# Comparative studies on *Paragonimus sadoensis* and *P. ohirai*. V. Comparison of susceptibility of *Assiminea japonica*, *Oncomelania hupensis chiui* and *Paludinella japonica*

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# Introduction

In review, Kawashima (1965) reported that five species of snails, i. e., Assiminea japonica, A. parasitologica, A. yoshidayukioi, Oncomelania hupensis nosophora and Paludinella japonica, were susceptible to P. ohirai infection. In addition, five other species of snails, i. e., Bythinella nipponica akiyoshiensis, O. h. chiui (syn. Tricula chiui), O. h. formosana (locality strain from Changhua County), O. h. quadrasi and O. minima, have also been reported susceptible to infection with P. ohirai although no cercarial formation, except for the first generation rediae, occurred in B. n. akiyoshiensis (Hashiguchi et al., 1968; Hashiguchi & Miyazaki, 1968; Chiu, 1969; Kawashima & Hamajima, 1969; and Yoshimura et al., 1970a).

On the other hand, investigations on the susceptibility of various snails to *P. sadoensis* infections have revealed that four species of snails, i. e., *O. minima* (Hamajima *et al.*, 1968; Hashiguchi *et al.*, 1968; Kawashima & Hamajima, 1969; and Yoshimura *et al.*, 1970a), *B. n. akiyoshiensis* (Hashiguchi *et al.*, 1968), *O. h. nosophora* (Hembree *et al.*, 1970) and *A. parasitologica* (Yoshimura *et al.*, 1970a) could easily be infected with the lung fluke. Thus, the first intermediate host specificity of *P. sadoensis* is similar in some respects to that of *P. ohirai*.

In this study, susceptibility experiments

were conducted on A. japonica, O. h. chiui and P. japonica snails against P. sudoensis and P. ohirai infections in order to determine whether significant differences are found in the first intermediate host specificity between the two lung flukes.

## **Materials and Methods**

Snail species examined for susceptibility were A. japonica, O. h. chiui and P. japonica. Simultaneously, O. minima and A. parasitologica snails served as controls for P. sadoensis and P. ohirai respectively.

A. japonica and A. parasitologica were collected near the mouth of the Asahi river, Shimoda-machi, Kamo-gun, Shizuoka Prefecture while P. japonica snails were collected at Shirahama, Shimoda-machi. Adult O. minima snails were collected from small streams near the mouth of the Okura river, and from small streams near Iwayaguchi, Aikawa-machi, Sado-gun, Niigata Prefecture. O. minima snails used for experimental infections were the F1 generation of fieldcollected snails reared in the laboratory. Mature O. h. chiui snails were collected at Alilao village, Shimen district, Taipei county, Taiwan, China, in 1968. O. h. chiui snails used for this study were also the F1 generation of field-collected snails.

Both O. h. chiui and P. japonica snails were maintained as previously described for raising O. minima snail whereas A. japonica was maintained similar to A. parasitologica (Yoshimura et al., 1970a).

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*P. japonica* snails were fed with a small amount of commercial tropical fish food (Kurorera Kagaku Kenkyusho) once or twice a month; the snail care of other four species was according to the methods described by Yoshimura *et al.* (1970a). Room temperature varied from 15-28°C as recorded on an automatic recording thermometer (Ota Seisakusho).

*P. sadoensis* and *P. ohirai* adult worms both of which were developed through their own first and second intermediate hosts were previously described in Part IV of this study (Yoshimura *et al.*, 1970b). *P. sadoensis* and *P. ohirai* eggs shed from these adult worms were incubated for 15 days and artificially hatched by the procedures previously described (Yoshimura *et al.*, 1970a).

Both A. japonica and A. parasitologica snails were maintained in the laboratory for 5 months prior to infection whereas P. japonica snails were maintained for 20-26 days in the laboratory before exposure. Snail exposure was made by immersing individual snails for 5 hours in a small dish (12 mm wide and 13 mm deep) which contained 5 miracidia in dechlorinated water.

Cercarial emergence was determined by examining individual snails in the same type of dish as used for exposure. Snails were immersed in dechlorinated water and examined for 1-4 hours.

Exposed snails were crushed to determine snail infection rates. Morphological observations and measurements of larval forms separated from infected snails were performed by the methods described by Ito *et al.* (1969).

