play a role in the actual first intermediate host for this lung fluke even if this snail was distributed on Sado Island.

P. ohirai infection rate was slightly lower in *O. minima* (66%) than in *A. parasitologica* snails (77%). However, the infection rate in *O. minima* snail is rather high when compared with the aforementioned infection rates obtained from several snail species infected with *P. ohirai*. The only significant difference observed between *A. parasitologica* and *O. minima* snails exposed to *P. ohirai* was that *O. minima* was infested with a smaller number of rediae. This fact suggests that intramolluscan *P. ohirai* larval propagation was better in *A. parasitologica* than in *O. minima* snails.

P. ohirai cercariae were first recovered from *O. minima* and *A. parasitologica* snails 95 and 103 days after exposure respectively. Thus, cercarial formation appeared to be more delayed than that of *P. sadoensis*. Snail examinations performed during the period of 60—80 days after exposure were made on less than 10 individuals of each snail species, therefore, it is uncertain whether *P. ohirai* cercarial formation occurs before 95 days after exposure.

Morphological features of *P. ohirai* sporocysts and the first generation rediae recovered from the two species of snails are identical with those described previously by Ikeda (1957) and Kawashima (1965). No appreciable differences were observed in morphology of *P. ohirai* larvae from *A. parasitologica* and *O. minima* snails, except for the first and second generation rediae from *O. minima* containing less germ balls than those from *A. parasitologica* (Tables 11 & 12). All of these results suggest that *O. minima* is susceptible to *P. ohirai* infection.

Previously, Kawashima & Hamajima (1969) reported that the differentiation of the two species by sporocyst and cercarial stages was difficult, but that differences in the ratios of intestinal length to body length of the first and second generation rediae between the two species were significant. The present observations revealed variation in intestinal length of the first generation rediae in both species but no statistically significant difference was seen in the ratios of intestinal

length to body length between the two species. Ito *et al.* (1969) reported that the morphological features of the second generation rediae and cercariae of the two species of *Paragonimus* and their measurements were very similar. Since the morphology and measurements of both sporocysts and the first generation rediae of *P. sadoensis* and *P. ohirai* are also closely allied, it would be difficult to distinguish *P. sadoensis* from *P. ohirai* in any of their larval stages.

Davis (1969) re-examined so-called *Tricula minima* from an anatomical point of view, stating that this snail species should belong to the genus *Oncomelania*. Kawashima & Miyazaki (1963a, b) reported that *O. h. nosophora* could easily be infected with both *P. ohirai* and *P. iloktsuenensis*. Recently, Hembree *et al.* (1970) reported that *O. h. nosophora* was also susceptible to infection with *P. sadoensis*. Thus, it is of interest to note that a snail species belonging to the genus *Oncomelania* actually plays a role in the natural first intermediate host for *P. sadoensis* which is considered to be closely allied to *P. ohirai*.

Kawashima & Miyazaki (1964) divided four lung flukes, *P. ohirai*, *P. iloktsuenensis*, *P. miyazakii* and *P. westermani*, into three groups based on differences in their affinities to *O. h. nosophora* snail. According to this classification, it can be concluded that *P. sadoensis* has an analogous host specificity to those of *P. ohirai* and *P. iloktsuenensis*.

The present results pertaining to the susceptibility of *O. minima* and *A. parasitologica* snails against *P. sadoensis* and *P. ohirai* suggest that the difference in ecological features of the first intermediate hosts may not necessarily be an essential differentiating character between the two *Paragonimus* species. The difference in the first intermediate hosts between the two lung flukes are similar to those of the Taiwanese and Chinese or Japanese strains of *P. iloktsuenensis*: the first intermediate host for the former is a fresh water—amphibious snail, *Oncomelania hupensis chiui* (syn. *Tricula chiui*) (Habe et Miyazaki, 1962) Davis, 1968 (Miyazaki & Chiu, 1962) while those for the latter are brackish water snails belonging to the genus *Assiminea* (Chen, 1940, Tomimura *et al.*, 1960).

In the present experiments, snail mortality during the course of experiments was very high in *A. parasitologica*. This high mortality may be caused by severe infestation of nematodes derived from the soil and/or laboratory rearing conditions since this snail is a brackish water inhabitant.

