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Some notes on differentiation of *Paragonimus kellicotti* and *P. miyazakii* in their eggs and larval stages

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Until 1955, there were three species of *Paragonimus* known to occur in Japan, *Paragonimus westermani* (Kerbert, 1878), *P. ohirai* Miyazaki, 1939, and *P. iloktsuenensis* Chen, 1940. In 1955, Miyazaki reported a fourth species of this genus in Japan, *P. kellicotti* from weasels. However, he stated in his paper that this *Paragonimus* might actually be a different species which could not be distinguished from *P. kellicotti* in the adult stage, but could be distinguished in their larval stages.

In 1960, Kamo *et al.* found a new type of metacercaria in *Potamon dehaani* in Yamaguchi Prefecture, Japan. In an experimental animal the adult worm maturing from this metacercaria was described by Kamo *et al.* in 1961 as *Paragonimus miyazakii* n. sp.. Since then, it has been believed that the *P. kellicotti* which was recovered from weasels by Miyazaki (1955) also must be the *P. miyazakii*. A small fresh water snail, *Bythinella* (*Moria*) *nipponica akiyoshiensis* (Kuroda et Habe, 1957), was reported by Hatsushika *et al.* in 1966, as the first intermediate host of *P. miyazakii*.

Since the differentiation between *P. kellicotti* and *P. miyazakii* has been difficult, not only as adults but also in the egg and larval stages, the present study was initiated in an attempt to answer the following questions: (1) Morphological differences in the eggs of *P. kellicotti* and *P. miyazakii* in fresh feces. (2) Susceptibility of some Japanese snails to the larvae (miracidia to cercaria) of *P. kellicotti* of the North American strain. (3) The possibility of *P. kellicotti* cercariae developing into metacercariae in the Japanese fresh water crab, *Potamon dehaani*, and the possible morphological differences of this metacercaria and that of *P. miyazakii* in this same intermediate host.

Materials and Methods

1. Comparative morphology of eggs of *P. kellicotti* and *P. miyazakii*.

A dog was given metacercariae of *P. kellicotti* which were collected from *Cambarus robustus* in Michigan, through the courtesy of Prof. H. van der Schalie, in 1963, and another dog was given metacercariae of *P. miyazakii* collected from *Potamon dehaani* in Kyoto Prefecture in 1965. Eggs of both species were obtained when needed from the feces of these two dogs. Study of the eggs was always performed on fresh (unfixed) material.

2. Experimental infection of some Japanese and American snails with miracidia of *P. kellicotti* and *P. miyazakii*.

Some Japanese snails which were known to serve as first intermediate hosts for *P. westermani*, *P. ohirai*, and *P. iloktsuenensis* were exposed to miracidia of *P. kellicotti*. *Pomatiopsislapidaria* which was kindly sent to us by Prof. H. van der Schalie was also studied in the same manner as a control.

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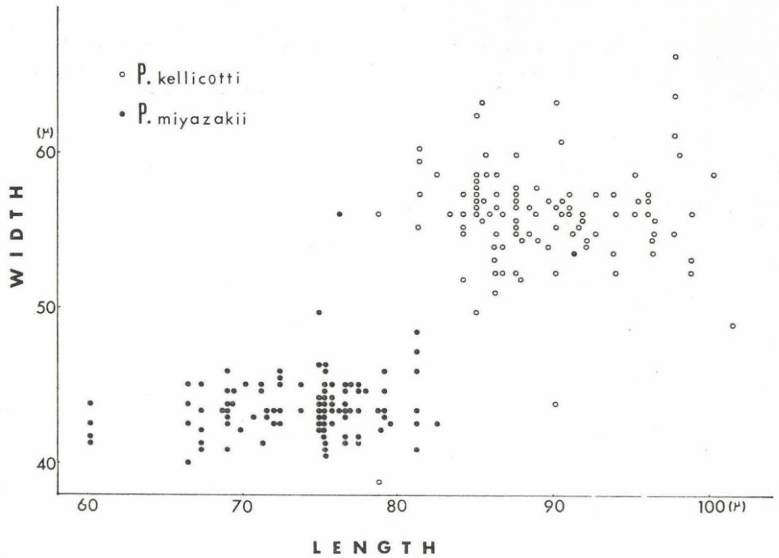


