Further Observations on the Morphology of Adult Lung Fluke, *Paragonimus philippinensis* Ito, Yokogawa, Araki and Kobayashi, 1978

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Introduction

In the Philippines the existence of human paragonimiasis had already been known by Musgrave (1907) who reported 17 cases among Filipinos. In 1957, Yogore and his co-workers studied the morphology of the Philippine lung fluke, and called it as Paragonimus westermani. But in 1978, a new name, Paragonimus philippinensis was proposed instead of P. westermani by the present authors, because of some distinct differences between the Philippine lung fluke and P. westermani from Japan. In the former report of us (1978), with regard to the adult morphology of P. philippinensis, it was noted that some more detailed study would be necessary to find a critical feature on the adult morphology between the Philippine lung fluke and P. westermani from Japan.

In the present paper, the result of further observations on 49 adult flukes of *P. philip-pinensis* was presented with some corrections of the former report. The holotype and paratypes of the adult fluke of *P. philippinensis* were deposited in Parasitology Collection,

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Materials and Methods

For obtaining the adult fluke of *P. philip-pinensis*, many crabs, *Sundathelphusa philip-pina* (Martens, 1868), were collected from Sorsogon and Leyte provinces, Philippines. Cysts isolated from these crabs were fed to dogs and rats experimentally. 60, 110–116 and 130 days after feeding dogs, and 70, 78–83 and 126 days after feeding rats, the flukes were obtained from the lungs and pleural cavity of these mammals. These flukes were fixed with 70% alcohol under pressure, stained with alum-carmine, and mounted with canada-balsam. Observations and measurements were made by such specimens.

Results

The special observation was focused on the size of body and suckers, and the shape of ovary, because the fundamental structure of remaining parts were almost as same as that in the former report by the present authors (1978).

1. Body size

In Table 1 and 2, results of measurements on each worm were shown with the mean of each group. The presence or absence of

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Table 1 Measurements of adult worms of P. philippinensis obtained from experimental dogs (unit is μ m except the body of mm)

Days after feeding	No.	Body l. w.	Oral sucker l. w.	Ventral sucker l. w.	Pharynx l. w.	Ova in uterus
60	1*	4.7×2.9	394×518	403×528	230×307	+
	2*	5.6×2.9	355×518	480×470	230×269	+
	3*	4.1×2.4	307×432	$403\!\times\!461$	$221\!\times\!202$	_
	4*	5.2×2.5	$403\!\times\!528$	$432\!\times\!451$	$250\!\times\!250$	_
	5*	4.6×2.1	365×451	$422\!\times\!422$	$211\!\times\!202$	_
	6*	4.6×2.5	355×499	403×403	$269\!\times\!221$	_
	mean	4.8×2.6	$363\!\times\!491$	$424\!\times\!456$	$235\!\times\!242$	
	1*	6.2×3.4	470×758	538×682	307×355	+
	2*	7.5×4.3	355×518	547×557	$-\times-$	+
	3*	7.9×4.3	576×710	$634\!\times\!662$	346×336	+
	4*	6.8×4.0	$461\!\times\!634$	518×576	259×336	+
	5*	6.5×4.2	$528\!\times\!653$	$490\!\times\!624$	$288\!\times\!307$	+
110-116	6**	8.4×4.3	614×806	691×701	317×326	+
110-110	7**	7.9×4.3	547×758	$605\!\times\!682$	317×374	+
	8**	6.2×3.6	$470\!\times\!653$	557×643	250×269	+
	9**	7.2×4.3	480×739	$614\!\times\!634$	$288\!\times\!374$	+
	10**	7.1×3.7	$-\times912$	$509\!\times\!653$	$154\!\times\!461$	+
	11**	6.6×3.4	$403\!\times\!691$	$643\!\times\!653$	384×336	+
	mean	7.1×4.0	$490\!\times\!712$	577×642	$291\!\times\!347$	
130	1**	10.2×5.8	547×787	672×758	307×403	+
	2**	8.6×5.5	595×720	$614\!\times\!662$	$346\!\times\!365$	+
	mean	$9.4{ imes}5.7$	571×754	643×710	$327\!\times\!384$	

note: * marks are Sorsogon-strain, ** marks are Leyte-strain.

ova in the uterus to show the maturity of each worm was also noted at the most right column. The average body size of six worms from dogs of 60 days after feeding was 4.8 mm long and 2.6 mm wide, and that of five worms from rats of 70 days after feeding was 4.9 mm long and 3.3 mm wide. So no remarkable difference on the body size between the worms from dogs and rats could be recognized in the early period of 60–70 days after infection.