Summary

The susceptibility of *Oncomelania minima* and *Assiminea parasitologica* snails to infection with both *Paragonimus sadoensis* and *P. ohirai* was studied to establish the similarities or differences in the first intermediate host specificity between the two lung flukes; analyses were made in terms of snail infection rates against the respective lung flukes, period necessary for the completion of cercarial formation, morphology of each larval stage, and of larval size. Results obtained are summarized as follows:

1) Snail infection rates of *P. sadoensis* ranged from 84 to 98% in *O. minima* and 38—55% in *A. parasitologica*.

2) *P. sadoensis* cercariae were recovered from both snails 70 days after exposure. These snails were parasitized with a large number of rediae and cercariae.

3) No appreciable differences were observed in larval morphology and size between *P. sadoensis* larvae developed in *O. minima* and *A. parasitologica* snails.

4) *P. ohirai* snail infection rates were 77% and 66% in *A. parasitologica* and *O. minima* snails respectively.

5) *P. ohirai* cercariae were found from *O. minima* 95 days after exposure and from *A. parasitologica* 103 days after exposure. *O. minima* snails were infested with relatively few *P. ohirai* rediae (less than one half of the rediae from *A. parasitologica*) and cercariae as compared with those of *A. parasitologica* snails.

6) No significant difference was observed in larval morphology and size between *P.*

ohirai larvae obtained from *A. parasitologica* and *O. minima* snails except for the first and second generation rediae from *O. minima* snail containing less germ balls than those from *A. parasitologica*.

7) Morphology of *P. sadoensis* larvae and their measurements were similar to those of *P. ohirai*. Thus, it would be difficult to distinguish *P. sadoensis* from *P. ohirai* by differences in their larval morphology or size.

Acknowledgments

The authors express their gratitude to Captain John K. Werner, Chief, Department of Medical Zoology, 406 th Medical Laboratory, and Dr. John I. Bruce, Jr., Chief, WRAIR Composite Drug Screening Unit, 406 th Medical Laboratory, for reviewing the manuscript. The authors are also indebted to Dr. George M. Davis for his helpful suggestions pertaining to the laboratory maintenance of *Oncomelania minima* and *Assiminea parasitologica* snails. Thanks are also due to Mr. Eikichi Miyasaka for his kind assistance in raising the snails.

Explanation of Figures

Fig. 1 *Paragonimus sadoensis* sporocyst (2 days after exposure) taken from *Oncomelania minima* snail. (Scale : 25 μ)

Fig. 2 *P. sadoensis* sporocyst (30 days after exposure) taken from *O. minima* snail. (Scale : 100 μ)

Fig. 3 Young form of *P. sadoensis* first generation redia (75 days after exposure) taken from *O. minima* snail. (Scale : 100 μ)

Fig. 4 Mature form of *P. sadoensis* first generation redia (75 days after exposure) taken from *O. minima* snail. (Scale : 100 μ)

Fig. 5 Young form of *P. sadoensis* second generation redia (90 days after exposure) taken from *O. minima* snail. (Scale : 100 μ)

Fig. 6 Young form of *P. sadoensis* second generation redia (94 days after exposure) taken from *Assiminea parasitologica* snail. (Scale : 100 μ)

Fig. 7 Premature *P. sadoensis* second generation redia (70 days after exposure) taken from *O. minima* snail. (Scale : 100 μ)

Fig. 8 Premature *P. sadoensis* second generation redia (70 days after exposure) taken from *A. parasitologica* snail. (Scale : 100 μ)

Fig. 9 Mature *P. sadoensis* second generation redia taken from *O. minima* snail. (Scale : 100 μ)

Fig. 10 Mature *P. sadoensis* second generation redia taken from *A. parasitologica* snail. (Scale : 100 μ)

Fig. 11 *P. sadoensis* cercaria taken from *O. minima* snail. (Scale : 100 μ)