Fig. 1 Showing non-overlapping in egg size between *P. kellicotti* and *P. miyazakii*

Table 1 Measurements (in microns) of eggs of *P. kellicotti* and *P. miyazakii*

Species	Length	Width	Thickness of egg shell	
			Lateral side	Aboperculated side
P. kellicotti	(A) 89.05±5.03*	56.46±2.85*	1.83	2.58
	(B) 87.29±3.64*	57.02±2.55*	2.69	3.11
P. miyazakii	(A) 73.87±3.76*	43.91±2.44*	1.02	1.09
	(B) 74.25±3.15*	49.79±2.20*	1.22	1.06

(A) : Data of the present investigation. (B) : Data shown by Terauchi *et al.* (1961).
* : Standard deviation.

The exposure of snails to miracidia was as follows: ten snails in a petri dish (10 cm diameter) containing tapwater of 1 cm depth were exposed to known numbers of miracidia for two hours at 25°C; following this the snails were removed to an aquarium where they were maintained under suitable conditions at 22-25°C.

3. Experimental infection of *Potamon dehaani* and *Procambarus clarkii* with *P. kellicotti* cercariae.

Cercariae of *P. kellicotti* were obtained from *Pomatiopsis lapidaria* which had been previously infected with the miracidia. Into a large petri dish (20 cm in diameter) containing either ten crabs or crayfishes in tapwater of about 5 mm in depth, approximately 300 of these cercariae were released for 24 hours. These crabs and crayfishes were then kept in the laboratory at 22-25°C under suitable conditions. The metacercariae that developed in these intermediate hosts were morphologically compared to those of *P. miyazakii* which were obtained from naturally infected *Potamon dehaani* from Kyoto Prefecture. Measurement of the metacercaria was carried out, under the microscope, without coverglass to avoid the pressure by it.

Results

1. Comparative morphology of eggs of *P. kellicotti* and *P. miyazakii*.

1) Size

Table 2 Experimental infection of some Japanese and American snails with miracidiae of *Paragonimus kellicotti* and *P. miyazakii*

Paragonimus	Snails	Number of miracidia exposed per one snail	Days after miracidial infection	Number of snails examined	Number of snails positive for rediae and cercariae
Paragonimus kellicotti	*Semisulcospira bensoni*	10–30	54–105	22	0
	Assiminea japonica	3	70	9	0
	Assiminea parasitologica	3	70	48	0
	Assiminea yoshidayukioi	3	70	50	0
	Oncomelania nosophora	3	70	30	0
	Physa acuta	3	70	3	0
	Pomatiopsis lapidaria	6	174	12	10
Paragonimus miyazakii	*Semisulcospira bensoni*	50–100	87–110	53	0
	Pomatiopsis lapidaria	6	98	2	0

One hundre peggs of each species from fresh feces were measured. The results are shown in Table 1 and are compared with the data obtained by Terauchi *et al.* (1961) from eggs preserved in formalin. From these it is evident that eggs of *P. kellicotti* are larger in size and have a thicker egg shell than those of *P. miyazakii* (Figures 1 through 5).

2) Shape

The location of the maximum width of eggs is sometimes useful in differentiating species of *Paragonimus*. For instance, the maximum width of *P. westermani* is usually located in the operculated half and that of *P. ohirai* usually is in the middle of the egg. The symmetry of eggs may be characteristic in some species of *Paragonimus*. *P. westermani* generally shows asymmetry, while *P. ohirai* is generally symmetrical. The present investigation showed that 84% of *P. kellicotti* eggs had their maximum width in the middle and 93% were symmetrical; and that 77% of *P. miyazakii* eggs were wider at the middle and 78% were symmetrical.

