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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

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Introduction

In 1956, Otsuru & Katagiri discovered the adult worms of a *Paragonimus* species from weasels (*Mustela itatsi itatsi*) captured on Sado Island, Niigata Prefecture, and morphologically identified the flukes as *Paragonimus ohirai* Miyazaki, 1939 (Otsuru & Katagiri, 1956; Otsuru *et al.*, 1957). Recently, this lung fluke was re-examined by Miyazaki *et al.* (1968) in detail and was designated as a new species, *Paragonimus sadoensis*. Miyazaki *et al.* (1968) stated that *P. sadoensis* differed from *P. ohirai* in several morphological features such as: 1) the general body shape of the adult, 2) the form of the metacercarial cyst, including red granule deposition in the larval body, 3) the length of the metacercarial stylet, and 4) the number of flame cells in the cercarial stage. They stated further that ecological differences in the first and second intermediate hosts for the two species plus the above factors validated the creation of a new species.

To the contrary, Yokogawa *et al.* (1968) stated that the differentiation of the two species based on the position of specific antigen-antibody precipitin systems using immunoelectrophoresis coupled with absorption tests was difficult. Yoshimura (1969) reported that the disc electrophoretic patterns of adult whole body proteins of *P. sadoensis* were essentially identical with those of *P. ohirai* with only minor differences.

From the above, a comparison of the morphological features of larval forms of *P. sadoensis* and *P. ohirai*, especially of their excretory systems, would contribute to our knowledge of the similarities or differences between the two species. The morphology of *P. sadoensis* redia and cercaria has been reported by Hamajima *et al.* (1968), however, no description of their excretory systems has been made. Thus, the morphology of the larval forms of both species was studied and compared with each other, special attention being given to a comparison of their excretory systems.

Materials and Methods

Potamon dehaani crabs naturally infected with *P. sadoensis* metacercariae, as well as *Tricula minima* snails used for infection experiments were collected from small streams near the mouth of the Okura river in Aikawa-machi, Sado-gun, Niigata Prefecture, Japan, September 1968. *Sesarma dehaani* crabs in-

fested with *P. ohirai* metacercariae and *Assiminea parasitologica* snails were collected at the mouth of the Asahi river, Shimoda, Izu Peninsula, Japan, October 1968.

Adult worms of the respective species were harvested from experimentally infected rats and cats 50 days after exposure. All of the adult worms were kept in physiological saline solution for 12 hours in an incubator at 28°C. Eggs shed from the adult worms were then incubated for 15–19 days at 28°C.

P. sadoensis larvae were obtained from *T. minima* snails experimentally exposed to the miracidia while larvae of *P. ohirai* were harvested from previously infected *A. parasitologica* snails. Rediae and cercariae of the respective species were collected by crushing infected snails.

Mature *P. sadoensis* rediae used for measurements, were obtained from snails 70–140 days after infection and corresponding rediae of *P. ohirai* were obtained form snails 103–130 days after exposure. Measurements of rediae were made on fresh materials under slight cover glass pressure. Mature cercariae utilized for measurements were obtained from the infected snails 166 and 140 days after exposure for *P. sadoensis* and *P. ohirai* respectively. Ten percent hot formalin was used to fix cercariae and they were measured under slight cover glass pressure.

Observations on morphology and excretory systems of larval forms were made on mature rediae and cercariae taken from exposed snails 146–149 days after exposure for *P. sadoensis* and on mature rediae and cercariae taken from exposed snails 124–127 days after exposure for *P. ohirai*; larvae were placed in a watch glass filled with 0.2% saline solution. Observations of detailed structures were made on preparations of fresh materials under heavy cover glass pressure.

Results

1. Morphology of *P. sadoensis* redia and cercaria

Redia: In the snails, rediae were found from the blood sinusoidal systems surrounding the stomach, intestine and liver. The rediae are light yellow or yellowish white in color, elongated, cylindrical in shape and with a more or less rounded posterior end. Neither collar nor appendages were observed. Transverse streaks were especially notable on the body surface of the cephalic region. A moderate number of sensory hairs, approximately 2 μ long, was recognizable in the vicinity of the anterior end of the body (Fig. 1). Measurements of the rediae are presented in Table 1. When the snails were parasitized with a small number of rediae, redial size was generally large. Conversely, rediae from snails infested with a great number of worms were usually small.

