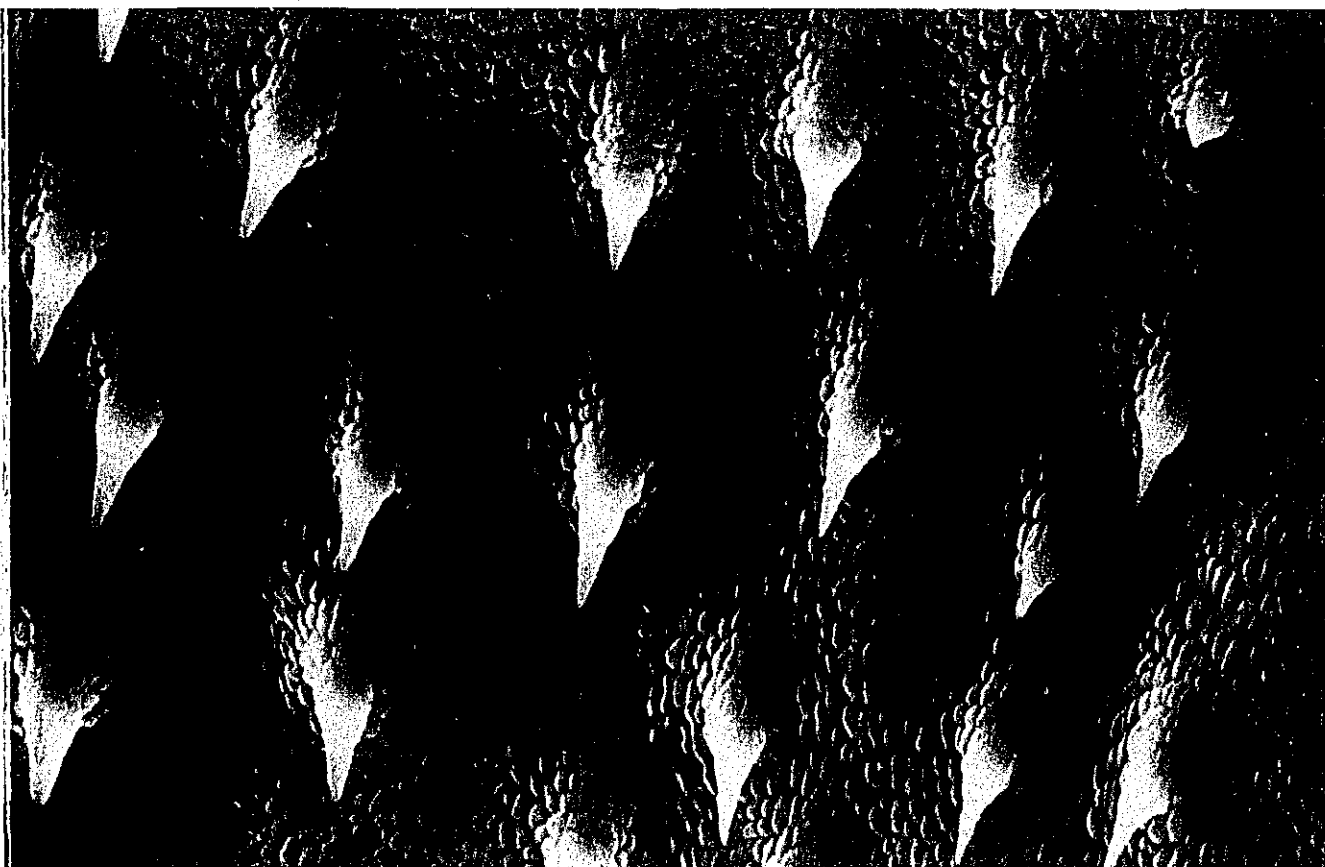


LUNG FLUKES
IN
THE WESTERN HEMISPHERE

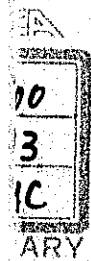
ICHIRO MIYAZAKI



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Scanning electron micrograph : Body surface
of adult *Paragonimus westermani*. × 2,000
(Photo by Dr. Y. Ishii)

Med. 72-11-80

LUNG FLUKES
IN
THE WESTERN HEMISPHERE

by

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December 1972

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Preface

In 1963 I attended the Seventh International Congresses on Tropical Medicine and Malaria in Brazil. On my way back to Japan, I visited "Instituto de Salubridad y Enfermedades Tropicales" in Mexico City, and by courtesy of the late Dr. Luis Mazzotti I had the opportunity to investigate *Paragonimus* eggs in the lung tissue of a Mexican male, who had already been reported by Martínez Báez and Jiménez Galán in 1961. Since that time, I have deeply been interested in the problem of paragonimiasis in Latin-American countries.

In the same year Dr. Mazzotti obtained adult lung flukes from a common opossum in Colima situated on the Pacific coast of Mexico, and sent them to me for taxonomic study. In 1965 he again collected many adult flukes from the same mammal in the same locality, and put them at my disposal. By his kind help a new lung fluke, *Paragonimus mexicanus* Miyazaki et Ishii, appeared in literature in 1968. Moreover, he introduced Peruvian parasitologists to me, who obtained adult lung flukes for the first time in Peru, and consequently another new lung fluke, *Paragonimus peruvianus* Miyazaki, Ibáñez et Miranda, was recorded in 1969. Since then, Peru has become very attractive for me, and I have already visited that country two times in order to make joint researches on *Paragonimus* with Peruvian collaborators.

This year the Japanese Government was requested by the Peruvian Government to send a specialist for giving technical guidance and lectures on *Paragonimus* to Peruvian scientists, and I was commissioned by the Overseas Technical Cooperation Agency (OTCA) of Japan to visit Peru for this purpose in September 1972. This booklet is the report of my joint researches on *Paragonimus* in Peru during the past three years, as well as a concise review of the lung fluke in other parts of the Western Hemisphere. In addition, some Asian species were briefly illustrated for comparison.