# Results

1. Experimental infection of five species of snails with *P. sadoensis* miracidia

At the completion of exposure, only one miracidium remained in a single dish of each of four species of snails, the exception being O. h. chiui in which no miracidia remained in any dishes.

|         | \$          | Snail exp                | osure                                    |                        | Examinatio | n of exposed s             | mails                  |
|---------|-------------|--------------------------|--|------------------------|------------|----------------------------|------------------------|
| Snails  | Date        | No. of<br>snails<br>used | Average No. of<br>Mir. exposed/<br>snail | Days after<br>exposure |            | No. of snails infected (%) | Larval stages<br>found |
|         |             |                          |  | 71-75                  | 5          | 0(0)                       |                        |
| A. j    | 3 Sept. '69 | 25                       | 5  | 79-83                  | 4          | 0(0)                       |                        |
|         |             |                          |  | Total                  | 9          | 0(0)                       |                        |
|         |             |                          |  | 71-75                  | 11         | 2(18)                      | 2nd G.R. & Cer.        |
| A. p    | 3 Sept. '69 | 25                       | 5  | <b>7</b> 9-83          | 10         | 2(20)                      | 2nd G.R. & Cer.        |
|         |             |                          |  | Total                  | 21         | 4(19)                      |                        |
|         |             |                          |  | 71-75                  | 9          | 3(33)                      | 2nd G.R. & Cer.        |
| 0. h. c | 3 Sept. '69 | 20                       | 5  | 79-83                  | 10         | 7(70)                      | 2nd G.R. & Cer.        |
|         |             |                          |  | Total                  | 19         | 10(53)                     |                        |
|         |             |                          |  | 71-75                  | 9          | 4(44)                      | 2nd G.R. & Cer.        |
| O. m    | 3 Sept. '69 | 25                       | 5  | 79-83                  | 10*        | 7(70)                      | 2nd G.R. & Cer.        |
|         |             |                          |  | Total                  | 19         | 11 (58)                    |                        |
| P. j    | 3 Sept. '69 | 25                       | 5  | 75                     | 2          | 1 (50)                     | 2nd G.R. & Cer.        |

Table 1 Experimental infection of five species of snails with Paragonimus sadoensis miracidia

Mir.=miracidia. A. j=Assiminea japonica. A. p=Assiminea parasitologica. O. h. c=Oncomelania hupensis chiui. O. m=Oncomelania minima. P. j=Paludinella japonica. 2nd G.R.=the 2nd generation redia. Cer.=cercaria.

\* Three of 10 snails released cercariae 68 days after exposure.

Results of experimental infections are summarized in Table 1. Snail infection rate was highest in *O. minima* (58%), followed by *O. h. chiui* (53%), *P. japonica* (50%), and *A. parasitologica* (19%). However, all of 9 *A. japonica* snails crushed were negative for *P. sadoensis* infection.

No cercariae emerged from either of five species of snails 56 days after exposure. Fifteen-45 cercariae emerged from 3 individuals of *O. minima* snails 68 days after exposure but not from the remaining four species of snails.

The mean numbers of rediae and cercariae found per snail were 111 (Range 74-130; N =3) and 154 (Range 4-438) respectively in A. parasitologica, similarly, 58 (Range 22-96; N=10) and 186 (Range 1-963) in O. h. chiai, and 66 (Range 29-114; N=10) and 168 (Range 13-641) in O. minima snail. A single positive snail of P. japonica harboured 34 rediae and 15 cercariae.

Morphology of *P. sadoensis* second generation redia and cercaria separated from *O. h. chiui* and *P. japonica* was identical with the descriptions made in Part I of this study (Ito *et al.*, 1969) (Figs. 1, 2, 3 & 4).

As previously described (Ito *et al.*, 1969), the extraordinarily large intestine (Fig. 5) was rarely noted in the second generation rediae separated from *O. minima* snail. As Ito *et al.* (1969) previously stated in *P. ohirai* second generation rediae taken from *A. para*- sitologica, intestinal contents of *P. sadoensis* rediae separated from *A. parasitologica* were more bright red brown in color than those from *O. minima* snails.

Comparisons of measurements of the second generation rediae and cercariae were made between O. h. chiui and O. minima snails (Tables 2 & 3). Ratios (%) of redial intestinal length to body length were  $13.6 \pm 3.05$  in the redia taken from O. h. chiui and  $15.9\pm6.79$ in that from O. minima snail. No significant difference was found between these values. Only one specimen of the second generation redia taken from P. japonica was available The measurements on for measurement. this individual are as follows: Body,  $643 \times$ 173  $\mu$ ; pharynx, 52×57  $\mu$ ; intestine, 112×81  $\mu$ ; No. of germ balls contained, 8 (one of them was cercaria).