The mouth is terminally located, followed by a well developed subglobular pharynx and a sac-like intestine with a comparatively thin wall. The intestine is more or less filled with numerous light yellow or brown colored fragments of variable size. The ratio of intestinal length to body length ranged from

Table 1 Comparison of the size of the second generation rediae of *Paragonimus sadoensis* and *P. ohirai* (in microns)

Species	No. of rediae measured	Body		Pharynx		Intestine		No. of germ balls	No. of cercariae
		Length	Width	Length	Width	Length	Width		
P. sadoensis	120	449*	102	34	39	55	23	1	1
		1250**	281	83	91	245	163	32	12
		750.9†	175.2	51.7	54.8	119.8	75.3	9.6	3.6
P. ohirai	42	490*	143	42	44	62	34	2	1
		1479**	265	62	65	163	114	22	8
		882.4†	208.6	51.8	53.4	106.7	64.2	10.3	4.0

* Minimum. ** Maximum. † Mean.

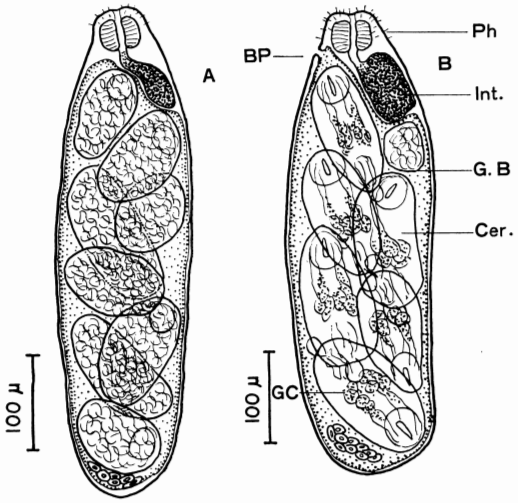

Fig. 1 Rediae of *Paragonimus sadoensis* from *Tricula minima* snails.
A. Immature 2 nd generation redia (70 days after infection).
B. Mature 2 nd generation redia containing many well-developed cercariae (90 days after infection).

7.2 to 35.9% (mean : 16.0±5.01). The birth pore is located adjacent to the pharynx. Rediae contain 1–32 (mean : 9.6) germ balls (developing cercariae) and 1–12 (mean : 3.6) fully developed cercariae. Two–twelve opalescent, round, germ cells were usually seen packed near the posterior end of the body.

The two excretory pores were located on the lateral body surface at a point approximately two-thirds of the body length from the anterior end. The excretory pores were never observed to fuse with each other. Both primary excretory canals originating from the excretory pores run anteriorly and divide into the anterior and posterior secondary excretory tubes at a point approximately on the midline of the body (Fig. 2). The anterior secondary excretory tube runs anteriorly and divides into two branches which bear 2–7 flame cells with their capillaries. Thus, the flame cells of the first tertiary excretory tube are distributed near the pharynx and intestinal region in the anterior one-fourth of the body while those of the second tertiary excretory tube are distributed in the anterior 2 nd quarter of the body. Similarly, the posterior secondary excretory tube runs posteriorly and usually divides itself into two branches which receive 2–7 flame cells with

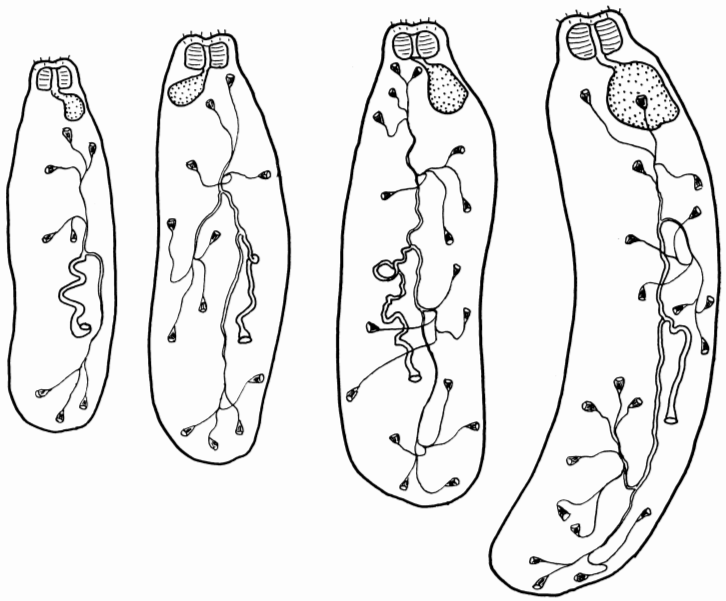

Fig. 2 Rediae of *Paragonimus sadoensis* from *Tricula minima* snails showing various modifications of the excretory system on one side.

