

ON THE MORPHOLOGICAL CHARACTERISTICS OF THE
MIRACIDIUM OF *PARAGONIMUS MIYAZAKII* KAMO
ET AL., 1961

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Accepted for Publication on September 3, 1979

Abstract

The morphological characters of the miracidium of *Paragonimus miyazakii* Kamo et al., 1961, are reported. The miracidium of *P. miyazakii* is readily distinguishable from that of other known species of *Paragonimus* such as *P. westermani*, *P. ohirai*, *P. kellicotti* and *P. pulmonalis* by the presence of 30 to 50 acidophile granules in the digestive gut. The granules are stained in a reddish blue color with the solution of acid dyes but they will not take dye with the solution of basic dyes.

INTRODUCTION

The lung flukes of the genus *Paragonimus* are known in about thirty species in the world, and five species of *Paragonimus* are reported in Japan, i. e., *P. westermani* (Kerbert, 1878), *P. ohirai* Miyazaki, 1939, *P. iloetsuenensis* Chen, 1940, *P. miyazakii* Kamo, Nishida, Hatsushika et Tomimura, 1961 and *P. sadoensis* Miyazaki, Kawashima, Hamajima et Otsuru, 1968. Quite recently, Miyazaki (1978)¹⁾ stressed that the typical adult worms of *P. pulmonalis* (Baelz, 1880) are mixed in the specimens which have been called colloquially "*P. westermani*".

The morphological differences in adult worms and metacercariae of known species of *Paragonimus* can be readily distinguished by their external or internal appearances, but the definite distinctions in the features of miracidia in the species present great difficulties in classification. During the morphological research on the miracidium of *P. miyazakii* the author has found that the miracidium of *P. miyazakii* differs entirely from that of other known species of *Paragonimus* in the stainability to some acid dyes. The author would like to report here about the prominent features on the morphology of the miracidium of *P. miyazakii* with some photographs.

MATERIALS AND METHODS

The miracidia of *P. miyazakii* used for the morphological observations were obtained from the eggs in feces of experimentally infected dogs. The

metacercariae of *P. miyazakii* used for infecting dogs were removed from the fresh water crabs, *Potamon dehaani* collected at the type locality of this fluke. The fresh eggs were incubated for several days at 25°C by the method of Harada-Mori culture.²⁾ The miracidia of other known species of *Paragonimus* such as so-called "*P. pulmonalis* (Baelz, 1880)" as reported by Miyazaki (1978),¹⁾ *P. ohirai* Miyazaki, 1939 and *P. kellicotti* Word, 1908, were also examined for the purpose of a comparative investigation. The metacercariae of *P. pulmonalis* used for infecting animals were removed from *Eriocheir japonicus* collected at the Shimanto River in Ehime Prefecture, Shikoku. The fresh eggs of *P. kellicotti* were donated by Prof. Yukio Yoshida of the Kyoto Prefectural University of Medicine. For the morphological study the liberated miracidia were fixed in a solution of Shaudin's fixative, and stained with several solutions of acid and basic dyes. The liberated miracidia were also examined under the microscope without staining.