Snail mortality was highest in *P. japonica* (92%), followed by *A. japonica* (64%), *O. minima* (24%), *A. parasitologica* (16%) and *O. h. chiui* (5%).

2. Experimental infection of five species of snails with *P. ohirai* miracidia

At the completion of exposure, only one living miracidium remained in a single dish of each of O. h. chiui and O. minima, and in 6 dishes of A. japonica snails. One-2 corresponding miracidia found in 12 dishes of P. japonica.

Results of experimental infection are presented in Table 4. Snail infection rate was

| Snail   | No. of             | Boo    | dy    | Phar   | ynx   | Intes  | stine | Ratio of<br>intestinal<br>length/ | No. of germ<br>balls (including |
|---------|--------------------|--------|-------|--------|-------|--------|-------|-----------------------------------|---------------------------------|
| species | rediae<br>measured | Length | Width | Length | Width | Length | Width | body length $\times 100$          | cercariae)                      |
|         |                    | 418*   | 133   | 39     | 42    | 52     | 26    | 8.9                               | 3(1)                            |
| 0. h. c | 31 -               | 951**  | 235   | 68     | 75    | 143    | 112   | 22.7                              | 18(5)                           |
|         | (                  | 641.7† | 179.0 | 47.8   | 57.5  | 85.6   | 60.8  | 13.6                              | 9.7(2.1)                        |
|         | (                  | 321*   | 143   | 34     | 44    | 44     | 34    | 8.1                               | 3(1)                            |
| 0. m    | 31                 | 1002** | 235   | 62     | 73    | 255    | 179   | 36.7                              | 21(7)                           |
|         |                    | 673.6† | 180.9 | 45.0   | 56.2  | 105.1  | 76.3  | 15.9                              | 12.4(2.0)                       |

 Table 2
 Size of mature second generation redia of Paragonimus sadoensis obtained from Oncomelania hupensis chiui and O. minima (in microns)

O. h. c=Oncomelania hupensis chiui. O. m=Oncomelania minima.

\* Minimum. \*\* Maximum. † Mean.

| Snail   | No. of                |        | ody  |      | sucker | Aceta | bulum | Sty  | let | T    | ail  |
|---------|-----------------------|--------|------|------|--------|-------|-------|------|-----|------|------|
| species | cercariae<br>measured | L      | W    | L    | W      | L     | W     | L    | W   | L    | W    |
|         |                       | ( 176* | 70   | 45   | 41     | 22    | 32    | 29   | 5   | 16   | 13   |
| 0. h. c | 22                    | 262**  | 114  | 55   | 51     | 39    | 42    | 33   | 7   | 24   | 20   |
|         |                       | 236.2  | 98.0 | 51.6 | 46.3   | 27.6  | 35.8  | 30.4 | 6.3 | 19.7 | 16.3 |
|         |                       | ( 189* | 59   | 40   | 42     | 23    | 28    | 29   | 6   | 17   | 13   |
| O. m    | 20                    | 286**  | 109  | 58   | 53     | 31    | 39    | 33   | 7   | 26   | 18   |
|         |                       | 240.1  | 95.1 | 50.9 | 48.2   | 27.5  | 35.1  | 30.5 | 6.7 | 21.9 | 15.4 |

 Table 3 Size of Paragonimus sadoensis cercaria obtained from Oncomelania hupensis chiui and O. minima (in microns)

L=length. W=width. O. h. c=Oncomelania hupensis chiui. O. m=Oncomelania minima. \* Minimum. \*\* Maximum. † Mean.