I wish to take this opportunity to express my sincere thanks to the late Dr. Luis Mazzotti in Mexico, who stimulated me to the research of lung flukes in Latin-American countries. I am also grateful to Drs. Hernán Miranda and Nicanor Ibáñez of "Universidad Nacional de Trujillo", Dr. Oscar Grados and Miss Norma Uyema of "Instituto Nacional de Salud Pública" in Lima, and Mr. César Mazabel of "Universidad Nacional Agraria de la Selva" in Tingo Maria for their continuous cooperation to my research in Peru. Thanks are also due to Dr. Martin D. Young, Director of Gorgas Memorial Laboratory in Panama, to Dr. Maurice D. Little of Tulane University in New Orleans,

USA., who supplied me with adult and larval specimens of *Paragonimus caliensis* from Colombia, and to Dr. Yoichi Ishii of Kyushu University who lent me several scanning electron micrographs from his collection.

Finally, I express my gratitude to the authorities concerned in Peru and Japan for close medical cooperation between the two countries, which made my third visit to Peru of this year particularly successful.

(Ichiro Miyazaki, December 1972)

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Historical Introduction

The lung fluke, *Paragonimus*, is one of harmful parasites, causing paragonimiasis among man and animals, but there have been many confusions and unsettled questions in taxonomy and ecology. In Japan, for example, *P. westermani* (Kerbert, 1878) had long been believed to be the only species of the genus until 1939, when the author found the second species, *P. ohirai* Miyazaki, at Yatsushiro City of Kyushu Island. After that time, another three species—*P. iloktsuenensis* Chen, 1940, *P. miyazakii* Kamo et al., 1961 and *P. sadoensis* Miyazaki et al., 1968—were added to the list of Japanese lung flukes by the author and his co-workers. In Thailand, on the other hand, some case reports of human paragonimiasis and an endemic area of the disease were recorded in literature, but the lung fluke itself had never been found until 1964, when Daengsvang et al. first recognized adults of *P. westermani* from two leopards captured in South Thailand. Soon after that, the author began to work on the lung fluke in cooperation with scientists in Thailand, and reported three new species—*P. siamensis* Miyazaki et Wykoff, 1965, *P. bangkokensis* Miyazaki et Vajrasthira, 1967 and *P. harinasutai* Miyazaki et Vajrasthira, 1968. In addition, he first recognized *P. macrorchis* Chen, 1962 and *P. heterotremus* Chen et Hsia, 1964, which were originally found in China. Thus, the number of Thai lung flukes has rapidly amounted to six species for several years, of which *P. heterotremus* was first reported from the skin of a Thai boy by Miyazaki and Harinasuta (1966) and then from the lung of a Laotian male by Miyazaki and Fontan (1970), showing its medical importance in those countries. Ultimately, valid species of *Paragonimus* has totaled 17 in Asia as shown in Table 1, of which 14 species were recorded during the past 30 years after the discovery of *P. ohirai*. Three of the 17 Asian lung flukes were removed from human body; they are *P. westermani*, *P. skrjabini* and *P. heterotremus*.

On the contrary, the Western Hemisphere has lagged behind Asia in the taxonomic study of *Paragonimus*, although the oldest lung fluke, *P. rudis* (Diesing, 1850), was found in Brazil. As shown in Table 2, the second species, *P. kellicotti* Ward, 1908, was found in the United States near 60 years later than *P. rudis*, and after that no other species were added to the list for another 60 years. During that period there were confusions in diagnosis of American lung flukes: most people erroneously reported *P. westermani* from man and animals without paying special attention to its morphology. Some persons thought *P. rudis* was the only species of the genus and others, including *P. westermani*, were all synonymous with *P. rudis* (Caballero, 1946, 1956; Caballero and Montero,

Table 1. Lung flukes in Asia

Species	Distribution
1. <i>Paragonimus compactus</i> (Cobbold, 1859)	India, Ceylon
2. <i>P. westermanni</i> (Kerbert, 1878) (syn. <i>P. ringeri</i> (Cobbold, 1880), etc.)	India, Ceylon, Thailand, Indonesia, Malaysia, Philippines, China, Taiwan, Japan, Korea, USSR, etc.
3. <i>P. ohirai</i> Miyazaki, 1939	Japan, China
4. <i>P. iloktsuenensis</i> Chen, 1940	China, Japan, Taiwan, Korea
5. <i>P. skrjabini</i> Chen, 1959 (syn. <i>P. szechuanensis</i> Chung et Tsao, 1962)	China
6. <i>P. yunnanensis</i> Ho et al., 1959	China
7. <i>P. miyazakii</i> Kamo et al., 1961	Japan
8. <i>P. macrorchis</i> Chen, 1962	China, Thailand, Ceylon
9. <i>P. fukienensis</i> Tang et Tang, 1962	China
10. <i>Euparagonimus cenocoptosus</i> Chen, 1962	China
11. <i>P. cheni</i> Hu, 1963	China
12. <i>P. heterotremus</i> Chen et Hsia, 1964 (syn. <i>P. tuanshanensis</i> Chung et al., 1964)	China, Thailand, Laos
13. <i>P. proliferus</i> Hsia et Chen, 1964 (syn. <i>P. menglaensis</i> Chung et al., 1964)	China
14. <i>P. siamensis</i> Miyazaki et Wykoff, 1965	Thailand, Ceylon
15. <i>P. bangkokensis</i> Miyazaki et Vajrasthira, 1967	Thailand
16. <i>P. harinasutai</i> Miyazaki et Vajrasthira, 1968	Thailand
17. <i>P. sadoensis</i> Miyazaki et al., 1968	Japan

Table 2. Lung flukes in the Western Hemisphere

Species	Distribution
1. <i>Paragonimus rudis</i> (Diesing, 1850)*	Brazil
2. <i>P. kellicotti</i> Ward, 1908	USA, Canada
3. <i>P. caliensis</i> Little, 1968	Colombia, Peru, Panama(?)
4. <i>P. mexicanus</i> Miyazaki et Ishii, 1968	Mexico, Guatemala(?), Costa Rica(?), Panama(?)
5. <i>P. peruvianus</i> Miyazaki, Ibáñez et Miranda, 1969	Peru, Panama, Ecuador(?)
6. <i>P. amazonicus</i> Miyazaki, Grados et Uyema, 1973**	Peru

* regarded as a *species inquirenda*.

** will be described in the near future.

1961), while other persons described "*P. rudis*=*P. kellicotti*" (Stewart and Jones, 1959; Thatcher, 1967). Miyazaki (1955 a) also presumed that Caballero's *P. rudis* from Guatemala might be the same with *P. kellicotti* from the United States, but he noticed that careful comparison of their metacercariae was very important.