Table 2 Comparison of size of *Paragonimus sadoensis* and *P. ohirai* cercariae (in microns)

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
P. sadoensis	40	194*	86	47	42	20	32	29	6	17	15	38	28
		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
P. ohirai	40	192*	92	50	45	21	33	27	6	16	14	47	31
		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

L=length. W=width. * Minimum. ** Maximum. † Mean.

their corresponding capillaries. As shown in Fig. 2, however, no branching from the posterior secondary tube was observed in some rediae, especially in premature ones. Therefore, no definite regularity of redial flame cell pattern was observed both in the "factor of division" and in the number of tertiary excretory tubes derived from the posterior secondary tube.

The flame cell pattern of the second generation redia of *P. sadoensis* can be represented as follows:

2[(n+n)+(n+n)] or 2[(n+n)+n], (n=2−7, usually 3−6)

Cercaria: Measurements of cercariae are presented in Table 2. The body is ellipsoidal in shape with a small, oval or ellipsoidal tail at the posterior end of the

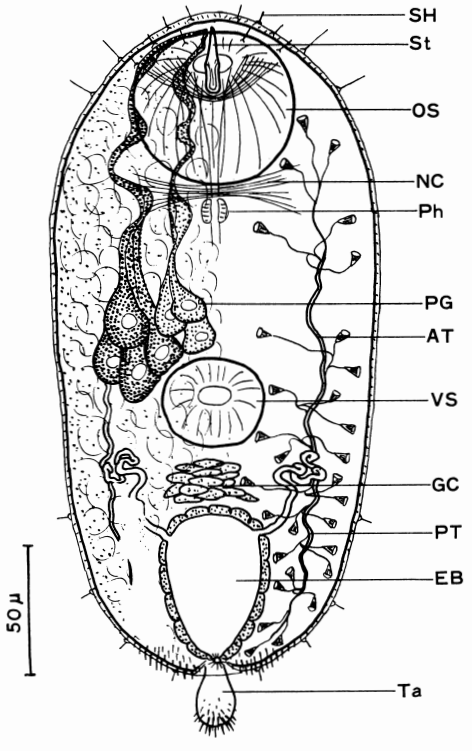

Fig. 3 Cercaria of *Paragonimus sadoensis* from *Tricula minima* snails showing the penetration glands on the left side, the excretory system on the right side.

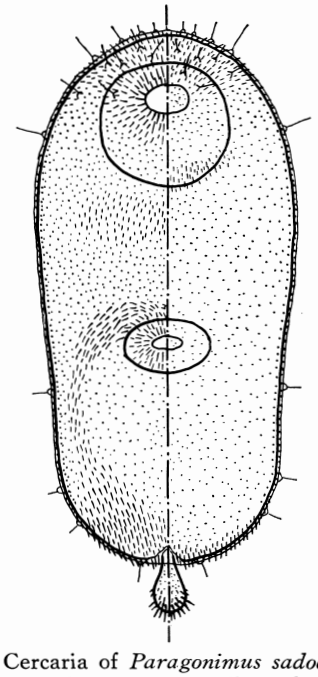

Fig. 4 Cercaria of *Paragonimus sadoensis* from *Tricula minima* snails indicating the sensory hairs and spines on the body surface. Ventral spines on the left half and dorsal spines on the right half. Note their locations and sizes.

body. Body shape will of course, vary greatly according to cercarial movement. The body is covered with minute spines which are longer and stouter in the vicinity of the posterior end of the body and posterior part of the tail (Fig. 4). On the ventral body surface, somewhat longer spines are seen in the following areas; the anterior margin of the body, on the oral sucker surrounding the mouth, on the body surface near the nerve commissure or pharynx, on the acetabulum, and on the arch-like area extending from the anterio-lateral side of the acetabulum to the posterior end of the body (Fig. 4). The dorsal body surface is uniformly covered with minute spines, except in the vicinities of the anterior margin of the body and of the posterior margin of the oral sucker where somewhat longer spines are present (Fig. 4). Several sensory hairs, approximately 5 μ long, were observed at the lateral margins of both the anterior and posterior parts of the body as well as near the anterior margin of the oral sucker as shown in Figs. 3 & 4. However, the definite number of sensory hairs distributed on the above areas could not be determined.