Table 4 Experimental infection of five species of snails with Paragonimus ohirai miracidia

|         | 5           | Snail exp                | osure                                    |                        | Examinatio                | n of exposed s             | snails                                |
|---------|-------------|--------------------------|--|------------------------|---------------------------|----------------------------|---------------------------------------|
| Snails  | Date        | No. of<br>snails<br>used | Average No. of<br>Mir. exposed/<br>snail | Days after<br>exposure | No. of snails<br>examined | No. of snails infected (%) | Larval stages<br>found                |
| A. j    | 9 Sept. '69 | 25                       | 5  | 78                     | 3                         | 0( 0)                      |                                       |
|         |             |                          |  | 70                     | 3*                        | 3(100)                     | Spor., 1st & 2nd<br>G.R., & Cer.      |
| A. p    | 9 Sept. '69 | 25                       | 5  | 83                     | 6                         | 4(67)                      | 2nd G.R., & Cer.                      |
|         |             |                          |  | 97-98                  | 8                         | 5(63)                      | 2nd G.R., & Cer.                      |
|         |             |                          |  | Total                  | 17                        | 12(71)                     |                                       |
|         |             |                          |  | 78                     | ĥ                         | 2(33)                      | 1st G.R.                              |
|         |             |                          | _  | 83                     | 3                         | 3(100)                     | Spor., & 1st G.R.                     |
| 0. h. c | 9 Sept. '69 | 20                       | 5  | 97                     | 5                         | 3(60)                      | Spor., 1st & 2nd<br>G.R. (young form) |
|         |             |                          |  | Total                  | 14                        | 8(57)                      |                                       |
|         |             |                          |  | 70                     | 9                         | 0(0)                       |                                       |
| O, m    | 9 Sept. '69 | 26                       | 5  | 78                     | 2**                       | 2(100)                     | 2nd G.R., & Cer.                      |
| 0. m    | 5 Sept. 05  | 20                       | 5  | 97                     | 5                         | 1(20)                      | 1st & 2nd G.R.                        |
|         |             |                          |  | Total                  | 16                        | 3(19)                      |                                       |
| P. j    | 9 Sept. '69 | 25                       | 5  | 78                     | 3                         | 1(33)                      | 2nd G.R., & Cer.                      |

Mir.=miracidia. Spor.=sporocyst. 1st G.R.=the 1st generation redia. 2nd G.R.=the 2nd generation redia. Cer.=cercaria. \* One of 3 snails released cercariae 69 days after exposure. \*\* One of 2 snails released cercariae 69 days after exposure.

highest in A. parasitologica (71%), followed by O. h. chiui (57%), P. japonica (33%), O. minima (19%) and A. japonica (0%; only 3 individuals were examined). Differences in the period necessary for intramolluscan P. ohirai larval development were found between O. h. chiui and the other three species of snails; cercarial formation occurred in O. minima, A. parasitologica and P. japonica snails 69-78 days after exposure, conversely, no cercariae appeared in O. h. chiui even 97 days after exposure although a few young forms of the second generation redia were found (Table 4).

Cercarial emergence was not observed in any species of snails up to 55 days after exposure but one individual of both *O. minima* and *A. parasitologica* snails released 3-4 cercariae 69 days after exposure.

The mean numbers of rediae and cercariae found per snail were 123 (Range 78-166; N =6) and 80 (Range 1-183) respectively in A. *parasitologica*, similarly, 111 and 52 in O. *minima*, and 98 and 117 in P. *japonica*.

Morphological features of *P. ohirai* second generation rediae and cercariae taken from *P. japonica* were analogous to those of *P. sadoensis* as previously described in Part I of this study (Ito *et al.*, 1969). *P. ohirai* second generation redia and cercaria separated from P. japonica are shown in Figs. 6 & 7.

Measurements of the second generation redia and cercaria taken from *P. japonica* are compared with those from *A. parasitologica* (Tables 5 & 6). The ratios (%) of redial intestinal length to body length were  $15.0\pm$ 2.28 in the redia taken from *P. japonica* and  $15.6\pm4.20$  in that from *A. parasitologica*. No significant difference was observed between these ratios.

Snail mortality was highest in both A. japonica and P. japonica (88%), followed by O. minima (38%), A. parasitologica (32%), and O. h. chiui (30%).

# Discussion

Contaminations of natural Paragonimus infection in A. parasitologica used for this