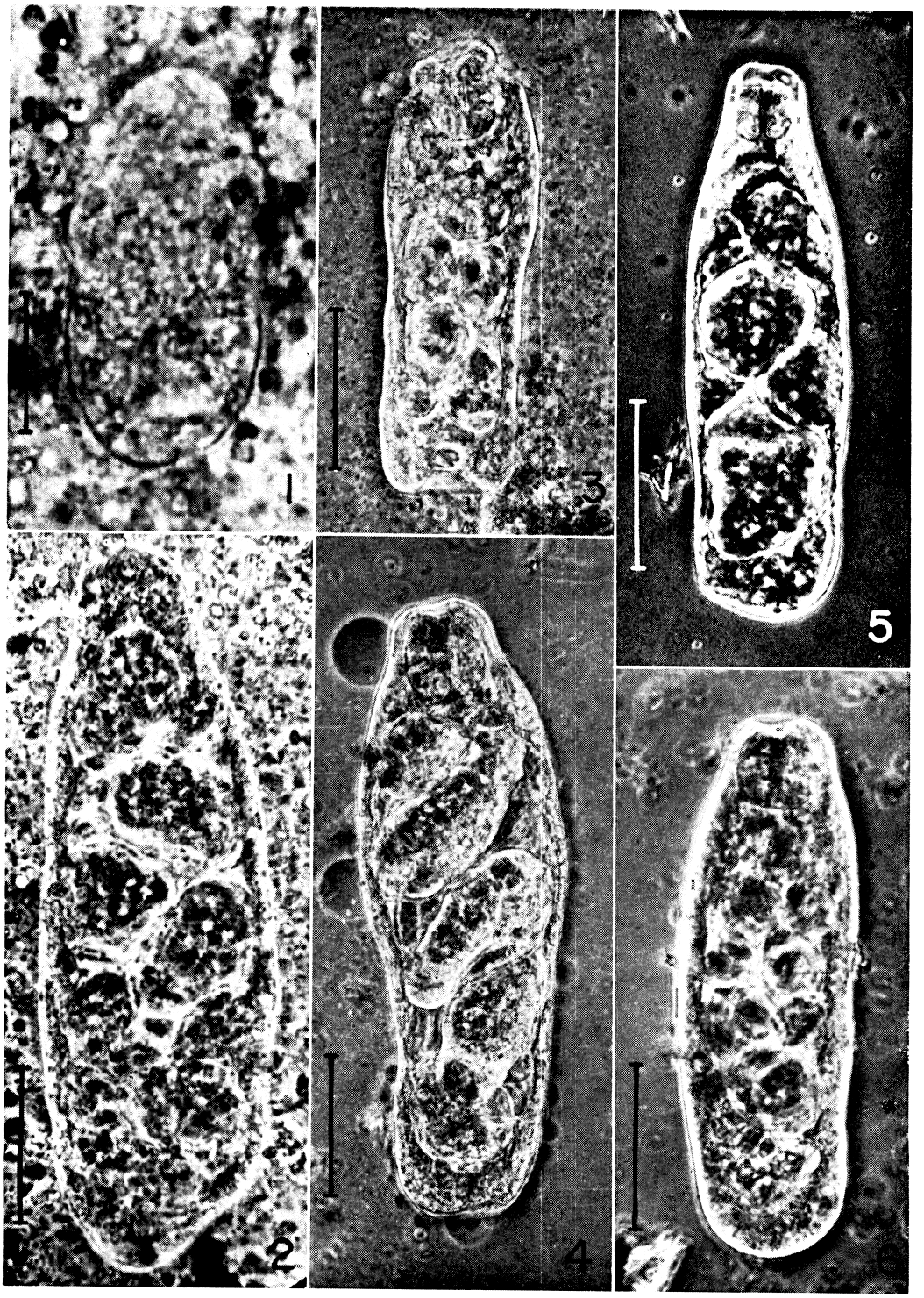
Fig. 12 *P. sadoensis* cercaria taken from *A. parasitologica* snail. (Scale : 100 μ)