Mazzotti and Miyazaki (1965) reported *Paragonimus* adults for the first time

in Mexico, which were collected from one of 11 common opossums, *Didelphis marsupialis*, in Colima situated in mountainous region on the Pacific coast of the country. They noticed that the Mexican species had more delicately branched ovary than *P. kellicotti*. Then, Miyazaki and Ishii (1968 a,b) demonstrated more clear-cut differences between them in their eggs, and proposed in their second paper a new specific name, *P. mexicanus*, for the former species.

In Costa Rica, Sogandares and Smalley (1965) briefly reported an unidentified *Paragonimus* metacercaria from a preserved specimen of a crab, *Pychohallus tristani*, and in 1967 the same authors collected three metacercariae of *Paragonimus* sp. from one of 15 *P. tristani* and two larvae from two of 13 crabs, *Potamocarcinus magnus*. Five metacercariae obtained were all found on or in the heart tissue of the affected crabs. The specific name of the lung fluke concerned was unknown. Brenes, Zeledón and Rojas (1968) communicated in the same country the finding of *Paragonimus* adults from mammals, such as four-eyed opossum, racoon, grey-fox and domestic cat. Moreover, they obtained *Paragonimus* cercariae from a kind of snail, *Pyrgophorus* sp. and metacercariae from 19 of 150 crabs, *P. tristani* and from one of five another crabs, *P. magnus*. The authors mentioned that the metacercariae appeared crawling free on the liver of the crabs at the moment of examining them. It is interesting that the habitat of metacercariae in the same crabs differs with the papers. Insofar as the author is aware, the specific name of *Paragonimus* has not definitely been decided in Costa Rica.

In Panama, Thatcher (1967) reported "*P. rudis* (= *P. kellicotti*)" adults from six kinds of mammals - the common opossum, four-eyed opossum, jaguar, coati, dog and cat, as well as its metacercariae from a crab, *Pseudohelphusa richmondi*. Most recently, Miyazaki (1972) noted the occurrence of *P. peruvianus* in the same locality where Thatcher's materials were originated.

In South America, *P. rudis* (Diesing, 1850), the oldest lung fluke in the world, was found in Brazil, but at present time it is regarded as a *species inquirenda*. Human paragonimiasis has been reported since around the beginning of this century especially in Peru and Ecuador, and the causative agent of the disease was considered to be *P. westermanni* which had been imported by immigrants from the Orient. But, there had been no taxonomic study on South American lung flukes until recent years. Little (1968) found a new species, *P. caliensis* in Colombia more than 100 years after the discovery of *P. rudis*. In addition, he reexamined some *Paragonimus* specimens from Peru, Ecuador and Panama, and reported interesting results of his investigation without showing their specific names.

Ibáñez and Miranda (1967) first found adult lung flukes from a cat in an endemic area of paragonimiasis in Department of Cajamarca, northern part of Peru and sent them to the author for taxonomic study. For these flukes a new specific name, *P. peruvianus* was proposed by Miyazaki, Ibáñez and Miranda (1969). Since that time the author has been continuing basic studies of Peruvian lung flukes, and in 1971 the metacercaria of this fluke was described in detail by the same authors. Then, Miyazaki, Arellano and Grados (1972) demonstrated two adult lung flukes from a male patient residing in Aguaytia, Department of Loreto for the first time in Peru. But, these flukes were not definitely identified, because their morphology changed by bithionol administered before operation. Miyazaki and Grados (1972) first recognized *P. caliensis* in Cajamarca as the second species in Peru. In September of this year the author visited Peru as the third trip and found another new species in Tingo Maria, Department of Huánuco, which will be named *P. amazonicus* in the near future.

Rodriguez (1963) reported the life cycle of *P. westermani* in Ecuador, but from his description and figures it seems that the fluke concerned was not *P. westermani* but was probably *P. peruvianus*. According to Little (1968) who examined Rodriguez' specimen, the adult fluke from man in Ecuador was not *P. westermani*.

As mentined previously, there have been many confusions in taxonomy of *Paragonimus* in the Western Hemisphere until recent years. These are due to the features of *Paragonimus* that the body of adults is fairly thick and is densely covered with vitelline glands. In the next chapter the author will show the method of preparing good specimens and the criteria for differentiating *Paragonimus*.

Morphology of *Paragonimus*

Generally speaking, it is important for species differentiation to investigate both adults and metacercariae. In some cases identification is very easy in adult forms, while in other cases it is much easier in larval forms. For example, *P. ohirai* and *P. iloktsuenensis* are hardly separated from each other in their adults (Fig. 1), while they are very easily distinguished in their metacercariae (Fig. 5). Differentiation in cercarial stage is not always easy, because the flame cell pattern is almost similar and the character of body surface is not easily recognized by the ordinary microscope. But, its surface structure is clearly demonstrated by the scanning electron microscope, as shown in Fig. 2.

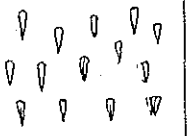



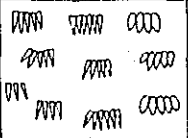

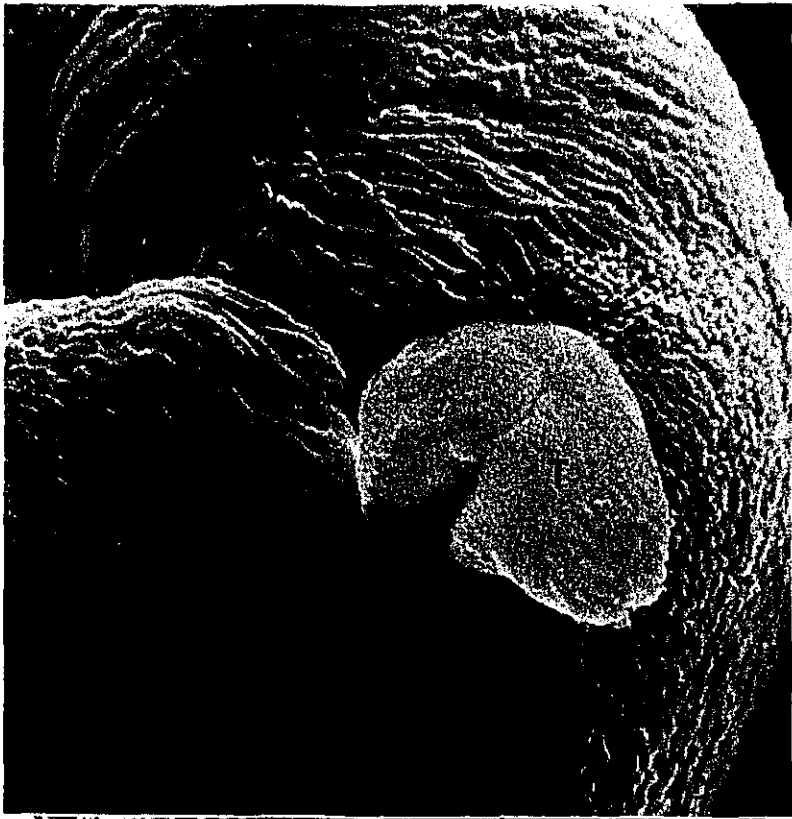
SPECIES	SPINES	OVARY
<i>P. westermani</i> (KERBERT)		
<i>P. miyazakii</i> KAMO <i>et al.</i>		
<i>P. ohirai</i> MIYAZAKI & <i>P. iloktsuenensis</i> CHEN		