 Table 5
 Size of mature second generation redia of Paragonimus ohirai obtained from Paludinella japonica and Assiminea parasitologica (in microns)

| Snail   | No. of<br>radiae |                   | Boo         | ły    | Phar   | ynx   | Intes  | stine | Ratio of<br>intestinal<br>length/ | No. of germ<br>balls (including |
|---------|------------------|-------------------|-------------|-------|--------|-------|--------|-------|-----------------------------------|---------------------------------|
| species | measured         |                   | Length      | Width | Length | Width | Length | Width | body length $\times 100$          | cercariae)                      |
|         |                  | (                 | 500*        | 168   | 44     | 47    | 65     | 42    | 11.5                              | 9(1)                            |
| P.~j    | 15               | $\left\{ \right.$ | 1079 * *    | 230   | 83     | 86    | 168    | 120   | 19.9                              | 21(2)                           |
|         |                  | (                 | 756.9†      | 201.1 | 53.2   | 60.1  | 113.0  | 83.6  | 15.0                              | 16.3(1.3)                       |
|         |                  | (                 | 583*        | 153   | 49     | 49    | 48     | 36    | 4.4                               | 3(1)                            |
| A. p    | 30               | $\frac{1}{2}$     | $1105^{**}$ | 296   | 65     | 78    | 245    | 179   | 23.8                              | 18(5)                           |
|         |                  | l                 | $829.7^{+}$ | 221.1 | 53.5   | 59.4  | 130.5  | 96.2  | 15.6                              | 10.6(1.6)                       |

P. j=Paludinella japonica. A. p=Assiminea parasitologica. \* Minimum. \*\* Maximum. † Mean.

Table 6Size of Paragonimus ohirai cercaria obtained from Paludinella japonica and<br/>Assiminea parasitologica (in microns)

| Snail   | No. of                | Bo      | dy    | Oral s | ucker | Aceta |      | Styl |     | Τa   |      |
|---------|-----------------------|---------|-------|--------|-------|-------|------|------|-----|------|------|
| species | cercariae<br>measured | L       | W     | L      | W     | L     | W    | L    | W   | L    | W    |
|         |                       | ( 221*  | 88    | 38     | 45    | 25    | 34   | 28   | 6   | 19   | 15   |
| P. j    | 10                    | 311**   | 120   | 56     | 53    | 34    | 40   | 31   | 7   | 27   | 20   |
|         |                       | (245.9† | 110.8 | 51.3   | 49.2  | 28.7  | 37.5 | 29.8 | 6.8 | 21.8 | 17.6 |
|         |                       | ( 224*  | 104   | 53     | 46    | 29    | 35   | 29   | 7   | 18   | 17   |
| A. p    | 20                    | 296**   | 135   | 59     | 53    | 41    | 46   | 32   | 8   | 30   | 24   |
|         |                       | (254.3† | 122.4 | 55.0   | 50.1  | 35.1  | 39.6 | 30.5 | 7.7 | 23.3 | 19.7 |

L=length. W=width. P. j=Paludinella japonica. A. p=Assiminea parasitologica.

\* Minimum. \*\* Maximum. † Mean.

