[Jap. J. Parasit., Vol. 19, No. 2, 199-205, 1970]

A New Molluscan Host for *Paragonimus iloktsuenensis* Chen, 1940 in Is. Amami-Oshima, Kagoshima Prefecture, Japan*

KENJIRÔ KAWASHIMA AND FUSANORI HAMAJIMA
Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

(Received for publication; April 30, 1970)

Introduction

Paragonimus iloktsuenensis, one of the mammalian lung flukes was first described by Chen (1940) on the basis of the specimens from experimental and natural hosts studied in Canton, China. In Japan, Miyazaki (1944 a & b, 1945) reported a kind of *Paragonimus* which was closely allied to *P. ohirai* Miyazaki, 1939, and he tentatively called it "small type of *P. ohirai*", because of the small size of the metacercaria. In 1950, Miyazaki identified it as *P. iloktsuenensis*, comparing this fluke with Chinese specimens. At the same time, its biology and geographical distribution were studied by Miyazaki and his co-workers (Miyazaki, 1947 a & b, 1950; Miyazaki, Mannoji & Arita, 1951; Mannoji, 1952). Mouths of the following three rivers were hitherto reported in Japan as the infested area with *P. iloktsuenensis:* the Shin-yodo in Osaka Prefecture, the Sendai in Kagoshima Prefecture and the Kako in Hyogo Prefecture. In 1962, it was reported by Miyazaki and Chiu that this fluke occurred at Alilao Village of Taipei County in the northern part of Formosa.

Most recently, Sato *et al.* (1969 a & b) found this fluke from the crab, *Sesarma dehaani*, and from the rats, *Rattus rattus* and *R. norvegicus* in Is. Amami-Oshima, Kagoshima Prefecture, which is situated about 400 km to the south of Kagoshima City. However, the molluscan host of this fluke in this island has not been found as yet.

From the 4th through the 6th of June, 1969, the authors had an opportunity of visiting the island and of investigating this fluke in different kinds of wild animals. Among them, a kind of gastropod molluscs was proved to be naturally infected with this fluke. This mollusc was identified by Dr. T. Habe of the National Science Museum, Tokyo as *Angustassiminea nitida* (Pease) var.[1] (Figs. 2 & 3). Subsequently, the authors experimentally proved that this fluke developed into cercariae in this mollusc. The present paper is concerned with *A. nitida* as a new molluscan host of *P. iloktsuenensis*.

Materials and Methods

Natural Molluscan Infection

This investigation was carried out in the mouth of Kawauchi River, Sumiyo Village, Is. Amami-Oshima (Figs. 1, 7 & 8). In June of 1969, the gastropod mollusc, *A. nitida* was found by the authors on the river banks of the area mentioned above, and more than 1,000 individuals of this mollusc were collected. The intramolluscan stages of *Paragonimus* were recovered from naturally infected molluscs by crushing the shell. The larval stages of the fluke were transferred to a slide and observed alive under a slight coverslip pressure.

* Supported in part by the U. S. Army Research and Development Group (Far East), Department of the Army under Contract No. DAJB 17-70-C-0105.

1) Dr. Habe considers, in a broad sense, this species should be referred to *Angustassiminea nitida* group, although he described in 1942 this mollusc as *Assiminea castanea satumana*.