Although the location of the maximum width and symmetry of the eggs were not useful in distinguishing *P. kellicotti* and *P. miyazakii*, the shape of the egg shell at the aboperculated end was observed to be distinctive in each species. As shown in Figures 2 through 5, the egg shell at the aboperculated end of *P. kellicotti* is smoothly rounded and has a shadow-like black thickening. The egg shell of *P. miyazakii*, on the other hand, generally does not have such a thickening, is not rounded, but is irregular in shape and always has a small concurve at the end of the eggs. Thus, *P. kellicotti* and *P. miyazakii* are distinguishable from one another by examining their eggs even though the adult worms are hardly distinguishable.

2. Experimental infection of some Japanese and American snails with miracidia of *P. kellicotti* and *P. miyazakii*.

Semisulcospira bensoni is known to be the first intermediate host of *P. westermani*. *Assiminea parasitologica*, *A. yoshidayukioi* and *A. japonica* are susceptible snail hosts of *P. ohirai* and *P. iloktsuenensis* in Japan. Although not known as snail hosts for species of *Paragonimus*, *Oncomelania nosophora* is an important snail as the intermediate host of *Schistosoma japonicum*, and *Physa acuta* is an experimental snail host of *Fasciola hepatica*.

Table 3 Experimental infection of *Procambarus clarkii* and *Potamon dehaani* with cercariae of *Paragonimus kellicotti* of the North American strain

Crayfish and crab	No. of crayfishes and crabs	Days after cercarial infection	Number of metacercariae recovered from					total
			heart & pericardium	inner membrane of carapace	liver	muscle	others	
Procambarus clarkii	1	53	3	1	0	0	3	7
	2	〃	0	0	0	0	0	0
	3	〃	0	3	0	0	2	5
	4	73	1	0	0	0	0	1
	5	100	3	0	0	0	0	3
	6	〃	2	0	0	0	0	2
	7	〃	1	0	0	0	0	1
	8	〃	1	0	0	0	0	1
Potamon dehaani	1	62	0	0	1	0	0	1
	2	〃	0	0	0	0	0	0
	3	〃	3	0	1	0	0	4
	4	〃	3	0	1	0	0	4
	5	99	1	0	0	0	0	1
	6	〃	0	0	0	0	0	0
	7	〃	9	0	0	0	0	9
	8	〃	0	0	0	0	0	0
	9	〃	0	0	0	0	0	0
	10	〃	1	0	0	0	0	1
	11	〃	2	4	0	0	0	6
	12	〃	0	0	0	0	0	0
	13	〃	0	0	0	0	0	0
	14	〃	11	0	0	0	0	11
	15	〃	3	0	0	0	0	3
	16	118	3	0	0	0	0	3
	17	203	4	0	0	0	0	4

These six species of Japanese snails, with *Pomatiopsis lapidaria* of North America as a control, were tested for their susceptibility to larval infection by *P. kellicotti*. *Pomatiopsis lapidaria* and *Semisulcospira bensoni* were also tested for their susceptibility to *P. miyazakii* miracidia.

As shown in Table 2, the larvae of *P. kellicotti* failed to develop in the six species of Japanese snails, although *Pomatiopsis lapidaria* harboured a number of rediae and cercariae about six months after miracidial infection. Attempts at experimental infection of *Pomatiopsis lapidaria* and *Semisulcospira bensoni* with *P. miyazakii* miracidia failed.

3. Morphological differences in metacercariae of *P. miyazakii* and *P. kellicotti* from experimentally infected *Procambarus clarkii* and *Potamon dehaani*.

Potamon dehaani is the only fresh water crab in Japan and is known to be the second intermediate host for *P. westermani* and *P. miyazakii*. *Procambarus clarkii* was originally imported from Louisiana, U. S. A. in 1930, and since then it has become widely distributed all over Japan. In 1952, Yokogawa found the metacercariae of *P. westermani* in this crayfish.

Miyazaki (1964) showed some morphological differences in the metacercariae of *P. kellicotti* in *Cambarus robustus* from North America and *P. miyazakii* in *Potamon dehaani* in Japan. The present study describes the morphology of *P. kellicotti* matacercaria that developed in *Potamon dehaani* after experimental infection.