|                    |                              | Paragonimus sadoensis            | us sadoens.                          | is                                 |                              | Para                             | Paragonimus ohirai                   | iirai                           |
|--------------------|------------------------------|----------------------------------|--------------------------------------|------------------------------------|------------------------------|----------------------------------|--------------------------------------|---------------------------------|
| Snail species      | No. of<br>snails<br>examined | No. of<br>snails<br>infected (%) | Final<br>forms of<br>larvae<br>found | Investigators<br>(year)            | No. of<br>snails<br>examined | No. of<br>snails<br>infected (%) | Final<br>forms of<br>larvae<br>found | Investigators<br>(year)         |
| Assiminea castanea | I                            |                                  | I                                    | I                                  | 97                           | 0(0)                             |                                      | Kawashima (1961)                |
|                    | 6                            | 0(0)                             |                                      | Present authors                    |                              | + +                              | Cer.<br>Cer.                         | Ogita (1954)<br>Ikeda (1957)    |
| A intenion         |                              |                                  |                                      |                                    | 33                           | 0(0)                             |                                      | Yokogawa et al. (1958)          |
| n undafne vez      |                              |                                  |                                      |                                    | 50                           | 1(2.0)                           | Cer.                                 | Yoshida & Miyamoto (1959)       |
|                    |                              |                                  |                                      |                                    | 72                           | 4(5.6)                           | Cer.                                 | Kawashima (1965)                |
|                    |                              |                                  |                                      |                                    | 3                            | 0(0)                             |                                      | Present authors                 |
| A. kushimotoensis  | 1                            | I                                | I                                    | I                                  | ۵.                           | (0)                              |                                      | Yoshida (1960)                  |
| A. latericea miya- | ł                            | I                                | I                                    | I                                  | 44                           | 0(0)                             |                                      | Kawashima (1961)                |
| zakii              |                              |                                  |                                      |                                    | 104                          | 0 (0)                            |                                      | Kawashima (1965)                |
|                    | 145                          | $66(46^*=38-55)$                 | Cer.                                 | Yoshimura <i>et al.</i><br>(1970a) | 30                           | 10(33.3)                         | Cer.                                 | Yokogawa et al. (1958)          |
|                    | 21                           | 4(19)                            | Cer.                                 | Present authors                    | 69                           | 42(60.8)                         | Cer.                                 | Yoshida & Miyamoto (1959)       |
| A. parasitologica  |                              |                                  |                                      |                                    | 14                           | 4(28.5)                          | Cer.                                 | Kawashima (1961)                |
|                    |                              |                                  |                                      |                                    | 48                           | 31(64.6)                         |                                      | Kawashima (1965)                |
|                    |                              |                                  |                                      |                                    | 48                           | 37(77)                           | Cer.                                 | Yoshimura et al. (1970a)        |
|                    |                              |                                  |                                      |                                    | 17                           | 12(71)                           | Cer.                                 | Present authors                 |
| V usehidzentisi    | -                            |                                  | Į                                    |                                    | 33                           | 19 (57.6)                        | Cer.                                 | Yoshida & Miyamoto (1960)       |
| 11. Younuguktor    |                              |                                  |                                      |                                    | 45                           | 26 (57.8)                        |                                      | Kawashima (1965)                |
| Bvthinella nibbo.  |                              | $(52.5\pm 9.6)$                  | 1st G.R.                             | Hashiguchi <i>et al.</i><br>(1968) |                              | $(87.5\pm16.1)$                  | 1st G.R.                             | Hashiguchi <i>et al.</i> (1968) |
| nica akiyoshiensis |                              |                                  |                                      |                                    | 228<br>997                   | $177(77.6\pm12.8)$               |                                      | (0301) :-[:]                    |
|                    |                              |                                  |                                      |                                    | 731                          | 182(/0.8)                        | Ist G.K.                             | Hashiguchi & Miyazaki (1908)    |

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| Clithon retropictus           |     |                       | 1    | I                                  | 50            | (0) 0                             |                                  | Yoshida & Miyamoto (1959)<br>Kawashima (1965)   |
|-------------------------------|-----|-----------------------|------|------------------------------------|---------------|-----------------------------------|----------------------------------|---|
| Oncomelania<br>hupensis chiui | 19  | 10(53)                | Cer. | Present authors                    | 88<br>14      | $46(52^* = 40.7 - 69.2)$<br>8(57) | Cer.<br>2nd G.R.<br>(young form) | Chiu (1969)<br>Present authors                  |
| O. h. formosana               | I   | 1                     | 1    | I                                  | 56**<br>46*** | $5(9^*=8.0-9.7)$<br>0(0)          | Cer.                             | Chiu (1969)<br>Chiu (1969)                      |
| O. h. nosophora               | 118 | 82 (69.5)             | Cer. | Hembree et al.<br>(1970)           | 66<br>22      | 66 (100)<br>22 (100)              | Cer.<br>Cer.                     | Kawashima & Miyazaki (1963)<br>Kawashima (1965) |
| O. h. quadrasi                | 1   |                       | Ι.   | 1                                  | 49            | 49 (100)                          | Cer.                             | Chiu (1969)                                     |
|                               | 21  | 11 (52.3)             | Cer. | Hamajima <i>et al.</i><br>(1968)   | 10            | 5(50)                             | Cer.                             | Hashiguchi et al. (1968)                        |
|                               | 10  | 10(100)               | Cer. | Hashiguchi <i>et al.</i><br>(1968) | n.            | (100)                             | Cer.                             | Kawashima & Hamajima<br>(1969)                  |
| O. minima                     | ۵.  | (100)                 | Cer. | Kawashima &<br>Hamajima(1969)      | 102           | 67 ( 66)                          | Cer.                             | Yoshimura et al. (1970a)                        |
|                               | 551 | $502(91^* = 84 - 98)$ | Cer. | Yoshimura et al.<br>(1970a)        | 16            | 3(19)                             | Cer.                             | Present authors                                 |
|                               | 19  | 11 (58)               | Cer. | Present authors                    |               |                                   |                                  |   |
| Paludinella<br>japonica       | 5   | 1(50)                 | Cer. | Present authors                    | <u>م.</u> ۳   | (30.0)<br>1 (33)                  | Cer.<br>Cer.                     | Yoshida (1960)<br>Present authors               |
| Semisulcospira<br>bensoni     | I   | ł                     | 1    | I                                  | 55            | (0)<br>(0)                        |                                  | Yoshida (1960)<br>Kawashima (1965)              |