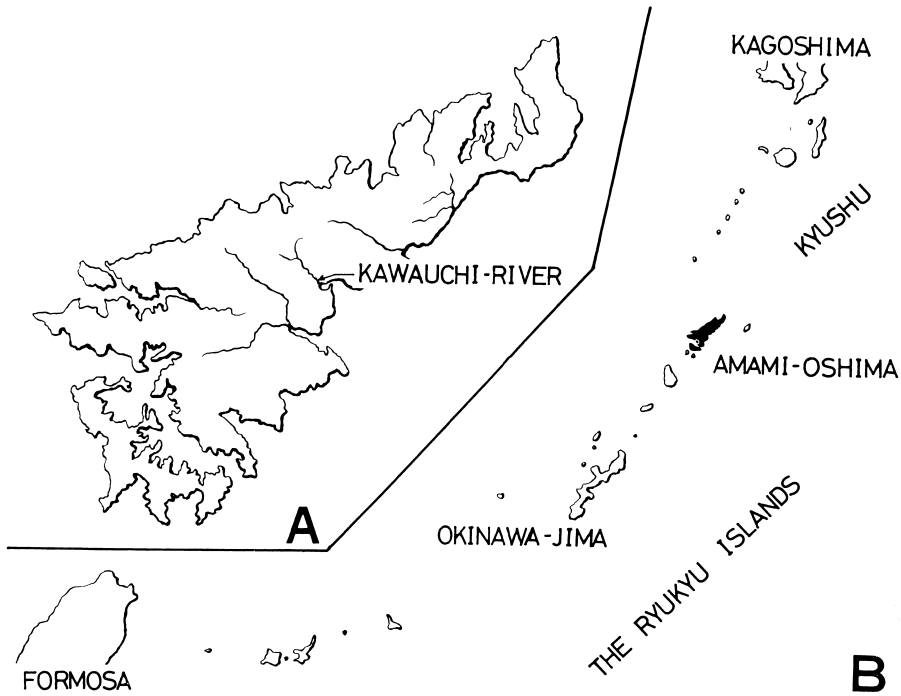


Fig. 1. A: Map of Is. Amami-Oshima. Arrow means the area where this investigation was carried out.
B: Map showing the location of Is. Amami-Oshima in Kyushu and the Ryukyu Islands.

Experimental Molluscan Infection

Eggs of *P. iloktsuenensis* were collected from the uterus of the mature flukes which were obtained from naturally infected rats in Is. Amami-Oshima as well as experimentally infected albino rats autopsied after several months, which were fed with the metacercariae collected from infected crabs in the same island. The eggs put in a water-filled watch glass were kept in the incubator at 27°C. The water was changed every other day and after 3–4 weeks the miracidia were hatched by cooling at 5°C for 15 minutes.

Out of *A. nitida* collected from Is. Amami-Oshima, non-infected molluscs with *Paragonimus* were selected by shedding of cercariae. These molluscs were exposed individually to 10 miracidia of *P. iloktsuenensis* in a small Petri dish (1 cm in diameter) for 4–6 hours. The larval stages were observed by the same method as those from naturally infected molluscs.

The measurements were made on 15 living specimens naturally infected and the average for each feature was compared with that of 10 living specimens from experimental hosts. At the same time, the adults from the mammalian host and the metacercariae from the crustacean host were studied.

Results

Natural and Experimental Molluscan Infection

Six of 892 molluscs of *A. nitida* examined were proved to be naturally infected with *Paragonimus* larvae (infection rate: 0.67%). On the other hand, in all of 60 molluscs of the same species examined during the period of 32–81 days after experimental exposure to the miracidia, a number of rediae and cercariae of this fluke were found (infection rate: 100%).

Table 1 Comparison of the sizes of the second generation rediae naturally and experimentally infected (in micron)

Specimens	Body		Pharynx		Intestine	Cercariae
	length	width	length	width	length	
naturally infected	837.1	163.9	46.8	48.0	87.7	3-8
experimentally infected	1,086.3	265.2	45.5	50.9	70.1	3-8

Table 2 Comparison of the sizes of cercariae naturally and experimentally infected (in micron)

Specimens	Body		Oral sucker		Acetabulum		Stylet	Tail
	length	width	length	width	length	width	length	length
naturally infected	245.0	88.8	49.7	52.8	35.7	39.8	26.9	26.9
experimentally infected	237.1	81.1	49.4	51.7	37.2	39.8	26.3	27.3

Intramolluscan Stages

As shown in Tables 1 and 2, there was no distinct morphological difference between the larvae naturally and experimentally infected, although the second generation rediae from experimental hosts were somewhat larger than those from natural hosts.

Crustacean and Mammalian Hosts in the Area Studied

S. dehaani collected on the river banks was heavily infected with larval *Paragonimus*. Fifty-three of 105 *S. dehaani* examined were proved to be naturally infected with the metacercariae (infection rate: 50.5 %). All of these were identified as *P. iloktsuenensis*. Fifty of them were fed into albino rats orally, in which 25 adult *Paragonimus* were obtained 46 days after inoculation. On the other hand, all of five *R. rattus* collected were proved to be naturally infected with adult *Paragonimus*.