The oral sucker is subglobular in shape, ventro-subterminally located, and provided with a conspicuous stylet which has a round base and sharply pointed tip. The acetablum, being subglobular in shape, is smaller than the oral sucker, and located in the ventro-anterior three-fifths of the body. The mouth, surrounded by the oral sucker, is subterminally located, followed by a prepharynx (38 μ long) and a globular pharynx (15×19 μ in size). The esophagus and intestinal tracts could not be traced. Two kinds of penetration glands are present in both lateral sides of the anterior half of the body; four outer pairs which contain coarse granules are located at approximately mid-level of the body whereas three inner pairs are finely granular in appaerance and located at almost the same level or slightly anterior to the outer glands. Individual ducts from the former gland cells run together anteriorly along the dorsal side of the body, around the oral sucker, and then open laterally beside the stylet in the vicinity of the anterior margin of the oral sucker. The ducts from the inner gland cells similarly run anteriorly inside of the former ducts and open much closer to the stylet at approximately the same level as the openings for the outer glands. A genital primordium consisting of a mass of several germinal cells was seen between the acetabulum and the excretory bladder.

The large wedge-shaped excretory bladder which is lined with a thick layer of cuboidal cells, occupies a wide area of the posterior body half behind the acetabulum. Two main excretory canals originate from the anterio-lateral corners of the bladder and run latero-obliquely. After several convolutions, they divide into the anterior and posterior secondary excretory tubes at a level slightly posterior to the acetabulum. The anterior secondary excretory tube runs anteriorly, successively giving off four anterior tertiary excretory tubes and finally ends in the first anterior tertiary tube near the lateral side of the oral sucker. Each of the five anterior tertiary tubes bears three flame cells with their own capillaries (Fig. 3). Similarly, the posterior secondary excretory tube successively gives off four posterior tertiary excretory tubes and finally divides into three capillaries with corresponding flame cells near the latero-posterior end of the body. Each of the above four posterior tertiary tubes bears three capillaries with their corresponding flame cells (Fig. 3). Thus, the flame cell pattern of the mature cercaria of *P. sadoensis* can be expressed as $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$.

2. Morphology of *P. ohirai* redia and cercaria

Redia: As described in *P. sadoensis*, rediae were recovered from the blood sinusoidal systems surrounding the digestive tracts and liver. The morphological features of *P. ohirai* rediae were closely allied to those of *P. sadoensis*. Measurements of *P. ohirai* rediae are shown in Table 1. The ratio of intestinal length to body length varied from 7.0 to 24.5% (mean : 12.6±3.14) ; this mean value was small as compared with the value from *P. sadoensis* rediae. A statistically significant

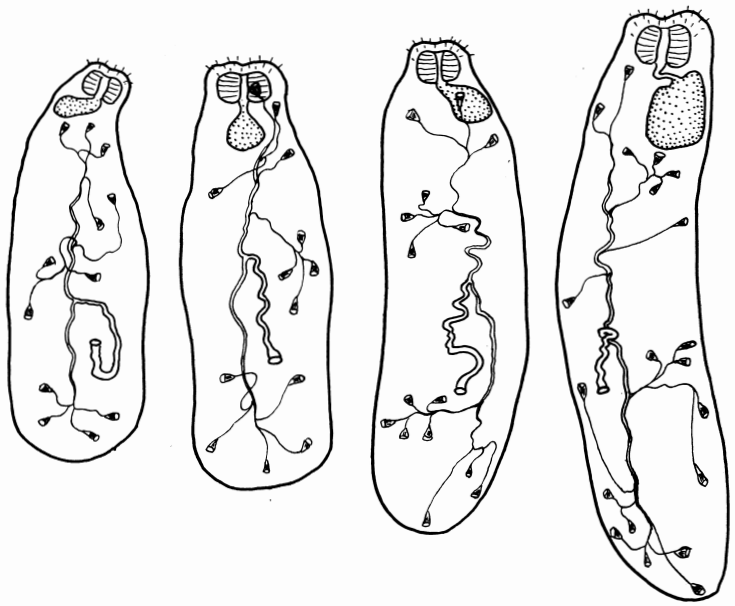

Fig. 5 Rediae of *Paragonimus ohirai* from *Assiminea parasitologica* snails showing modifications of the excretory system on one side. Note there is no distinct difference between *P. sadoensis* and *P. ohirai* (cf. Fig. 2).

difference was found in the intestinal length between the rediae of the two species ($p<1.0\%$). Furthermore, it was noted that *P. ohirai* rediae never had intestine of which the ratio of intestinal length to body length was over 30% (see Table 1). The intestinal contents of *P. ohirai* rediae were usually bright red-brown or somewhat red in color as compared with the light yellow or brown colored contents of *P. sadoensis* rediae.