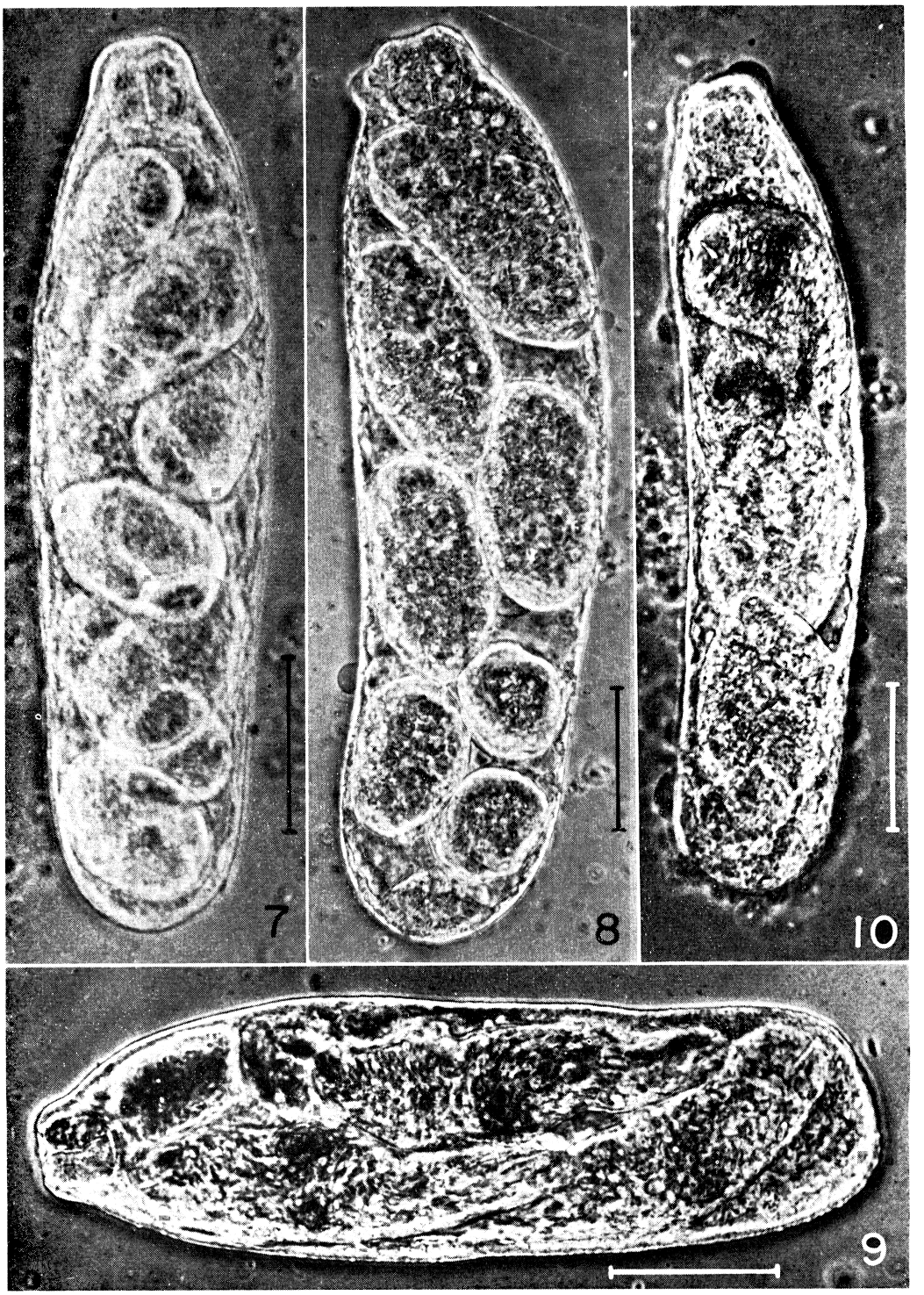
Fig. 13 Mature *Paragonimus ohirai* second generation redia taken from *A. parasitologica* snail. (Scale : 200 μ)