Fig. 1. Diagrammatic representation of two criteria for separating four species of Japanese lung flukes in their adult stage. *P. sadoensis*, the newest fluke in Japan, belongs to the *ohirai-iloktsuenensis* group.

Morphology of adult flukes: General structure is illustrated in Fig. 3 by means of *P. westermani*, the most well-known species of medical importance in Asia. It is worthy to note that *situs inversus* of female reproductive organs frequently appears in all species of *Paragonimus*. It is quite necessary for species differentiation to make sufficiently flattened specimens, because the body is fairly thick in all kinds of *Paragonimus*. The author recommends to fix living adults with 70% alcohol under pressure, and to stain them with alumcarmin. If necessary, fast green is used for staining of cuticular spines. For species differentiation the following items are important criteria: 1) shape of whole body (mostly oval, occasionally elongated), 2) arrangement of cuticular spines (single or grouped), 3) shape and size of ovary and testes, 4) comparison of transverse diameter between oral and ventral sucker, 5) shape and size of eggs, and 6) character of eggshell. Cuticular spines are inclined to split longitudinally in case of old flukes. Therefore, some spines that were originally single look like grouped spines. In such a case, young flukes should be examined. The shape of each individual spine is generally unsuitable for species differentiation.

As stated previously, the body is densely covered with vitelline glands, which frequently keep the ovary and testes from sight. Accordingly, it is not rarely indispensable before mounting to remove the cuticle and vitelline glands over and/or under the gonads. The author utilizes sewing and injec-



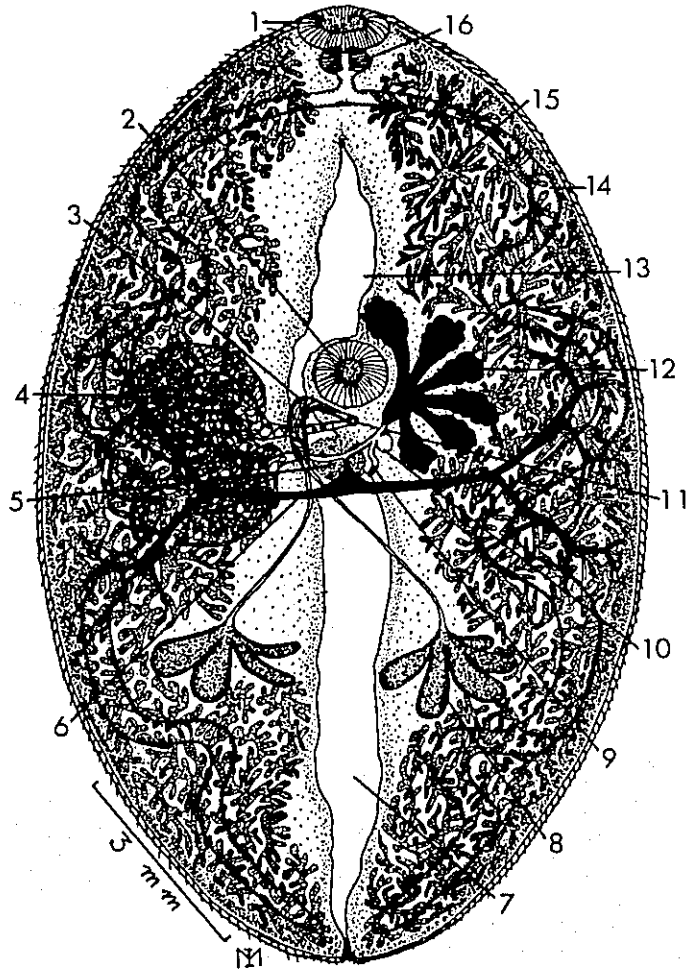


Fig. 3. Morphology of adult *P. westermanni*. 1: oral sucker, 2: ventral sucker, 3: seminal vesicle, 4: uterus, 5: Mehlis' gland, 6: vitelline duct, 7 & 13: excretory bladder, 8: testis, 9: Laurer's canal, 10: seminal receptacle, 11: genital pore, 12: ovary, 14: intestine, 15: vitellaria, 16: pharynx

←Fig. 2. Scanning electron micrographs of terminal surface of cercarial body. Dorsum on the right. T: short tail. *P. sadoensis* (upper, $\times 3,600$) is provided with long hairs on ventral side, while *P. miyazakii* (lower, $\times 6,000$) is covered with short spines only, those on ventral surface (black arrow) being much thicker.

(Photo by Dr. Y. Ishii)