RESULTS

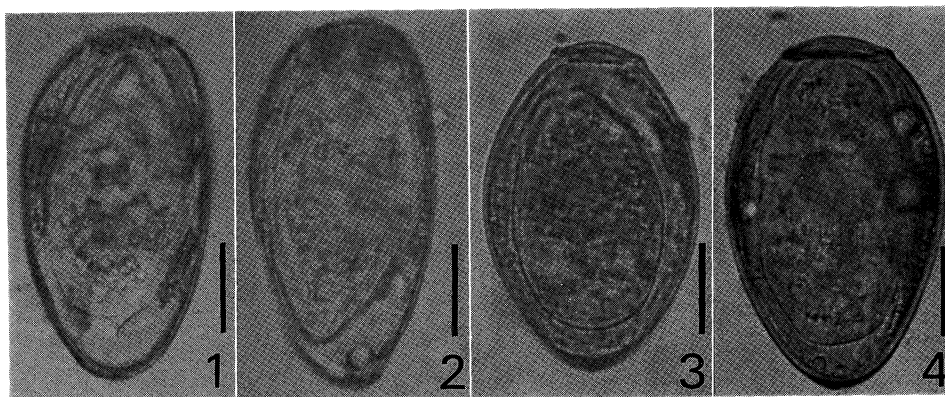
The miracidia of *P. miyazakii* in the eggs began an active movement for expansion and contraction of the body on the 23rd day after incubation at 25°C (Fig. 1). Hatching of the miracidia of *P. miyazakii* was enhanced considerably by the proper management of low-temperature in the same manner as those of other known species of *Paragonimus* (Ameel, 1934³⁾; Chen, 1940⁴⁾). In other words, if the incubation tubes are kept at a low-temperature (about 10°C) for half hour period, then the tubes are brought back to the room temperature, and when the feces-smear filter papers from the tubes are placed in water at 25°C, the matured miracidia in the eggs are prompted to hatch, and the liberated miracidia swarm out into water. The liberated mira-

TABLE I. Comparison of the size of miracidia of known species of *Paragonimus* (in microns)

Species	Length	Width	Average length X width		Author
<i>P. westermani</i> *	81 - 99	36 - 54			Nakagawa (1915)
<i>P. westermani</i> *	62 - 92	35 - 56			
<i>P. westermani</i>	51 - 68	31 - 40	63	X 37	Watanabe (1935)
<i>P. kellicotti</i>	60 - 102	31 - 48	81	X 41	
<i>P. iloktsuenensis</i>			61	X 29	Chen (1940)
<i>P. miyazakii</i> *	63 - 83	30 - 45	65	X 38	Present author
<i>P. miyazakii</i>	48 - 85	20 - 50	60	X 29	
<i>P. westermani</i> *°	63 - 85	40 - 55	77	X 48	
<i>P. westermani</i> °	48 - 80	20 - 43	60	X 36	

* Living specimens.

° Probably *P. pulmonalis* by Miyazaki (1978).



Figs. 1-4. Fully developed eggs of *Paragonimus* spp., twenty days incubation at 25°C. (Scale=0.02mm)

Fig. 1. *Paragonimus miyazakii*.