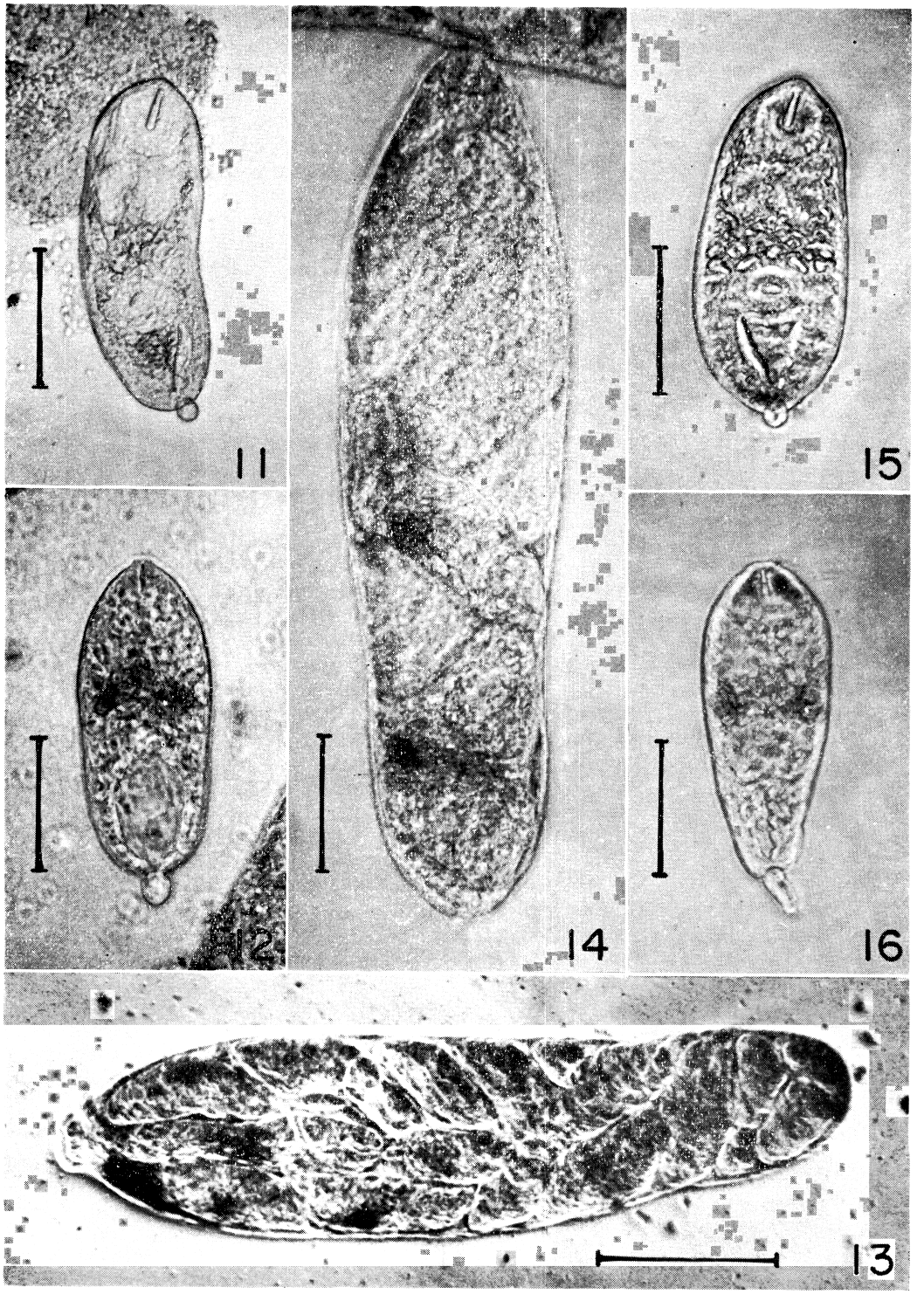
Fig. 14 Mature *P. ohirai* second generation redia taken from *O. minima* snail. (Scale : 100 μ)

Fig. 15 *P. ohirai* cercaria taken from *A. parasitologica* snail. (Scale : 100 μ)

Fig. 16 *P. ohirai* cercaria taken from *O. minima* snail. (Scale : 100 μ)