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study can not be excluded because of collecting these snails from an endemic area of paragonimiasis ohirai. However, the chances of natural infection in *A. parasitologica* are minimized for reasons previously pointed out by Yoshimura *et al.* (1970a). *P. japonica* was also field-collected. However, natural *Paragonimus* infection of this snail has never been recorded and the distribution of the snail has no relation to the endemic area of paragonimiasis ohirai as previously pointed out by Miyazaki *et al.* (1960).

Present results revealed that both O. h. chiui and P. japonica snails could be easily infected with P. sadoensis and yielded the cercariae whereas A. japonica seemed to be insusceptible to P. sadoensis infection. Since only 9 A. japonica snails were examined, however, it is uncertain whether this snail is insusceptible to infection with this lung fluke.

As previously reported by Chiu (1969), O. h. chiui was found easily infected with P. ohirai ; our infection rate (57 %) of O. h. chiui against P. ohirai is analogous to those (40.7-69.2 %) obtained by Chiu for P. ohirai. As far as the present results are concerned, however, intramolluscan development of P. ohirai larvae differed from that of P. sadoensis in the same snails; no P. ohirai cercariae, except for the young form of the second generation rediae, occurred in O. h. chiui snails even 97 days after exposure, conversely, the formation of P. sadoensis cercariae occurred 71 days after exposure in O. h. chiui which had been maintained in the same room as P. ohirai exposed O. h. chiui snails. This fact implies that P. ohirai larval development in O. h. chiui is slower than that of P. sadoensis. Chiu (1969) examined P. ohirai infected O. h. chiui snails 3-4 months following exposure, reporting that the cercariae were recovered from the snails. Therefore, it is surmised that P. ohirai cercarial formation could have occurred in O. h. chiui if the infected snails in this study had been maintained for a longer period than in our experiment (97 days).

When one considers the fact that the first

intermediate host of P. sadoensis is O. minima which belongs to the genus Oncomelania, it is reasonable to assume that O. h. chiui snails could be infected with P. sadoensis. This study also showed that the morphology and size of larvae formed in O. h. chiui resembled those formed in O. minima.

Yoshida (1960) reported that *P. japonica* could be infected with *P. ohirai* (infection rate: 30.0%) and yielded the cercariae. Our results confirmed the data reported by Yoshida. Measurements of *P. ohirai* larvae taken from *P. japonica* were also similar to those separated from *A. parasitologica* (Tables 5 & 6).

As summarized in Table 7, experimental infection of A. japonica with P. ohirai has beed made by Ogita (1954), Ikeda (1957), Yokogawa et al. (1958), Yoshida & Miyamoto (1959) and Kawashima (1965). All investigators, except for Yokogawa et al., reported that A. japonica was positive for P. ohirai infection. Susceptibility of this snail to infection with P. ohirai could not be determined with certainty in this experiment since mortality of A. japonica (88%=22/25) was so high that only 3 snails exposed could be examined.

Kawashima & Hamajima (1969) reported that both P. sadoensis and P. ohirai miracidia could develop to the cercarial stage in O. minima snail within 40 days after exposure. Yoshimura et al. (1970a) reported that P. sadoensis cercariae were first found in O. minima snails 70 days after exposure while P. ohirai cercariae were recovered from the same snails 95 days after exposure. In the present study, however, both P. sadoensis and P. ohirai cercariae were released from O. minima 68-69 days after exposure. This suggests that periods necessary for intramolluscan cercarial formation were similar between the two lung flukes. Discrepancies in periods necessary for intramolluscan larval development, reported by the aforementioned investigators, may be due to differences in temperature for maintaining exposed snails as previously suggested by Kawashima & Miyazaki (1963).

Many susceptibility studies of various molluscs to infection with *P. ohirai* and *P. sadoensis* have been made as summarized in Table 7. This table suggests that the first intermediate host specificity of *P. sadoensis* is closely allied to that of *P. ohirai*; six species of snails, i. e., *A. parasitologica*, *B. n. akiyoshiensis*, *O. h. chiui*, *O. h. nosophora*, *O. minima* and *P. japonica*, can be infected with both lung flukes.