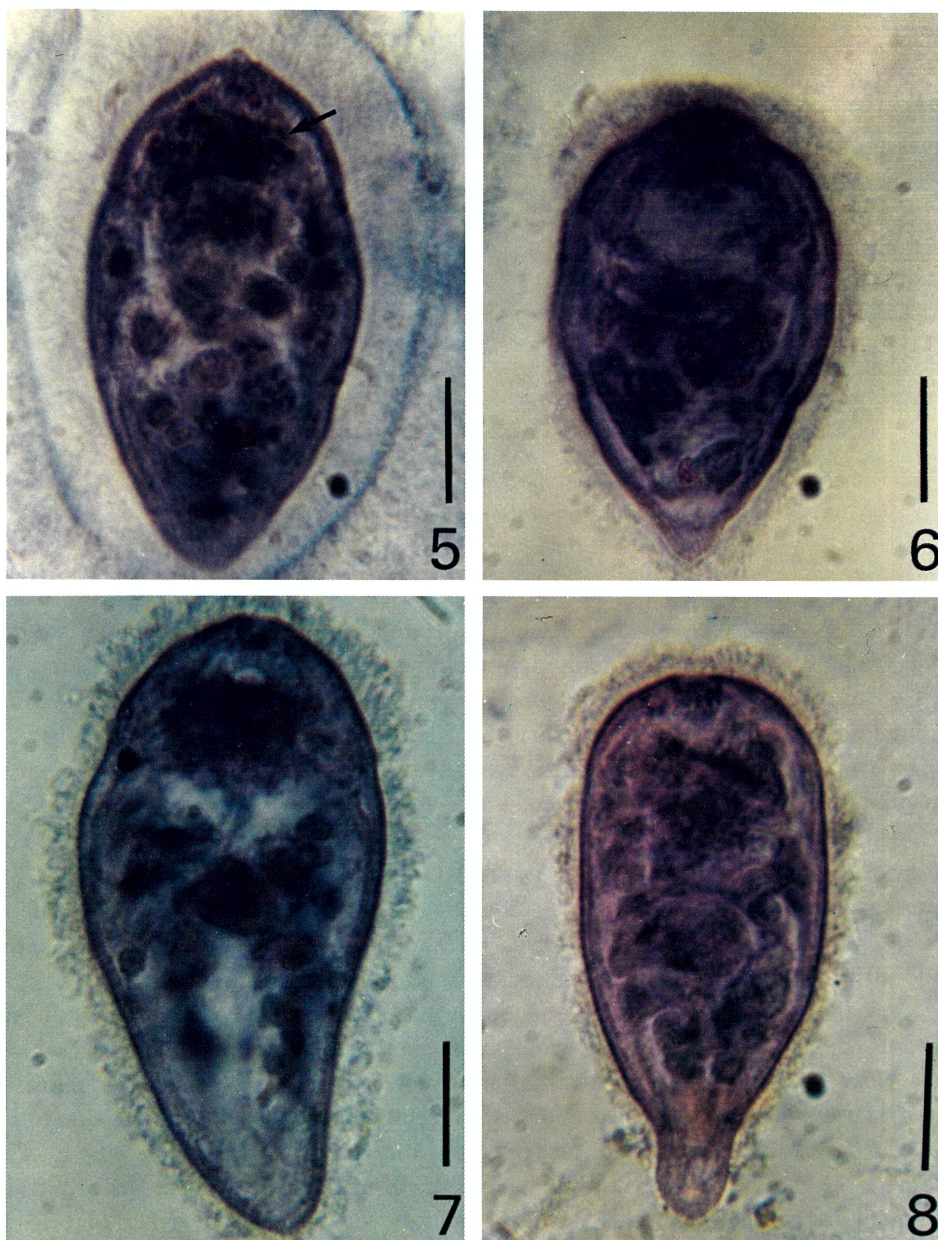
Fig. 2. *P. westermani* (probably *P. pulmonalis*).

Fig. 3. *P. ohirai*.

Fig. 4. *P. kellicotti*.

cidia of *P. miyazakii* swarm while rotate their body actively in the water, and the behaviors of the liberated miracidia of *P. miyazakii* are the same as those of other known species of *Paragonimus*. All of the eggs including miracidium on the filter paper survived for a long period of time so long as they are left standing in the culture tube, and the hatching of the miracidium occurs only when they are placed in the water.

Measurements of the miracidia of *P. miyazakii* and *P. pulmonalis* are shown in Table I. Fifty fixed miracidia of *P. miyazakii* measure 48 to 85 μm (av. 60) in length, and 20 to 50 μm (av. 29) in width. The miracidia of *P. pulmonalis*, on the other hand, measure 48 to 80 μm (av. 60) in length, and 20 to 43 μm (av. 36) in width. The body of the miracidium of *P. miyazakii* is typically pyriform in shape, and epidermal cells are covered with cilia of about 10 μm long. The epidermal cells can be divided into four zones which are clearly noticed by three distinct transverse stripes. The first row consists of six triangular cells, the second seven rectangular cells, the third three inverted trapezoid cells, and the posterior end individual cells arranged like a cap, respectively. The excretory system consists of one pair of flame cells and is situated in a line near the posterior margin of the second row, and each excretory duct winds up to down in several loops and opens to the outside of the body between the second and third rows. The morphological features of the miracidium of *P. miyazakii* are very similar to those of other known species of *Paragonimus*, but in the staining preparations the notable difference



Figs. 5-8. Liberated miracidia of *Paragonimus* spp., stained with anilin blue. (Scale=0.015 mm)

Fig. 5. Miracidium of *Paragonimus miyazakii*, showing acidophile granules in the digestive gut (arrow).

Fig. 6. Miracidium of *P. westermanni* (probably *P. pulmonalis*).

Fig. 7. Miracidium of *P. ohirai*.