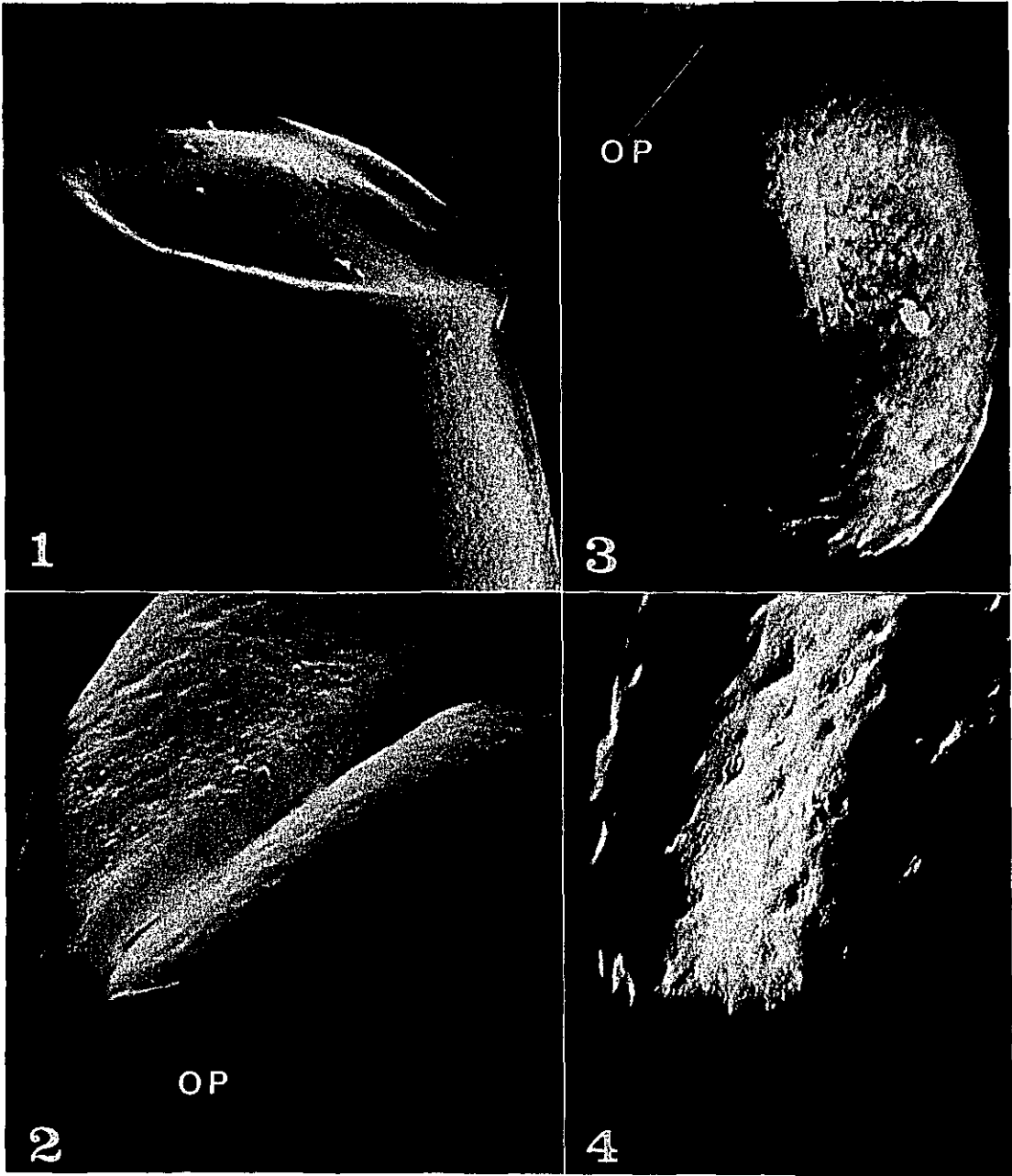


Fig. 4. Scanning electron micrographs of eggshell in three species of American lung flukes. OP: operculum
 1. *P. kellicotti*; surface smooth. ($\times 3,000$) 2. *P. mexicanus* (two eggs); surface slightly undulated. ($\times 2,000$) 3 and 4. *P. peruvianus*; surface highly undulated. ($\times 1,200$ and $\times 3,000$)
 (Photo by Dr. Y. Ishii)

tion needles to cut off the cuticle and glands, and a small wire-ring to press down the worm body. In order to know the character of eggs, it is recommended to investigate mature eggs laid in saline solution by each individual worm. If laid eggs are not available, it is also possible to get mature eggs from the terminal part of uterus. Eggs laid in the worm cyst of the lungs are also useful, provided that worms in the cyst are the same species. For detailed observation of the eggshell, the scanning electron microscope is very reliable, as shown in Fig. 4.

Morphology of metacercariae: Species differentiation is usually more easily performed by features of living metacercariae, and for this purpose the following items are significant: 1) encysted or non-encysted, 2) number of cyst, 3) shape and size of inner cyst, 4) thickness of inner cyst membrane, 5) body size of excysted larva, 6) comparison of transverse diameter between oral and ventral sucker, 7) length of stylet in oral sucker, 8) extent of excretory bladder, 9) red granules in larval body, and 10) color of intestines. In case of *P. westermanni*, for instance, the larval body is enveloped with two kinds of cysts. The inner cyst is spherical in shape, approximately 0.4 mm in diameter, and its membrane is fairly thick, measuring about 20 micra. Asian species ever reported are all encysted, some of them being shown in Figs. 5 and 6; while *P. peruvianus* is entirely naked without encystation (Fig. 19). Intestines of the latter species are usually yellowish in color and red granules are distributed exclusively in ventral side of the body, whereas in case of *P. westermanni* intestines are colorless and red granules are recognized also in dorsal side. The outer cyst, which anchors the metacercaria to the host tissue at one point, is not useful for differentiation, because its size and shape are easily changeable and its membrane is thin and fragile.

For fixation of excysted larvae the author uses 10% formalin under cover-glass pressure. The red granules rapidly disappear by formalin, but the yellow color of intestines usually remains unchanged fairly long time. In most species the excretory bladder reaches to the intestinal bifurcation (Fig. 7, A), but in some case it reaches midway between the bifurcation and ventral sucker (Fig. 7, B); while in another case such as *Euparagonimus cenocopiosus* Chen, 1962, it reaches only the posterior margin of ventral sucker. Generally speaking, the stained and mounted specimen of excysted metacercaria is less suitable for identification than the living larva. In the following chapters, important American lung flukes will be explained concisely.

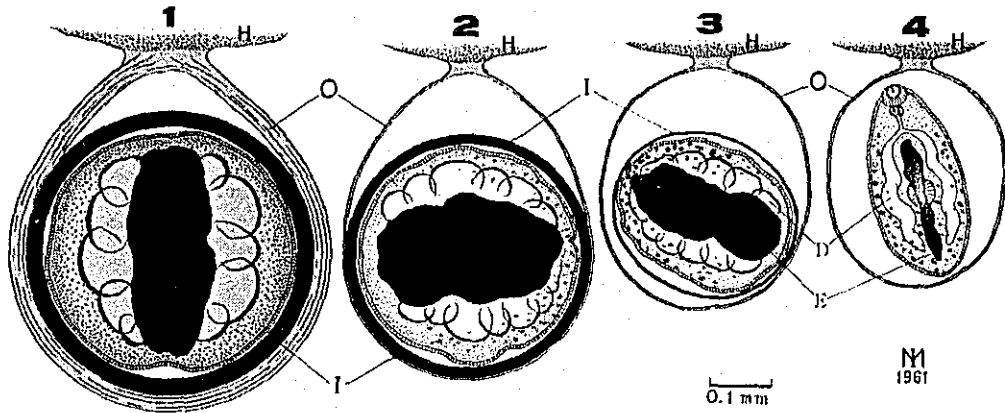


Fig. 5. Differences of metacercariae in four species of Japanese lung flukes. Same magnification.