Rediae contain 2–22 (mean : 10.3) germ balls (developing cercariae) as well as 1–8 (mean : 4.0) mature cercariae. The flame cell pattern of the mature *P. ohirai* rediae was identical with that of *P. sadoensis* (Figs. 2 & 5) ; the pattern was $2[(n+n)+(n+n)]$ or $2[(n+n)+n]$, $(n=2-7$, usually $3-6)$.

Cercaria : The morphological characteristics of cercarial internal organs were identical with those of *P. sadoensis* and no significant differences were observed between the two species (see Figs. 3 & 6). The distribution of both body spines and sensory hairs was also analogous to that of *P. sadoensis.* Measurements of cercariae are presented in Table 2 and these were also very close to those of *P. sadoensis.*

The flame cell pattern of the mature cercaria of *P. ohirai* is identical with that of *P. sadoensis* (Fig. 6) ; the pattern can be expressed as $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$.

Discussion

The morphological features of the larval forms of both *P. sadoensis* and *P. ohirai,* including both their excretory systems and body measurements, were almost identical. As far as the present observations are concerned, the mean ratio of intestinal length to body length in rediae was the only significant difference observed between the two species. Rediae with extraordinarily large intestines rarely appeared in *P. sadoensis.* It would, however, be difficult to distinguish *P. sadoensis* from *P. ohirai* by differences in their intestinal lengths due to the variability in both species. The intestine of *P. ohirai* rediae was usually bright red-brown in color.

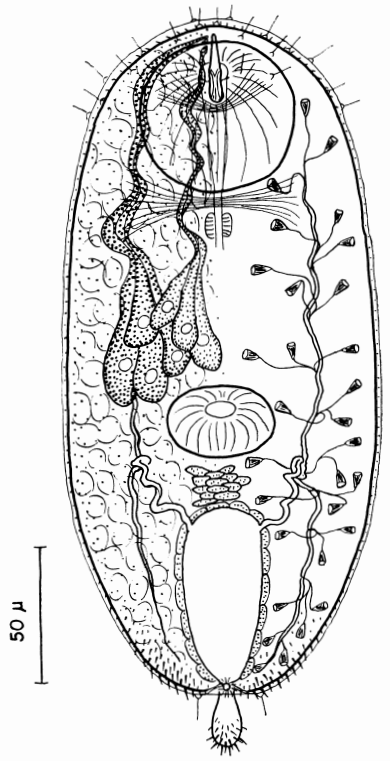

Fig. 6 Cercaria of *a Pragonimus ohirai* from *Assiminea parasitologica* snails. Note there is also no distinct difference between *P. sadoensis* and *P. ohirai* (cf. Fig. 3).

This is possibly due to the difference in color of food debris derived from the snail host. The present data concerning the morphology of *P. sadoensis* rediae and cercariae are nearly identical with those previously described by Hamajima *et al.* (1968).

Ishii & Miyazaki (1968) studied the body surface of *P. sadoensis* cercariae using a scanning electron microscopic technique, stating that cilia like hairs and spines found on the body surface were different in their distribution and the shape in each position. In the present observations, spines found from the ventro-posterior end of the body near the base of the tail, the posterior region of the tail, and from other several areas depicted in Fig. 4 are surmised tocorrespond to "cilia like hairs and spines" described by Ishii & Miyazaki.

Descriptions of the larval forms of *P. ohirai* have been made by Ogita (1954), Ikeda (1957), Yokogawa *et al.* (1958), Yoshida & Miyamoto (1959), Yokogawa *et al.* (1960) and Kawashima (1965). The present data for *P. ohirai* were identical with their results except for the cercarial flame cell pattern.

Kawashima (1965) has expressed the flame cell pattern of *P. ohirai* redia as $2[(2+2)+(2+2)]=16$. In the present study, however, no regularity in the "factor of division" of redial flame cell patterns was observed as previously indicated in the redia of *P. westermani* (Komiya & Ito, 1950) and in that of *P. iloktsuenensis* (Komiya *et al.*, 1960). In addition to this, two types of patterns were found in the number of branches from the posterior secondary excretory tube in the rediae of both species.