Comments

The molluscan host of this fluke was first confirmed by Chen (1940) in China to be *Assiminea lutea*. In Japan, however, the molluscan host had not been found for a long time. In 1960, it was experimentally proved by Yoshida that this fluke developed into cercariae in the following four species of the Japanese gastropod molluscs: *Assiminea parasitologica*[2], *A. yoshidayukioi*[3], *A. japonica* and *Paludinella japonica*, of which the first species was found to be naturally infected with this fluke (Yoshida, 1959; Tomimura, Terauchi & Tarumoto, 1960). In Formosa, the larvae of this fluke were found by Chiu (1965) in the mollusc, *Tricula chiui*[4]. In 1963, *Oncomelania nosophora*[5] was experimentally proved by Kawashima and Miyazaki to be infected with this fluke.

As to the crustacean host, *S. dehaani* and *S. sinensis* in Canton, China, *S. dehaani* and *Helice tridens tridens* in Japan and *Potamon miyazakii*[6] in Formosa have been proved to be naturally infected with this fluke by Chen (1940), Miyazaki (1944 a), Mannoji (1952) and Miyazaki and Chiu (1962 a & b), respectively.

2), 3) These species are referred to the genus *Angustassiminea*.

4) This mollusc is recognized as *Oncomelania hupensis chiui*.

5) This mollusc is recognized as *O. h. nosophora*.

6) This scientific name was defined by Miyake and Chiu (1965): A new potamonid crab, *Potamon (Geothelphusa) miyazakii* sp. nov., as an intermediate host of the lung fluke from Formosa. J. Fac. Agr. Kyushu Univ., 13, 595-600.

Rats, weasels and dogs have been reported as the mammalian host of this fluke in Japan. In spite of the wide distribution of mammalian and crustacean hosts of this fluke, the geographical distribution of this fluke is considerably limited. This fact seems to be mainly influenced by the distribution of the molluscan host, especially *A. parasitologica*. The results of the field investigation on the molluscan host of *P. iloktsuenensis* by Miyazaki, Kawashima and Yoshida (1960) and by Yoshida and Kawashima (1961) indicated clearly the fact mentioned above.

As *A. parasitologica* could not been found in Is. Amami-Oshima, the authors supposed the molluscan host of this fluke might be *A. nitida*, living in numbers on the river banks. In the present study, the authors' suppoition was proved as follows: (1) The intramolluscan stages from naturally infected *A. nitida* were identical with those of *P. iloktsuenensis* described by earlier workers. (2) In the experimental study by the use of *A. nitida*, this fluke developed into cercariae. (3) The intramolluscan stages from experimentally infected molluscs were identical with those from natural molluscan hosts. (4) *S. dehaani*, the dominant species of the crabs in the area studied, was heavily infected with the metacercariae of *P. iloktsuenensis*. (5) A number of adults of the same fluke were found in all of *R. rattus* collected in the area studied. (6) *A. nitida* was found in the area where infected crabs and rats with *P. iloktsuenensis* inhabit. (7) There was not found other species of *Paragonimus* in this area.

Then, it was definitely proved that *A. nitida* can serve as the molluscan host of *P. iloktsuenensis* in Is. Amami-Oshima.

Conclusion

The authors studied the role of *A. nitida* as the molluscan host of *P. iloktsuenensis* and found that six of 892 molluscs were naturally infected with this fluke in Is. Amami-Oshima, and simultaneously obtained all positives of 60 molluscs in the experimental molluscan infection. Because of them, it was concluded here that *A. nitida* was a new molluscan host of *P. iloktsuenensis*.

Acknowledgments

The authors express their gratitude to Prof. Ichiro Miyazaki, Head of the Department of Parasitology, Faculty of Medicine, Kyushu University for his comments on the manuscript. The authors' thanks are also due to Dr. Tadashige Habe, National Science Museum, Tokyo for his identification of the molluscan host and to Dr. Isao Tada, Department of Medical Zoology, Faculty of Medicine, Kagoshima University for his suggestions on the field investigation in Is. Amami-Oshima.