1 : *P. miyazakii*, 2 : *P. westermanni*, 3 : *P. ohirai*, 4 : *P. iloktsuenensis*
 I : inner cyst, O : outer cyst, D : intestine, E : excretory bladder, H : host tissue

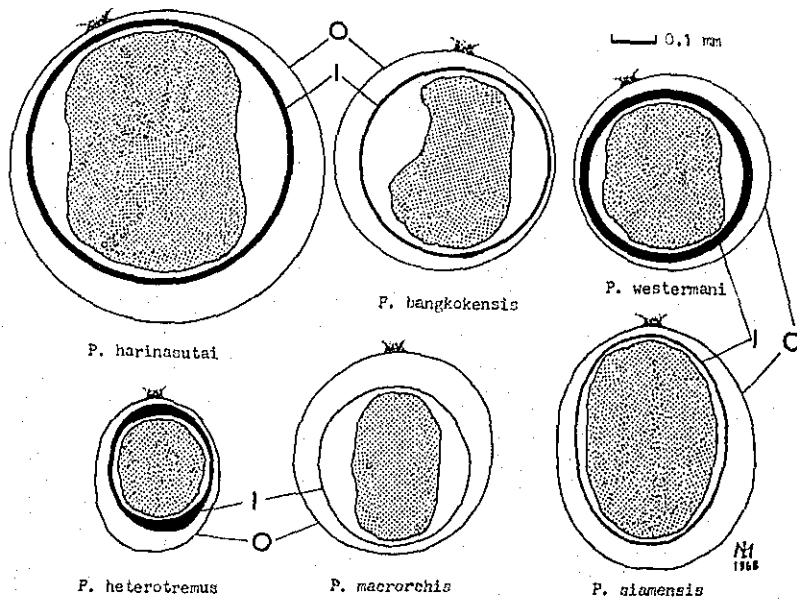


Fig. 6. Diagrammatic representation of six species of *Paragonimus* metacercariae occurring in Thailand. Same magnification. Dotted part means the larval body. I : inner cyst, O : outer cyst

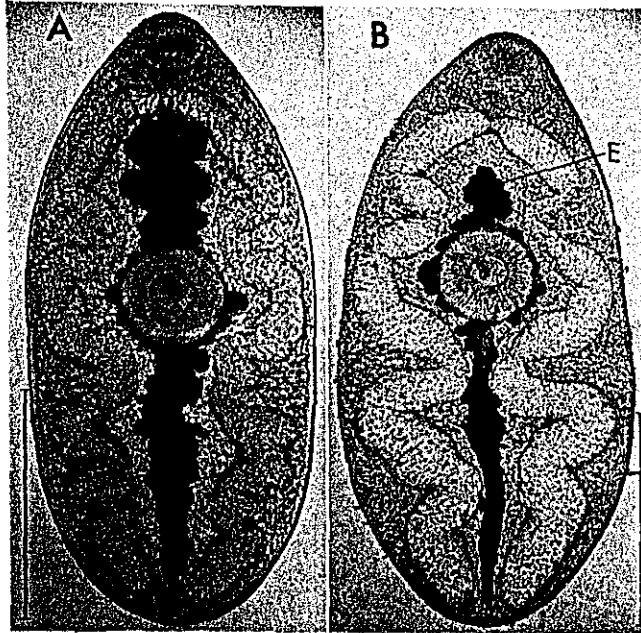


Fig. 7. Two kinds of metacercariae from a crab, *Pseudothelphusa richmondi* in Panama. Fixed with formalin, unstained. Scales : 0.5 mm. A. *P. peruvianus* ; dorsal view. Intestines yellow. B. Unknown species ; ventral view. Intestines colorless. Note the short excretory bladder (E).

Paragonimus kellicotti Ward, 1908

This well-known species was first found in a cat from Michigan, USA.; thereafter it was obtained from many kinds of mammals such as the pig, dog, goat, mink, muskrat, bobcat, raccoon, opossum, skunk, etc. Only one case of human infection with this fluke was reported by Abend (1910), but this causative agent should be reexamined from new point of view. *P. kellicotti* is widely distributed in the eastern half of the United States and Canada. Thatcher (1967) reported this fluke from Panama as the same species with *P. rudis*, but Miyazaki (1972) did not encounter it in his study at the same locality. Vevers (1923) and Miyazaki (1955 b) erroneously reported *P. kellicotti* in a Malaysian tiger and in Japanese weasels, respectively. Ultimately, this fluke is distributed in North America at present, and it needs further taxonomical study in order to confirm the occurrence of this fluke in Central and South America. According to Ameel (1934), the snail and the crustacean host of this fluke are respectively *Pomatiopsis lapidaria* and various species of crayfish belonging to the

genus *Cambarus*. Another two kinds of snails, *P. cincinnatiensis* and *Oncomelania nosophora* were experimentally proved by Basch (1959) to be potential hosts of this lung fluke. Yoshida and Nishimura (1968) reported that a Japanese freshwater crab, *Potamon dehaani* was highly susceptible to this fluke.