The flame cell pattern of *P. ohirai* cercaria was determined by Yokogawa *et al.* (1960) as $2[(1+1+1+1+1)+(1+1+1+1+1)]=20$ which had been previously confirmed by Yokogawa *et al.* (1958) and Yoshida & Miyamoto (1959). Kawashima (1965) has further confirmed this pattern in *P. ohirai* cercariae from experimentally exposed *Oncomelania nosophora* snails. However, the present observations revealed that the "factor of division" of *P. ohirai* cercaria was consistently 3 instead of 1. It is interesting to note that, among the five species of lung flukes of which the cercarial flame cell patterns have been established, four species, *P. westermani* (Yamaguti, 1943, Komiya & Ito, 1950), *P. iloktsuenensis* (Komiya *et al.*, 1960), *P. sadoensis* and *P. ohirai*, all have the same flame cell pattern represented as $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$, except for *P. kellicotti* (Ameel, 1934). As previously pointed out by Komiya *et al.* (1960), this probably suggests that the number and arrangements of the tertiary excretory tube derived from the anterior and posterior secondary excretory tubes are common to all species of the genus *Paragonimus*.

Komiya (1938) reported that the flame cell patterns of young or immature cercariae were different from that of the mature ones. The

disagreement of the data pertaining to the flame cell pattern of *P. ohirai* cercaria obtained by the aforementioned investigators with the present results could possibly be attributed to the degree of maturity of cercariae utilized for observations.

In conclusion, the differentiation of the larval forms between *P. sadoensis* and *P. ohirai* is difficult since not only their morphological features, including the excretory system, but also their size, is very similar.

Summary

The morphology of the rediae and cercariae of both *Paragonimus sadoensis* and *P. ohirai*, especially their excretory systems, were examined. The flame cell pattern and other morphological features of larval forms of the two species were found to be closely allied. In *P. sadoensis*, the redia of which the ratio of intestinal length to body length was over 30%, was very rarely observed. A statistically significant difference in ratio of redial intestinal length to body length was found between the two species. However, due to the variability in redial intestinal length, it is rather doubtful whether such a character can be used as a definitive species characteristic.

The flame cell patterns of rediae of *P. sadoensis* and *P. ohirai* were represented as $2[(n+n)+(n+n)]$ or $2[(n+n)+n]$, $(n=2-7$, usually $3-6)$. The flame cell pattern of cercariae of both species was expressed as $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$

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佐渡肺吸虫ならびに大平肺吸虫の比較に関する研究 I. 2種肺吸虫レジアおよびセルカリアの形態，特にそれらの排泄系について

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佐渡肺吸虫幼虫の形態，とりわけその排泄系統については未だ報告がない．そこでこの肺吸虫の第2代レジアおよびセルカリアの形態，特にその排泄系を明らかにし，本種の近似種であると考えられる大平肺吸虫のそれと比較した．

佐渡肺吸虫のレジアおよびセルカリアはあらかじめ実験的にミラシジアを感染させたナタネミズツボから，また大平肺吸虫のそれは同様に感染させたムシヤドリカワザンショウ貝から得たものである．

2種のレジアおよびセルカリアの形態はその排泄系をも含めて互いに酷似していることが解った．ただ佐渡肺吸虫では極めて稀ではあるが「腸長/体長」の比が36%(平均：16.0±5.01)に達する大きな腸を持つレジアが観察された．また佐渡肺吸虫レジアの「腸長/体長」比と大平肺吸虫レジアのそれ(平均：12.6±3.14)との間には推計学的有意差が認められた．しかしながら，両種ともにレジアの腸長にはかなりの変動が見られるため，「腸長/体長」の比が2種鑑別にあたっての決定的区別点になるかどうかについてはさらに検討が必要と思われる．

2種のレジアの焔細胞式は $2[(n+n)+(n+n)]$ または $2[(n+n)+n]$ (ただし $n=2\sim7$，通常 $3\sim6$) で表わされ，またセルカリアの焔細胞式は2種ともに $2[(3+3+3+3+3)+(3+3+3+3+3)]=60$ であった．

結論として，佐渡肺吸虫と大平肺吸虫の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かなり困難であると思われる．