Fig. 8. Miracidium of *P. kellicotti*.

can be recognized in the digestive organ. The digestive organ, a large sac-like gut, is situated at the anterior end of the body, and the gut of the miracidium of *P. miyazakii* is about 1/5 of the whole length of body, while that of the miracidium of *P. pulmonalis* is about 2/5. The noteworthy is the fact that 30 to 50 granules are found in the digestive gut of the miracidium of *P. miyazakii* in the staining specimens. The granules are small globular in shape, measuring about 1 μ m in diameter. These granules react vigorously in the solution of several acid dyes such as anilin blue, eosin, haematoxylin as well as fast green, and they stain in a reddish blue color (Fig. 1), but the granule's reactions to the solution of basic dyes such as Janus green, methylen blue and rhodamin B etc. do not at all occur. These acidophile granules revealed in *P. miyazakii*, on the other hand, are insufficiently recognized in the miracidia of other three species of *Paragonimus* examined in this investigation.

DISCUSSION

Although the morphology of the miracidia of the genus *Paragonimus* has been reported by various workers, the miracidia of *Paragonimus* show a great resemblance to each other in the external appearance and internal anatomy. The size of miracidial body of *P. miyazakii* resembles very closely that of *P. westermanni* from Nakagawa (1915)⁵⁾ and Watanabe (1935),⁶⁾ *P. iloktsuenensis* from Chen (1940),⁴⁾ and *P. pulmonalis* from the present author though the range of values is slightly smaller than *P. kellicotti* from Ameel (1934)³⁾ (Table I).

Moreover, the arrangement of ciliated epidermal cells in the miracidium of *P. miyazakii* is very similar to that of the miracidia of other known species of *Paragonimus* except that there is a subtle difference in number of the second row. The second row of epidermal cells in the miracidia of the genus *Paragonimus* seems to be 6 in number under ordinary occasion. Ameel (1934)³⁾ reported that the epidermal cells in the miracidium of *P. kellicotti* are in 3 transverse rows, 6 in the first row, 6 (rarely 7) in the second, and 3 in the third, with a single terminal cell. Watanabe (1935),⁶⁾ on the other hand, reported the number of the epidermal cells in *P. westermanni* to be 17 in all; 6 in the first row, 7 in the second, 3 in the third and a single terminal cell. Chen (1940)⁴⁾ also reported the epidermal cells in the miracidium of *P. iloktsuenensis* to be recognized by three distinctive transverse markings, and the second row consisting of six triangular cells. Therefore, the number of the second row in *P. miyazakii* is exactly the same as that of *P. westermanni*

from Watanabe (1935)⁶⁾ and from Tang (1940),⁷⁾ and *P. pulmonalis* in the present investigation.

The structures of the miracidium of *P. miyazakii* in the stained specimens resemble very closely those of three species of *Paragonimus* in the present investigation, but the presence of the acidophile granules in the digestive gut is one of the distinctive characteristics of the miracidium of *P. miyazakii*, though the function and the reason why they are there are not known in the present state of knowledge. There is no report on the presence of the acidophile granules in any miracidia of the genus *Paragonimus* though Chen (1940)⁴⁾ had described that the coarsely granules might be observed in the digestive gut of the miracidium of *P. iloktsuenensis* with non-stained material. The granules of the miracidium of *P. miyazakii*, however, cannot be distinguished in fresh raw materials, but if the materials are stained with the solution of acid dyes the presence of the granules become distinct in the digestive gut. Therefore, the miracidium of *P. miyazakii* can be readily distinguished as that of other species of *Paragonimus* by the presence of the granules in the digestive gut so long as the miracidia are stained with acid dye. Even if the miracidia of four species of *Paragonimus* are mixed on a slide glass, the miracidium of *P. miyazakii* is sharply distinguishable from that of other species of *Paragonimus* after staining with the solution of acid dye. This fact seems to suggest that the presence of the acidophile granules is the morphological characteristics of the miracidium of *P. miyazakii*.

Acknowledgment

The author would like to thank to Prof. Yukio Yoshida of the Kyoto Prefectural University of Medicine for providing the ova of *Paragonimus kellicotti* for the present investigation.

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