Adults (Figs. 8 and 9): Cuticular spines are originally single; ovary and testes are moderately branched. Oral sucker is usually a little smaller than ventral sucker. Eggs are biggest of all American lung flukes ever reported;

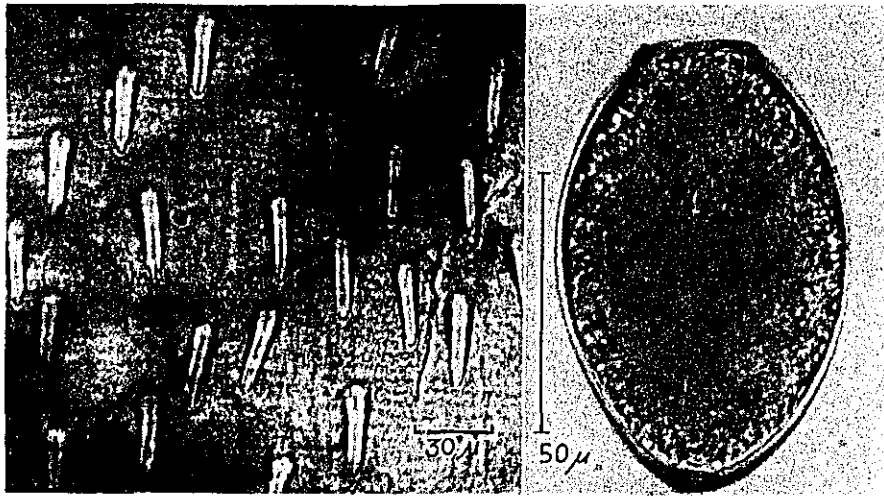


Fig. 8. Cuticular spines (left) and uterine egg of *P. kellicotti* adult.

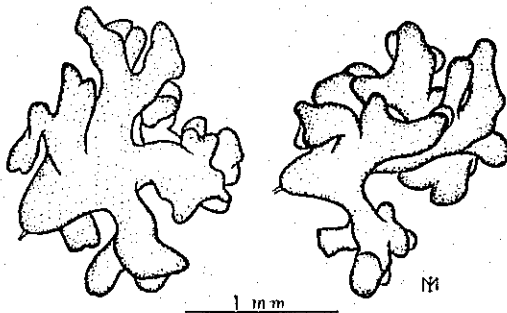


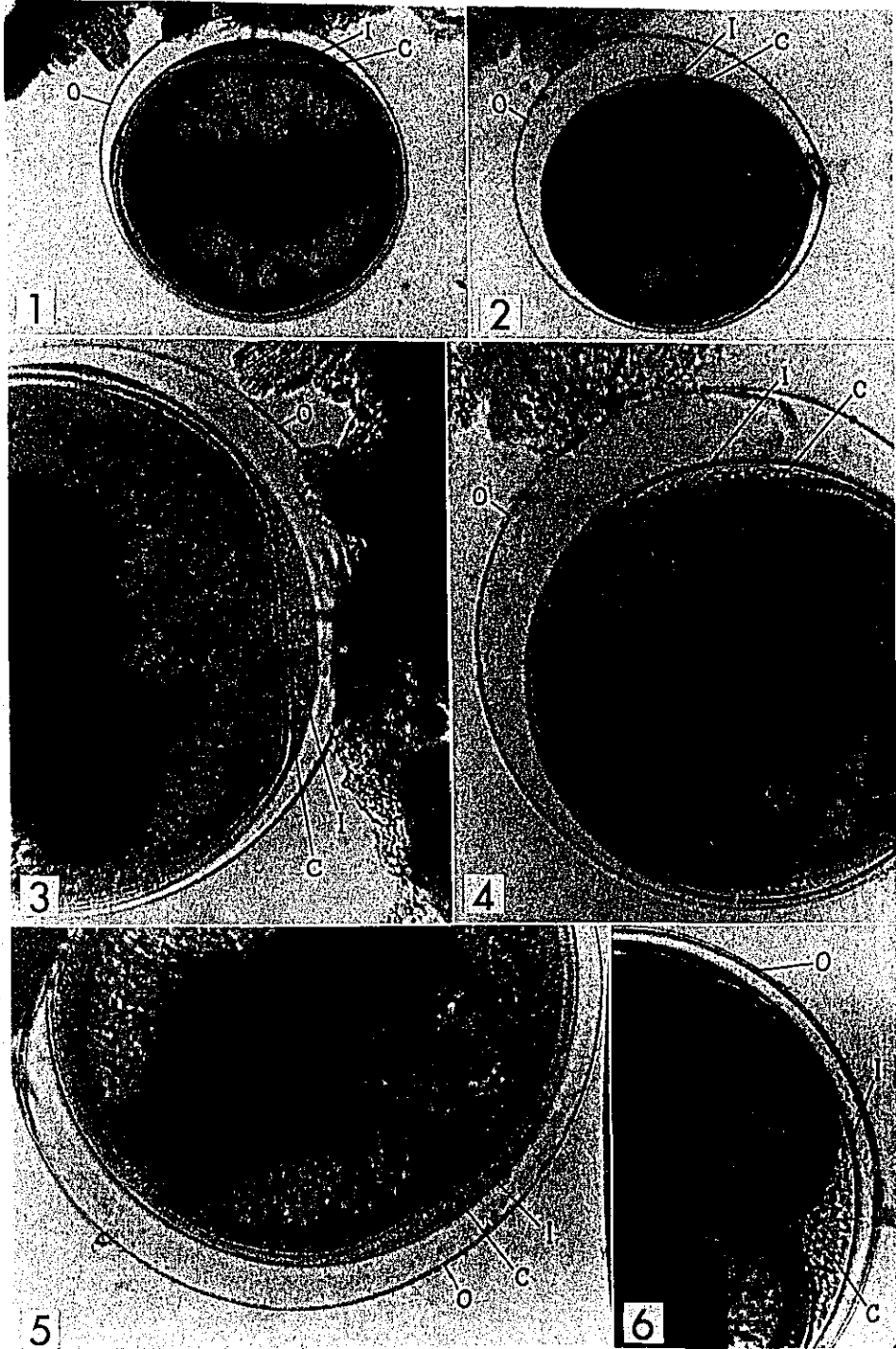
Fig. 9. Ovaries of *P. kellicotti* adults obtained from an experimental cat 197 days after infection. Dorsal view, anterior end at top.

100 eggs measured 82.3 to 99.8 by 54.3 to 61.3 (av. 91.2 by 56.7) micra, and eggshells were smooth and thick, measuring 1.68 to 2.68 (av. 2.27) micra on the lateral side (Miyazaki and Ishii, 1968 a, b). These features of eggs are good criteria for separating this fluke from other known species in America (Fig. 4).

Metacercariae (Fig. 10): They

Fig. 10. Four living metacercariae of *P. kellicotti* from a crayfish, *Cambarus robustus* — in Ann Arbor, Michigan, USA. Various magnification.