Then in the case of worms from dogs, the body size became larger as the time elapsed. The average body size of worms from dogs of 110–116 days after feeding was 7.1 mm long by 4.0 mm wide, and that of 130 days after feeding was 9.4 mm long and 5.7 mm wide. Whereas in the case of worms

from rats, the body size was comparatively smaller than that from dogs, being only 6.6 mm long and 4.2 mm wide in worms of 120 days after infection.

2. Sizes of oral and ventral suckers

The size of sucker became larger as the body grew larger in both hosts of dogs and rats. The main purpose of the present observation was to compare the size between oral and ventral suckers.

In table 1 and 2, all figures of measurements of both suckers were shown except two worms which could not be determined the length of oral sucker. Among 47 worms observed, if the length was used as a criterion, 37 had a smaller oral sucker, 7 had a larger oral sucker than the ventral one, and the remaining 3 had both suckers of

Table 2 Measurements of adult worms of P. philippinensis obtained from experimental rats (unit is μ m except the body of mm)

Days after feeding	No.	Body l. w.	Oral sucker l. w.	Ventral sucker l. w.	Pharynx l. w.	Ova in uterus
70	1*	5.4×3.6	384×509	422×518	307×259	+
	2*	4.9×3.5	$-\times442$	374×432	192×240	+
	3*	4.5×2.9	374×432	374×451	278×256	_
	4**	4.1×3.2	336×480	346×461	259×326	+
	5**	4.8×3.2	336×509	394×518	269×269	+
	mean	4.9×3.3	$357\!\times\!474$	$382\!\times\!476$	$261\!\times\!270$	
	1**	4.3×2.0	432×432	509×461	221×221	+
	2**	5.8×4.3	518×538	$461\!\times\!528$	288×269	+
	3**	3.4×1.9	259×374	432×432	$211\!\times\!173$	+
	4**	4.3×2.1	374×461	461×470	259×288	_
	5**	6.4×3.8	499×577	480×557	336×307	-
	6**	4.7×3.0	317×528	384×480	259×288	_
	7**	5.9×3.4	$422\!\times\!557$	480×557	298×307	_
	8**	5.0×3.7	432×499	432×499	269×355	_
	9**	3.6×2.0	$221\!\times\!403$	413×461	$192\!\times\!211$	_
78-83	10**	3.6×2.0	$346\!\times\!432$	$422\!\times\!480$	$240\!\times\!202$	_
70 00	11**	4.3×3.0	470×538	403×499	$288\!\times\!269$	_
	12**	4.9×3.6	528×538	432×518	278×307	_
	13**	4.9×3.2	$384\!\times\!528$	$451\!\times\!480$	$240\!\times\!240$	_
	14**	4.6×3.4	394×538	451×470	221×269	
	15**	4.7×2.8	259×461	442×509	$211\!\times\!230$	_
	16**	4.4×2.6	259×451	413×461	$230\!\times\!221$	_
	17**	3.8×2.3	365×470	403×413	230×211	_
	18**	3.8×2.9	307×451	$422\!\times\!442$	$192\!\times\!230$	_
	19**	4.8×3.8	528×595	$442\!\times\!528$	317×336	_
	mean	4.6×2.9	$385\!\times\!493$	$439\!\times\!487$	$252\!\times\!260$	
	1**	6.4×4.5	595×662	528×624	211×202	+
126	2**	6.7×4.3	$451\!\times\!586$	518×682	$365\!\times\!384$	+
	3**	5.8×2.8	$449\!\times\!643$	547×586	$288\!\times\!298$	+
	4**	7.4×4.4	518×605	576×595	$384\!\times\!365$	+
	5**	6.6×4.8	518×624	557×653	403×432	+
	6**	6.4×4.1	557×653	557×653	384×403	+
	mean	6.6×4.2	515×629	547×632	$339 \!\times\! 347$	

note: * marks are Sorsogon-strain, ** marks are Leyte-strain.

the same length. But on the contrary, if the width was pointed as a criterion, 15 had a smaller, 28 had a larger oral sucker than the ventral one, and 4 had both suckers of the same width. As a combination of the length and the width, if the product of length and width was adopted as a criterion, the conclusion would be brought that among 47 worms observed, 30 had a smaller oral sucker, 14 had a larger oral sucker than the ventral one, and 3 had the same sized oral and ventral suckers.

3. Ovary

As to the situation of the ovary, among 49 worms observed, 23 was on the right side, 21 was on the left side, 4 was on the posterolateral and 1 was on the antero-lateral to the ventral sucker. With regard to the shape of the ovary, 31 worms showed an easily distinguishable typical six lobed shape. 14 worms had also six lobed shape, though the shape was so irregular that it is rather difficult to recognize as a six lobed one. The remaining 4 worms had more irregular shaped ovary which might be counted the number of lobes as five or seven. However it would be fundamentally concluded that the ovary consists of a small central mass and six lobes, some of which being shortly subdivided again.