As shown in Table 3, a considerable number of mature metacercariae of *P. kellicotti* was recovered from 7 out of 8 crayfishes and from 11 out of 17 crabs after cercarial exposure. It is interesting that the Japanese fresh water crab, *Potamon dehaani*, is highly susceptible to the North

Table 4 Measurement of metacercariae of *Paragonimus kellicotti* in *Procambarus clarkii*, *Potamon dehaani* and *Cambarus robustus*, and those of *Paragonimus miyazakii* in *Potamon dehaani*

Paragonimus in crabs and crayfishes	Days after cercarial exposure	Number of measured	Size of inner cyst		Thickness of cyst wall	
			length	width	inner wall	outer wall
P. kellicotti in *Procambarus clarkii* (experimental infection)	53	9	436.6	367.7	5.6	3.3
	100	6	427.5	388.8	6.9	2.8
	(average)		433.0	376.2	6.0	3.1
P. kellicotti in *Potamon dehaani* (experimental infection)	99	10	391.4	380.5	4.2	1.8
	203	4	380.0	370.0	5.6	1.3
	(average)		388.0	377.6	4.6	1.4
P. kellicotti in *Cambarus robustus* (natural infection) (Miyazaki, 1964)			408.0	398.0	6.1	3.6
P. miyazakii in *Potamon dehaani* (natural infection)		10	489.9	475.5	16.8	10.3

American species of *Paragonimus*.

Table 4 shows the size of metacercariae of *P. kellicotti* obtained from experimentally infected crayfishes and crabs when compared to metacercariae of *P. kellicotti* from naturally infected *Cambarus robustus* (Miyazaki, 1964) and *P. miyazakii* from *Potamon dehaani*.

Table 4 shows that there is no difference in size of metacercariae of *P. kellicotti* developed in *Potamon dehaani* from those found in *Procambarus clarkii* and in *Cambarus robustus*.

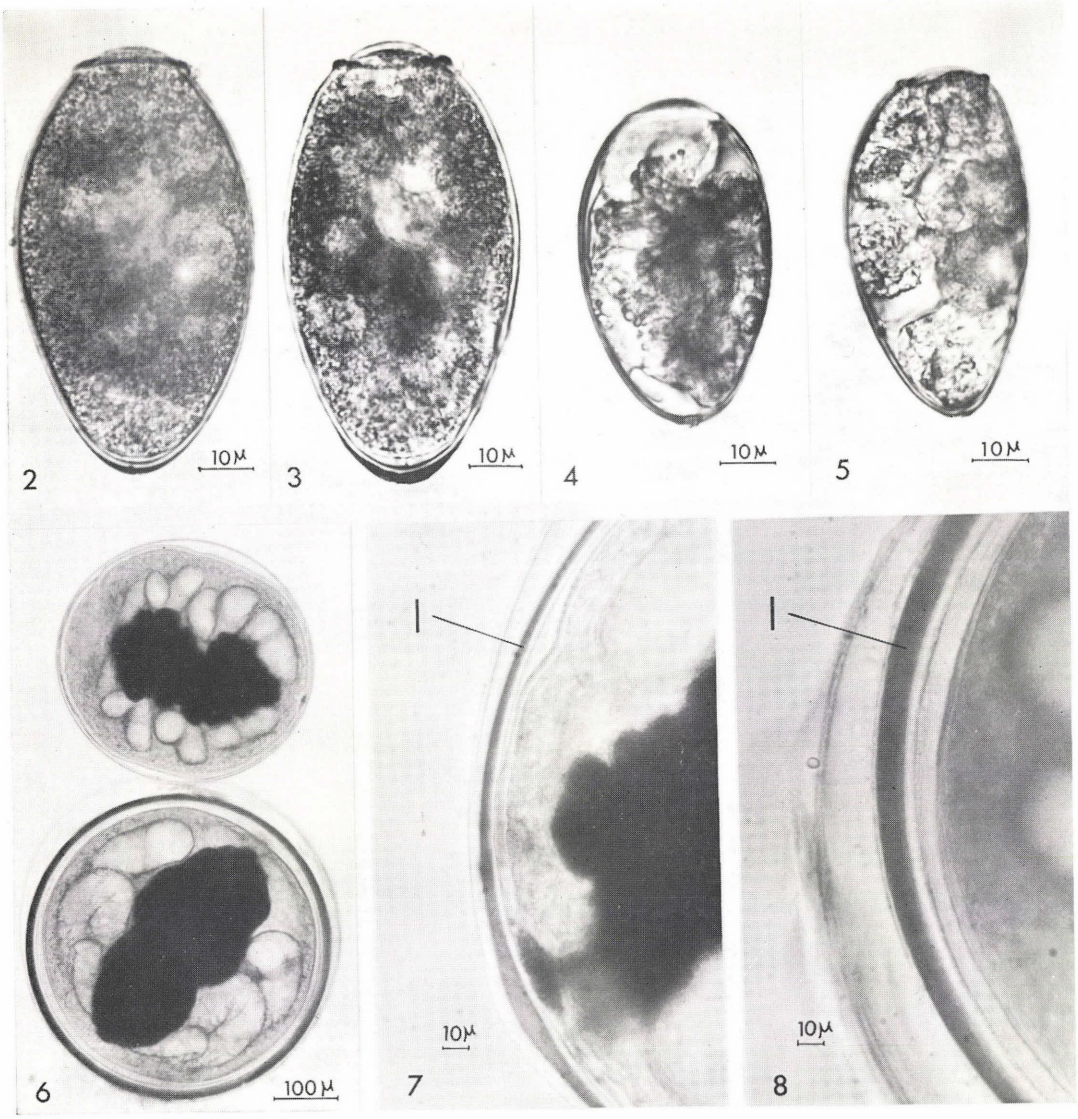
In other words, *P. kellicotti* metacercariae developed in *Potamon dehaani* are distinguishable from those of *P. miyazakii* in the same intermediate host by the following points (Figures 6-8): (1) The diameter of inner cyst of the *P. miyazakii* metacercaria is larger than that of *P. kellicotti*. (2) Inner cyst-wall (or inner membrane) of the former species is much thicker than the latter. (3) The pinkish globules in the parenchyma of metacercariae are scarcely visible in *P. miyazakii* whereas they are usually present in *P. kellicotti*.