References

1) Chen, H. T. (1940): Morphological and developmental studies of *Paragonimus iloktsuenensis* with some remarks on other species of the genus (Trematode: Troglotrematidae). Ling. Sci. J., 19, 429–530.
2) Chiu, J. K. (1962): Two species of *Paragonimus* occurring at Alilao Village of Taipei County, Taiwan (Formosa). Kyushu J. Med. Sci., 13, 51–66.
3) Chiu, J. K. (1965): *Tricula chiui* Habe et Miyazaki, 1962: a snail host for *Paragonimus iloktsuenensis* Chen, 1940 in Taiwan. Japan. J. Parasitol., 14, 269–280 (with Japanese summary).
4) Kawashima, K. and Miyazaki, I. (1963): The experimental infection of larval lung flukes to Japanese *Oncomelania* snails II. Experimental infection of *Paragonimus iloktsuenensis* Chen, 1940 to *Oncomelania nosophora* (Robson, 1915). Japan. J. Parasitol., 12, 159–161 (in Japanese with English summary).
5) Mannoji, N. (1952): Further notes on the lung fluke, *Paragonimus ohirai* Miyazaki, 1939 Part I. A study on the second intermediate host of the lung flukes, *P. ohirai* Miyazaki, 1939 and *P. iloktsuenensis* Chen, 1940. Acta Medica, 22, 1183–1190 (in Japanese with English summary).
6) Miyazaki, I. (1944 a): On the geographical distribution of *Paragonimus ohirai* Miyazaki, 1939 I. Medicine & Biology, 6, 23–26 (in Japanese).
7) Miyazaki, I. (1944 b): The third species of *Paragonimus* found in Japan. Medicine & Biology, 6, 197–201 (in Japanese)
8) Miyazaki, I. (1945): On the third species of

lung fluke from Japan "small type of *Paragonimus ohirai*". Report of Kagoshima Medical School, No. 1, 19–25 (in Japanese).

9) Miyazaki, I. (1947 a): Morphological differences of metacercariae among three species of *Paragonimus* in Japan. Medicine & Biology, 10, 223–225 (in Japanese).

10) Miyazaki, I. (1947 b): On the ovary of "small type of *Paragonimus ohirai*". Report of Kagoshima Medical School, No. 3, 5–8 (in Japanese with English summary).

11) Miyazaki, I. (1950): Lung flukes (Summary of the lecture at annual meeting of Japanese Society of Parasitology). Tokyo Medical Journal, 67, 21–23 (in Japanese).

12) Miyazaki, I. and Chiu, J. K. (1962 a): On the lung fluke, *Paragonimus iloktsuenensis* Chen first recognized in Taiwan. Japan. J. Parasitol., 11, 285 (in Japanese).

13) Miyazaki, I. and Chiu, J. K. (1962 b): First report of the lung fluke *Paragonimus iloktsuenensis* Chen from Formosa. J. Parasitol., 48, Section 2, 23–24.

14) Miyazaki, I., Mannoji, N. and Arita, M. (1951): On the distribution of lung flukes, *Paragonimus ohirai* Miyazaki, 1939 and *P. iloktsuenensis* Chen, 1940. Medicine & Biology, 20, 121–123 (in Japanese).

15) Miyazaki, I., Kawashima, K. and Yoshida, Y. (1960): Studies on the snail hosts of *Paragonimus ohirai* Miyazaki, 1939 and *P. iloktsuenensis* Chen, 1940. Kyushu J. Med. Sci., 11, 261–275.

16) Sato, A., Tada, I., Nagano, K., Otsuji, Y. and Fukushima, H. (1969 a): On a lung fluke found in Amami-Oshima Is., Kagoshima, Japan. Japan. J. Parasitol., 18, 28–33.