Discussion

For the comparison of body size between *P. philippinensis* and *P. westermani* from Japan, a recent report of Habe (1978) yields quite sufficient data, because he noted the body size of *P. westermani* of Kyushu, Japan,

from several kinds of experimental hosts at various days after feeding. In table 3, the summary of our data with that of Habe (1978) were shown to compare the body size.

In the case of worms from dogs, as shown in Table 3, it is clear that the body size of *P. philippinensis* is always smaller than *P. westermani* from Japan in any corresponding days after feeding. In the summary of our former report (Ito *et al.*, 1978), it was noted that "as to the morphology of the adult fluke, *P. philippinensis* seems to be slightly smaller than *P. westermani*". But now it will be concluded that *P. philippinensis* is significantly smaller than *P. westermani* of Kyushu, Japan, in the case of worms from dogs.

In the case of worms from rats, so far as shown in Table 3, the body size of *P. philippinensis* is generally larger than *P. westermani* from Japan. Moreover many worms of *P. philippinensis* from rats attained their maturity. With regard to the differences of worm development in rats between *P. philippinensis* and *P. westermani* from Japan, Yokogawa and his co-workers are

Table 3 A comparison of measurements of adult worms between P. philippinensis and P. westermani

(unit is µm except the body of mm)

Exptl. host	Days after feeding	No. of worms examined	Body l. w.	Oral sucker l. w.	Ventral sucker l. w.	No. of worms with eggs in uterus (%)		
P. philipp	P. philippinensis (after the present data by materials from the Philippines)							
dog	60	6*	4.8×2.6	$363\!\times\!491$	424×456	2/6 (33.3%)		
dog	110-116	11*	7.1×4.0	490×712	$577\!\times\!642$	11/11(100 %)		
dog	130	2*	9.4×5.7	571×754	643×710	2/2 (100 %)		
rat	70	5*	4.9×3.3	357×474	382×476	4/5 (80.0%)		
rat	78-83	19***	4.6×2.9	385×493	439×487	3/19(15.8%)		
rat	126	6*	6.6×4.2	$515\!\times\!629$	$547\!\times\!632$	6/6 (100 %)		
P. westermani (after Habe, by materials from Kyushu, Japan)								
dog	60	19*	8.78×3.48	$-\times$ 742	639×669	10/19(52.6%)		
dog	100	24*	10.63×5.11	$-\times$ 950	854×836	22/24(91.7%)		
dog	185	30*	12.89×7.35	$-\times1056$	748×838	30/30(100 %)		
rat	100	26**	1.20×0.53	$-\times$ 121	132×153	0/26		
rat	100	5*	$4.50{\times}2.64$	$-\times$ 566	$622\!\times\!588$	0/5		

note: * marks are from cyst in lungs, ** mark is from muscle, and *** mark is from pleural cavity and cyst in lungs.

going to report in details on the Southeast Asian J. Trop. Med. Pub. Hlth. in 1979.

As to the size of oral and ventral suckers, Kerbert (1878) stated as ".... sind gleich gross, mit einem Durchmesser von 0.78 mm" in materials from India, Baelz (1883) noted as ".... sind fast gleich gross" in materials of Japan, and Nagayoshi (1942) reported as the oral sucker had a diameter of 0.75 mm and the ventral sucker 0.80 mm in materials of Formosa. But according to our present data, the majority of both suckers are not spherical but ellipsoidal in shape, with a shorter length and a longer width. To compare the size of both suckers in general, if the length is marked merely, the oral sucker was smaller than the ventral one, on the while if only the width is marked, the oral sucker was larger than the ventral one. And from a viewpoint of the product of length and width, it would be concluded that the oral sucker is slightly smaller than the ventral one, even if it is nearly the equal size.

With regard to the shape of ovary, in the discussion of the former report of us (Ito et al., 1978), it was noted as "the typical sixlobed ovary was seen in only a few specimens, and the majority worms has less than six lobes in the ovary ". But the present careful observation of 49 worms including the former 14 materials revealed that 31 worms had a typical six-lobed ovary, 14 had irregular but six-lobed one, and only 4 had more irregular shaped ovary which might be counted the nember of lobes as five or seven. Consequently the former discussion must be corrected as "in general the ovary consists of a small central mass and six lobes, some of which being shortly subdivided again, though a few of them being rather irregular in shape".

Recently a comment to the proposition of a new name, *P. philippinensis* Ito, Yokogawa, Araki *et* Kobayashi, 1978, was offered by Miyazaki (1978b, c) who wishes to think it as a synonym of *P. westermani* (Kerbert, 1878). His comment seems to be derived from his theory reported by himself (Miyazaki, 1978a, c). In his paper of 1978

c, he noted as "the lung fluke found by Kerbert from tigers in India and the worm found by Baelz from man in Japan were different from the beginning. Accordingly, the author wishes to call only the former, i. e. the bisexual type, *P. westermani* (Kerbert, 1878), and the latter, i. e. the parthenogenetic type, *P. pulmonalis* (Baelz, 1880) In the Philippines, on the other hand, the main cause of human infection is probably *P. westermani*, because *P. pulmonalis* has never been found there as yet."