1
2
3
4
5


7
8
10
9


11
12
14
15
16
13

References

1) Chen, H. T. (1940) : Morphological and developmental studies of *Paragonimus iloktsuenensis* with some remarks on other species of the genus (Trematoda : Troglotrematidae). Lingnan Sci. J., 19, 429-530.
2) Davis, G. M. (1968) : A systematic study of *Oncomelania hupensis chiui* (Gastropoda : Hydrobiidae). Malacologia, 7, 17-70.
3) Davis, G. M. (1969) : Reproductive, neural and other anatomical aspects of *Oncomelania minima* (Prosobranchia : Hydrobiidae). Venus (Jap. J. Malacol.), 28, 1-36. (in English with Japanese abstract)
4) Hamajima, F., Kawashima, K. and Miyazaki, I. (1968) : *Tricula minima* (Bartsch, 1936), a new snail host for *Paragonimus* sp. found on Sado Island, Niigata Prefecture, Japan. Jap. J. Parasit., 17, 46-52. (in Japanese with English abstract)
5) Hashiguchi, Y., Hamajima, F. and Miyazaki, I. (1968) : Studies on the susceptibility of two snails, *Bythinella* (*Moria*) *nipponica akiyoshiensis* and *Tricula minima* to *Paragonimus ohirai* Miyazaki, 1939 and *Paragonimus* sp. on Is. Sado. Jap. J. Parasit., 17, 294. (abstract in Japanese)
6) Hembree, S. C., Miyasaka, E. and Williams, J. E. (1970) : A preliminary report on the experimental infection of *Oncomelania nosophora* (Robson, 1915) with *Paragonimus sadoensis* Miyazaki et al., 1968. Res. Bull. Meguro Parasit. Mus., (No. 3, 18-20.)
7) Ikeda, A. (1957) : The experimental study on the larval development of *Paragonimus ohirai* Miyazaki, 1939 in the first intermediate host, *Assiminea japonica*. Jap. J. Parasit., 6, 312-313. (abstract in Japanese)
8) Ito, J., Yoshimura, K. and Hishinuma, Y. (1969) : Comparative studies on *Paragonimus sadoensis* Miyazaki, Kawashima, Hamajima, et Otsuru, 1968 and *P. ohirai* Miyazaki, 1939. I. Morphology of the rediae and cercariae, with special reference to the excretory systems. Jap. J. Parasit., 18, 530-538.
9) Kawashima, K. (1961) : An experiment on the host specificity of *Paragonimus ohirai* Miyazaki, 1939 to several snails of the genus *Assiminea*. Jap. J. Parasit., 10, 161-164. (in Japanese with English abstract)
10) Kawashima, K. (1965) : Experimental studies on the intramolluscan development of an oriental lung fluke, *Paragonimus ohirai* Miyazaki, 1939. Jap. J. Med. Sci. Biol., 18, 293-310.
11) Kawashima, K. and Hamajima, F. (1969) : Comparative studies on the larval development of *Paragonimus sadoensis* and *P. ohirai* in the snail, *Tricula minima*. Jap. J. Parasit., 18, 385. (abstract in Japanese)
12) Kawashima, K. and Miyazaki, I. (1963a) : The experimental infection of larval lung-flukes to Japanese *Oncomelania* snails. I. Experimental infection of *Paragonimus ohirai* Miyazaki, 1939 to *Oncomelania nosophora* (Robson, 1915). Jap. J. Parasit., 12, 94-97. (in Japanese with English abstract)
13) Kawashima, K. and Miyazaki, I. (1963b) : The experimental infection of larval lung-flukes to Japanese *Oncomelania* snails. II. Experimental infection of *Paragonimus iloktsuenensis* Chen, 1940 to *Oncomelania nosophora* (Robson, 1915). Jap. J. Parasit., 12, 159-161. (in Japanese with English abstract)
14) Kawashima, K. and Miyazaki, I. (1964) : The experimental infection of larval lung-flukes to Japanese *Oncomelania* snails. IV. Experimental infection of *Paragonimus westermani* (Kerbert, 1878) to *Oncomelania nosophora* (Robson, 1915). Jap. J. Parasit., 13, 459-463. (in Japanese with English abstract)
15) Miyazaki, I. and Chiu, J. K. (1962) : On the lung fluke, *Paragonimus iloktsuenensis* Chen first recognized in Taiwan. Jap. J. Parasit., 11, 285. (abstract in Japanese)
16) Tomimura, T., Terauchi, J. and Tarumoto, I. (1960) : Stuides on the first intermediate host of lung fluke, *Paragonimus iloktsuenensis* Chen, 1940 in the mouth of the River Shinyodo, Osaka Prefecture. Med. & Biol., 54 (2), 45-51. (in Japanese)
17) Yokogawa, M., Yoshimura, H., Koyama, C., Sano, M., Tsuda, M., Suzuki, J. and Tsuji, M. (1958) : On a new first intermediate host, *Paludinella devilis* (Gould, 1861) Habe, 1942, of *Paragonimus ohirai* Miyazaki, 1939. Tokyo Iji Shinshi, 75, 67-72. (in Japanese)
18) Yoshida, Y. (1960) : Experimental studies on the first intermediate hosts of *Paragonimus westermani*, *P. ohirai* and *P. iloktsuenensis*. Jap. J. Parasit., 9, 377-378. (abstract in Japanese)
19) Yoshida, Y. and Kawashima, K. (1961) : On the distribution of the snail hosts of *Para-*