Discussion

Morphological investigations of *P. kellicotti* egg was carried out in detail, by Ameel (1934), Yokogawa (1955), Terauchi *et al.* (1961) and Isshiki (1962), in which the third authors noticed that the egg of *P. kellicotti* is larger in size and has a thicker shell than does *P. miyazakii*. The present investigation has shown that the shape of aboperculated end of the eggs is also useful in differentiation of the two species.

In the course of searching for the snail host of *P. miyazakii*, Kawashima and Miyazaki (1964) succeeded in obtaining rediae and cercarae in *Oncomelania nosophora* after experimental miracidial infection, and Hatsushika *et al.* (1966) demonstrated *Bythinella* (*Moria*) *nipponica akiyoshiensis* to be the first intermediate host in nature. In the present study, the writers could not experimentally infect *Pomatiopsis lapidaria* and *Semisulcospira bensoni* with *P. miyazakii*. The present study has also shown that six species of Japanese snails (Table 2) were not susceptible to *P. kellicotti* miracidia although numerous rediae and cercariae were found in *Pomatiopsis lapidaria* which had been exposed in a similar fashion.

The possibility of differentiating both of these species of *Paragonimus* in their metacercarial stage was shown by Miyazaki



Explanation of Photos

Figs. 2 & 3: Egg of *Paragonimus kellicotti*. Figs. 4 & 5: Egg of *Paragonimus miyazakii*. Fig. 6: Metacercaria of *Paragonimus kellicotti* (top) and *Paragonimus miyazakii* (bottom) both developed in the Japanese fresh water crab, *Potamon dehaani*. Fig. 7: Cyst wall of metacercariaof *Paragonimus kellicotti* showing thin inner membrane (I). Fig. 8: Cyst wall of metacercaria of *Paragonimus miyazakii* showing thick inner membrane (I).