# Summary

1. Both Oncomelania hupensis chiui and Paludinella japonica snails were easily infected with Paragonimus sadoensis, producing the cercariae. Conversely, 9 Assiminea japonica snails were all negative for P. sadoensis infection.

2. O. h. chiui snail could be easily infected with *P. ohirai*. However, *P. ohirai* larval development in this snail was slow, i. e., no cercarial formation occurred up to 97 days after exposure.

3. The first intermediate host specificity of *P. sadoensis* is similar to that of *P. ohirai*.

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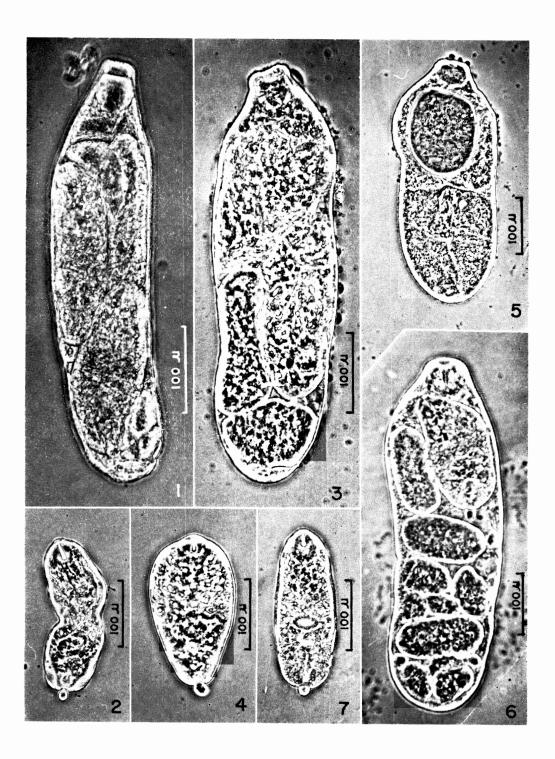
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#### **Explanation of Figures**

- 1. Paragonimus sadoensis second generation redia separated from Oncomelania hupensis chiui snail.
- 2. P. sadoensis cercaria separated from O. h. chiui snail.
- 3. P. sadoensis second generation redia separated from Paludinella japonica snail.
- 4. P. sadoensis cercaria separated from P. japonica snail.
- 5. Premature form of *P. sadoensis* second generation redia separated from *Oncomelania* minima snail. Note the large intestine.
- 6. Paragonimus ohirai second generation redia separated from Paludinella japonica snail.
- 7. P. ohirai cercaria separated from P. japonica snail.



# 佐渡肺吸虫ならびに大平肺吸虫の比較に関する研究. V. Assiminea japonica, Oncomelania hupensis chiui および Paludinella japonica の2種肺吸虫感染に対する感受性の比較

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佐渡肺吸虫と大平肺吸虫の第1中間宿主特異性に差異 が見られるかどうかを究明することは2種肺吸虫の種差 や類似点の解明に重要な手がかりを提供するものと思わ れる.そこで今回は、従来大平肺吸虫感染に感受性があ るとされている Assiminea japonica, Oncomelania hupensis chiui (syn. Tricula chiui) ならびに Paludinella japonica に2種肺吸虫を感染させ、これらの貝 の佐渡肺吸虫と大平肺吸虫に対する感受性を比較した.

その結果, 佐渡肺吸虫は *P. japonica* (感染率: 50 %=1/2) ならびに *O. h. chiui* (感染率: 53 %=10/1) によく感染し, セルカリアにまで発育する ことが 解つ た. これに対して *A. japonica* では被検9 個体の総て が陰性であつた. 佐渡肺吸虫が *O. h. chiui* によく感染 することは本種の第1中間宿主が Oncomelania 属に属 する Oncomelania minima であることを考えると極め て興味深い.

大平肺吸虫は O. h. chiui (感染率: 57 %= $^{8}$ / $^{4}$ )によ く感染したが、少なくとも今回の実験に関する限りで は、この貝における大平肺吸虫幼虫の発育は遅く、感染 後 97 日に至つてもセルカリアの形成は認められず、わ ずかに第2代レジアの幼若型を認めたにすぎない. P. japonica の大平肺吸虫感染率は 33 % ( $^{1}$ / $^{8}$ ) であつたが、 A. japonica では被検3 個体の総てが陰性であつた.

以上の成績から、2種肺吸虫はその第1中間宿主特異 性においても類似点の多いことが推察される.