gonimus ohirai Miyazaki, 1939 and *Paragonimus iloktsuenensis* Chen, 1940 in Japan. Jap. J. Parasit., 10, 152-160. (in Japanese with English abstract)

20) Yoshida, Y. and Miyamoto, M. (1959): Studies on a first intermediate host, *Assiminea parasitologica* Kuroda, 1958 ("*Paludinella devilis*", of Yokogawa et al. non Gould), of *Paragonimus ohirai* Miyazaki, 1939. Jap. J. Parasit., 8, 122-129. (in Japanese with English summary)

21) Yoshida, Y. and Miyamoto, M. (1960): Another new first intermediate host, *Assiminea yoshidayukioi* Kuroda, 1959, of *Paragonimus ohirai* Miyazaki, 1939. Jap. J. Parasit., 9, 211-216. (in Japanese with English abstract)

佐渡肺吸虫ならびに大平肺吸虫の比較に関する研究 II. ナタネミズツボおよびムシヤドリカワザンショウ貝の2種肺吸虫感染に対する感受性について

吉村堅太郎　菱沼良正　佐藤ミツ子

(第406医学研究所医動物学研究部)

佐渡肺吸虫と大平肺吸虫の種差を明らかにするための1つの試みとして，まず2種肺吸虫の第1中間宿主特異性に差異が見られるかどうかを検討した．すなわちナタネミズツボおよびムシヤドリカワザンショウ貝にこれらの肺吸虫を実験的に感染させ，これらの貝の2種肺吸虫感染に対する感受性を比較した．成績の分析に当つては，これらの貝における2種肺吸虫の感染率，セルカリア形成迄に要する期間，ならびに幼虫各期の形態と大きさなどに重点をおいた．得られた成績は次の通りである．

1) ナタネミズツボにおける佐渡肺吸虫の感染率は84～98% であり，ムシヤドリカワザンショウのそれは38～55% であつた．

2) 佐渡肺吸虫セルカリアは2種の貝のいずれにおいても感染後70日に初めて見い出された．2種の貝に寄生する佐渡肺吸虫レジアの数は類似しており，またセルカリアも多数検出された．

3) 2種の貝で発育した佐渡肺吸虫幼虫各期の形態は全く一致し，その計測値も類似していた．

4) ムシヤドリカワザンショウにおける大平肺吸虫の感染率は77%，ナタネミズツボのそれは66%であつた．

5) 大平肺吸虫セルカリアはナタネミズツボでは感染後95日に，またムシヤドリカワザンショウでは103日に初めて見い出された．ナタネミズツボに寄生する大平肺吸虫レジアの数はムシヤドリカワザンショウのそれに較べて少なく，また寄生セルカリア数も一般に少なかつた．

6) ナタネミズツボから見い出される大平肺吸虫レジアの包蔵胚球数はムシヤドリカワザンショウに寄生するレジアのそれより一般に少ない様であつた．しかしこのことを除けば，2種の貝から見い出される大平肺吸虫幼虫各期の形態には有意な差異が認められなかつた．

7) 佐渡肺吸虫ならびに大平肺吸虫幼虫各期の形態ならびにその計測値は酷似している．

以上の成績から，ムシヤドリカワザンショウが佐渡肺吸虫の感染に対して感受性を有すること，またナタネミズツボが大平肺吸虫感染に感受性のあることが解る．すなわち2種肺吸虫の第1中間宿主特異性は類似している．また2種肺吸虫幼虫を形態学的特徴，あるいは大きさの差異によって区別することは困難であると思われる．