(1964) who compared the metacercariae of *P. miyazakii* from *Potamon dehaani* in Japan and those of *P. kellicotti* from *Cambarus robustus* in Michigan. Results of the present study indicate that successful infection of *Potamon dehaani* with *P. kellicotti* cercariae is possible and the metacarcariae of both species are distinguishable even if they occur in the same intermediate host.

Summary

The present study adds further knowledge on the morphological differences between *Paragonimus kellicotti* and *P. miyazakii*. It is reaffirmed that in the former species the egg is larger and has a thicker shell than those of the latter. In addition, it was noticed in the present investigation that the morphology of the aboperculated end of the eggs is useful in distinguishing the two species.

The experimental infection of Japanese fresh water crabs, *Potamon dehaani*, with cercariae of *P. kellicotti* of the North American strain showed that this crab is highly susceptible to this species. Metacercariae of *P. kellicotti* which developed in *Potamon dehaani* were morphologically identical to those found in naturally infected *Cambarus robustus* in North America. Thus, the morphological differences in metacercariae of *P. kellicotti* and *P. miyazakii* as shown by Miyazaki (1964) are not affected by the difference of the intermediate host but are essential characters.

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虫卵および幼虫におけるケリコット肺吸虫と宮崎肺吸虫の差異について

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ケリコット肺吸虫と宮崎肺吸虫とはその形態上類似点が多く，特に成虫では鑑別が困難とされている．併し，虫卵（寺内ら，1961），およびメタセルカリア（宮崎，1964）では両者の間に差異が認められると報告されている．今回の観察はこれらの差異をさらに明確にする為に行われた．

まずケリコット肺吸虫と宮崎肺吸虫の虫卵を新鮮な状態で比較観察した．虫卵の大きさは従来の報告にほぼ一致し（表1，図1），前者は明らかに後者より大きく，且卵殻の厚さも前者の方が大であった．さらに今回の観察により両種虫卵の卵殻の無蓋端の形態に差のあることが認められた．即ち，前者においては卵殻は厚く，往々にして黒色時計皿状の肥厚を有し，且，スムースな丸味を有する．一方，後者においては卵殻は薄く，黒色時計血状の肥厚を有するものは殆どなく，且，輪廓は不規則で，小さな凹みを有することが多い（図 2-5）．

次に，両種肺吸虫の虫卵を培養してミラシジウムを得て，これを数種の日本産淡水貝および北米ミシガン産 *Pomatiopsis lapidaria* に接触させ，第1中間宿主の感受性の面から両種肺吸虫の差異をしらべた．その結果，ケリコット肺吸虫のミラシジウムは *Pomatiopsis lapidaria* の体内でよく発育してレヂアおよびセルカリアを生ずるが，宮崎肺吸虫は同じ貝の体内で発育するものをみなかった．又，ケリコット肺吸虫のミラシジウムを日本産のカワニナ，カワザンショウガイ，ムシヤドリカワザンショウ，ヨシダカワザンショウ，ミヤイリガイ，サカマキガイ等に接触させたがセルカリアを得ることが出来なかった（表2）．

宮崎（1964）は北米ミシガン産の *Cambarus robustus* に寄生しているケリコット肺吸虫のメタセルカリアを検査し，宮崎肺吸虫のそれとの間に著明な差のあることを報告した．今回の我々の実験は，北米産ケリコット肺吸虫のセルカリアを日本産のサワガニに感染させることが出来るかどうかを試み，もしメタセルカリアを生じた場合，その形態は *Cambarus robustus* 体内で発育したものと同じかどうか，換言すれば，同じ日本産サワガニを第2中間宿主とした場合，ケリコット肺吸虫と宮崎肺吸虫とはメタセルカリアの形態においてやはり著明な差があるかどうか，等の点を知る為に行われた．その結果，まず表3に示したように，ケリコット肺吸虫は，実験的に，アメリカザリガニのみならず，日本産サワガニによく感染してメタセルカリアを生ずることが明らかとなり，その形態は表4および図6-8に示したように，ケリコット肺吸虫メタセルカリアの特徴をよくそなえ，宮崎肺吸虫のそれと鑑別が可能であることが明らかとなった．