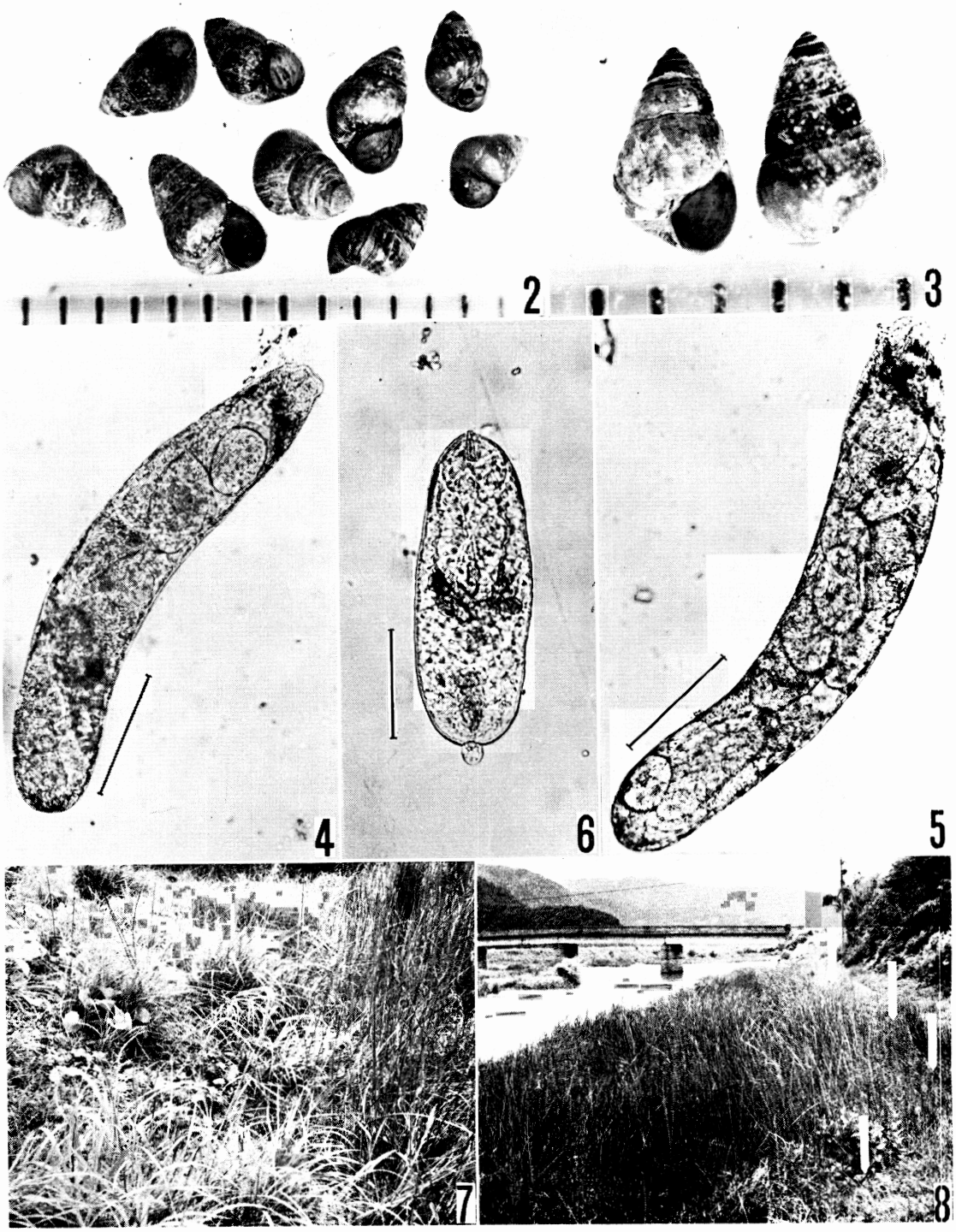
17) Sato, A., Tada, I., Nagano, K., Otsuji, Y., Ando, M., Fukushima, H. and Mitsudome, S. (1969 b): On the distribution of *Paragonimus iloktsuenensis* Chen, 1940 in Amami-Oshima Is., Kagoshima, Japan and the results of experimental infections. Japan. J. Parasitol., 3, 281–288 (in Japanese with English summary).