The proposition of a new name of P. philippinensis by the present authors in 1978 was based on the morphological and biological differences between the Philippine lung fluke and P. westermani from Japan. With regard to these differences there is no disagreement between Dr. Miyazaki and the However, as far as the present authors. authors are aware, there seems to be no sufficient proof that P. philippinensis is truly identical with P. westermani (Kerbert, 1878). In other words, there seems to be no decidable data whether the Philippine lung fluke prevalent among the Philippine people is truly identical with the Indian lung fluke from the tiger or not. At least it is notable that, about the cuticular spine of P. westermani, Kerbert (1881) described as "Die soliden Stacheln stehen in dichten Querreihen, in Gruppen von 2-7 auf der Basalmembran und ". On the while, as already noted in the former report of us (Ito et al., 1978), the cuticular spines of P. philippinensis are usually placed singly and arranged in diagonal rows, although occasionally in groups of two or three.

Anyway, much more detailed morphological and biological knowledges on the Indian lung fluke from the tiger should be indispensable in order to accept the opinion of Dr. Miyazaki who emphasizes to regard *P. philippinensis* as a synonym of *P. westermani* (Kerbert, 1878).

Summary

Further observations on 49 adult worms

- of *P. philippinensis* Ito *et al.*, 1978 were made by the present study. Some corrections of the former report of the present authors (1978) and some discussions about the comment of Miyazaki (1978b, c) were also given. The results were as follows:
- 1. With regard to the body size, in the case of worms from dogs, *P. philippinensis* is significantly smaller than *P. westermani* of Kyushu, Japan. Whereas in the case of worms from rats, *P. philippinensis* is seemed to be larger in general than *P. westermani* of Kyushu, Japan.
- 2. From a viewpoint of the product of length and width, the oral sucker is slightly smaller than the ventral one, even if they are nearly the equal size.
- 3. As to the shape of ovary, the former report of the present authors was corrected as "in general the ovary consists of a small central mass and six lobes, some of which being shortly subdivided again, though a few of them being rather irregular in shape."
- 4. Much more detailed morphological and biological knowledges on the Indian lung fluke from the tiger should be indispensable in order to accept the comment of Dr. Miyazaki who regards *P. philippinensis* as a synonym of *P. westermani*.

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フィリピン肺吸虫 (Paragonimus philippinensis) の成虫の形態に関する知見補遺

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フィリピンの肺吸虫は従来、日本の肺吸虫と同様にウェステルマン肺吸虫と呼ばれていたが、筆者らの研究によつて、セルカリア、メタセルカリア、成虫のいずれの時期も両者の間に形態的差異が認められ、フィリピンの肺吸虫はウ肺吸虫と呼ぶよりも、むしろ独立種としてParagonimus philippinensis と呼ぶのが妥当であろうと提案された(伊藤、横川、荒木、小林、1978)。その際成虫の形態についてはさらに今後も検討されなければならないことも付記されていた。

筆者らはその後もつづけてフィリピン産肺吸虫について感染実験をくりかえし、犬およびラットから得た成虫49匹についてその形態的観察をおこない、次のような結果をえた、

- 1. 前回の報告ではフィリピン肺吸虫は日本のウ肺吸虫よりやや小さいようであるとのべたが、今回の追加観察で、犬から得た虫体での比較ではフィリピン肺吸虫が日本産ウ肺吸虫より明らかに小形であつた.
 - 2. しかしラットからの虫体の比較ではフィリピン肺

吸虫が日本産ウ肺吸虫よりもやや大きいようであつたが、この点についてはさらに今後の検討にまちたい.

- 3. フィリピン肺吸虫の口吸盤と腹吸盤の大きさを比較すると、一般に前後径では口吸盤が小さく、左右径では口吸盤が大きく、さらに前後径と左右径の積では腹吸盤の方がやや大きいという結果がえられた.
- 4. 子宮の形態について前回の報告では、六葉に分岐しているものが少ないと論議したが、今回の追加観察によると六葉に分岐しているものが大部分であり、49虫体のうちわづかに4虫のみが、五葉から七葉に見えるような分岐状態であつた。すなわち一般的には六葉に分岐しているといつて差支えないであろう。
- 5. P. philippinensis は P. westermani のシノニム であるとする異論に対しては慎重に考慮しなければならない. そのためにはインド産のトラに 寄生する 肺吸虫と,フィリピンの流行地に存在する肺吸虫の異同をたしかめることが必要であると考えられる.