18) Tomimura, T., Terauchi, J. and Tarumoto, I. (1960): Studies on the first intermediate host of lung fluke, *Paragonimus iloktsuenensis* Chen, 1940 in the mouth of the River Shinyodo, Osaka Prefecture. Medicine & Biology, 54, 45–51 (in Japanese).

19) Yoshida, Y. (1959): Studies on the first intermediate host of *Paragonimus iloktsuenensis* Chen, 1940 in Japan (I) Experimental infection with the larva of *P. iloktsuenensis* to *Assiminea parasitologica* Kuroda, 1958 and *Assiminea japonica* von Martens, 1877. Japan. J. Parasitol., 8, 822–828 (in Japanese with English summary).

20) Yoshida, Y. (1960): Experimental studies on the first intermediate hosts of *Paragonimus westermani*, *P. ohirai* and *P. iloktsuenensis*. Japan. J. Parasitol., 9, 377–378 (in Japanese).

21) Yoshida, Y. and Kawashima, K. (1961): On the distribution of the snail hosts of *Paragonimus ohirai* Miyazaki, 1939 and *Paragonimus iloktsuenensis* Chen, 1940 in Japan. Japan. J. Parasitol., 10, 152–160 (in Japanese with English summary).


2
3
4
6
5
7
8

Explanation of Figures

Fig. 2. The molluscs, *Angustassiminea nitida* (Pease) var. collected from the banks of Kawauchi River, Sumiyo-Village, Is. Amami-Oshima. (Scale 1 mm)

Fig. 3. Enlargement of the molluscs, *A. nitida* (Pease) var. (Scale 1 mm)

Fig. 4. The second generation redia of *P. iloktsuenensis* Chen removed from naturally infected molluscs. (Scale 0.2 mm)

Fig. 5. The second generation redia of *P. iloktsuenensis* Chen removed from experimentally infected molluscs. (Scale 0.2 mm)

Fig. 6. Cercaria removed from naturally infected molluscs. (Scale 0.1 mm)

Fig. 7. The habitat of the mollusc, *A. nitida* (Pease) var. at the mouth of Kawauchi River. The molluscs were found under the stones, the fallen leaves and/or on stems of grasses.

Fig. 8. The distant view of the habitat of the molluscs. Arrows mean the areas where the molluscs were found.

鹿児島県奄美大島における小形大平肺吸虫の新しい第1中間宿主について

川島健治郎　浜島房則
(九州大学医学部寄生虫学教室)

佐藤ら (1969 a, b) は鹿児島県奄美大島数地区のクロベンケイから肺吸虫のメタセルカリアを，ネズミ類からその成虫を証明し，それを小形大平肺吸虫 *Paragonimus iloktsuenensis* Chen, 1940 として報告した．しかし，今日までその第1中間宿主は全く不明のままであつた．そこで，それを明らかにする目的で著者らは1969年6月4日から6日まで奄美大島に滞在し，住用村川内川の河口周辺で調査を行なつた．その結果，同地区において小形大平肺吸虫の第1中間宿主になると考えられるカイを発見し，これは国立科学博物館の波部忠重博士によりサツマクリイロカワザンショウと同定された．このカイは同氏により *Assiminea castanea satumana* Habe, 1942 と命名されていたものであるが，現在，同氏はこのカイを分類上 *Angustassiminea nitida* (Pease) の1亜種にすべきであると考えている．著者らは同地区において採集したこのカイのうち892個体を検査して，そのうち6個体 (0.67 %) に肺吸虫のレジアやセルカリアの自然感染を証明した．更にこのカイ60個体を用いて同島産肺吸虫の実験感染を行なつたところ，その100 %にレジア又はセルカリアへの発育を証明し，実験感染の成立することを明らかにした．得られた幼虫は何れも既知の小形大平肺吸虫の幼虫に形態上一致したこと，このカイにおける自然感染，実験感染が共に証明されたこと，更に，同地区で採集したクロベンケイの約50%に小形大平肺吸虫メタセルカリアを，捕獲したクマネズミの100 %にその成虫を証明したこと，又，この地区には，全く他種の肺吸虫が認められなかつたことなどからサツマクリイロカワザンショウを小形大平肺吸虫の新しい第1中間宿主として報告した．