1 & 3 and 2 & 4 are respectively the same larva. C : cuticle of larval body, II : heart tissue of crayfish, I : inner cyst, O : outer cyst



are always found in the pericardial cavity of the crayfish, and are attached to the heart tissue by a small portion of the outer cyst. The inner cyst is spherical in shape and measures approximately 0.4 mm in diameter, when free from pressure. Its membrane is thin, measuring 5.2 to 7.8 (av. 6.1) micra in thickness (Miyazaki, 1964). According to Ishii (1966), the inner cyst averaged 0.38 by 0.39 mm in diameter and its membrane was 2 to 6 (av. 3) micra in thickness. It feels strange that Ameel (1934) reported, "The cyst wall is thick, being about 0.056-0.067 mm in section". The larvae commonly contain many red granules in their body, sometimes appearing as pinkish globules to the naked eye. Ultimately, the metacercaria of *P. kellicotti* is similar to that of *P. westermanni*, but they are readily separated by the thickness of the inner cyst membrane.

Paragonimus caliensis Little, 1968

This fluke was found by Little (1968) from the common opossum, *Didelphis marsupialis* and the four-eyed opossum, *Philander opossum* in Cali, Colombia. Recently, Miyazaki and Grados (1972) recognized the same species from an experimental dog in Department of Cajamarca, northern part of Peru. Little (1968) and Miyazaki and Ishii (1968 a, b) suggested the occurrence of *P. caliensis* in Panama and Mexico, respectively. Therefore, this fluke seems to be widely distributed in South and Central America. As the crab host, *Strengeria* sp. was decided by Little in Colombia, and *Pseudothelphusa chilensis* was suspected to be the host in Peru. The snail host has never been reported as yet. Human infection is unknown, but it is presumed that some patients may harbor this fluke in Cajamarca area, since the suspected crab host is frequently eaten by inhabitants.

Adults (Figs. 11 and 12): Cuticular spines are originally single. Oral sucker is usually slightly larger than ventral sucker. Ovary is simply branched; it has large central mass and several short and broad lobes, some of them being shortly subdivided. Testes are also simply branched; testis on the same side as the ovary has usually six lobes and the opposite testis five, most lobes being terminally swollen like clubs with or without small processes. According to Little, formalin-fixed eggs measured 70 to 92 by 38 to 54 (av. 78 by 45) micra; eggshell was very thin (1 to 1.5), having irregular undulation, just like that of *P. peruvianus* (Fig. 17).

Metacercariae (Fig. 13): According to Little, they were found in elongate cysts in the liver of crabs. The cyst wall was single and very thin. Their body was pink in color due to small red granules in parenchyma. Measurements

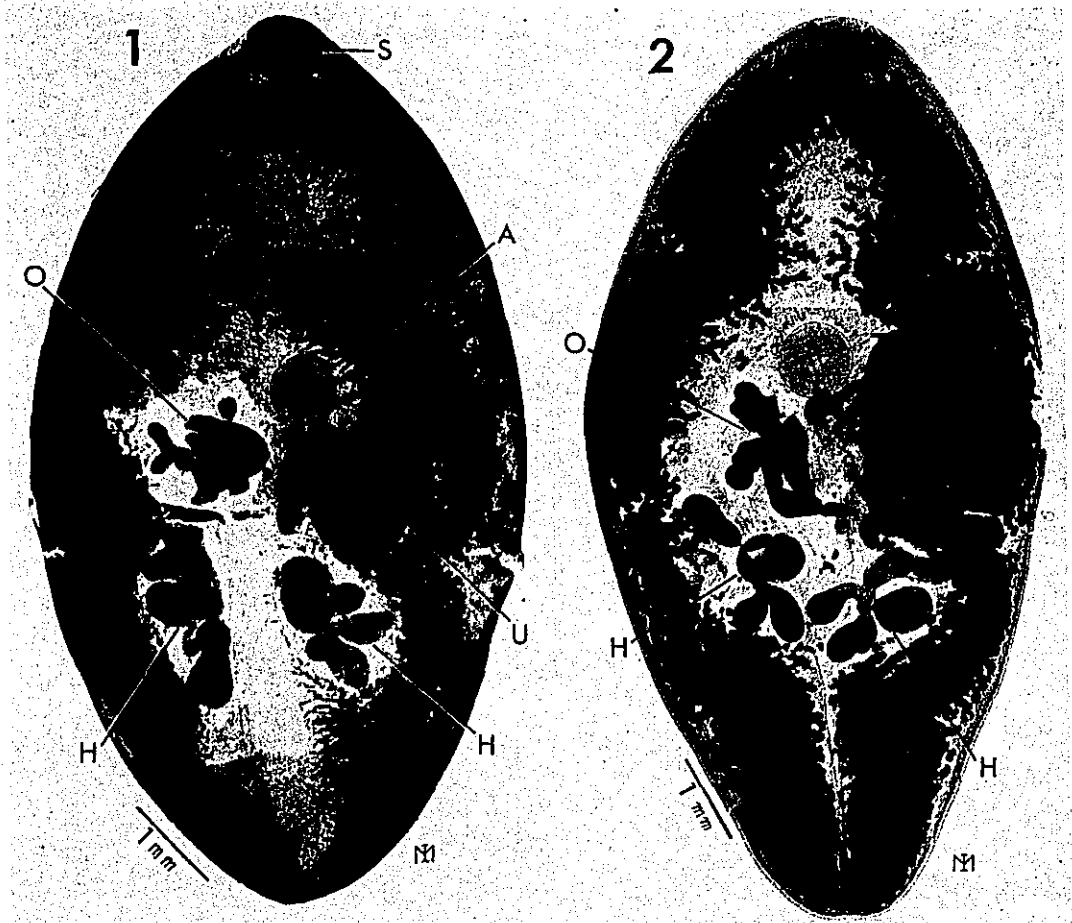


Fig. 11. *P. caliensis* adults. Ventral view. 1. From Peru. 2. From Colombia. (By the courtesy of Dr. M. D. Little) S : oral sucker, A : ventral sucker, O : ovary, U : uterus, H : testis.

(in mm) of 17 excysted and heat-killed metacercariae were as follows: body 0.85 to 1.05 (av. 0.96) by 0.40 to 0.55 (av. 0.48); oral sucker 0.10 to 0.15 (av. 0.12) in diameter; ventral sucker 0.15 to 0.20 (av. 0.19) in diameter. Length of stylet was about 25 micra. Flame cell formula was $2 \times [(3+3+3+3+3+3+3+3) + (3+3+3+3+3+3+3+3)] = 96$. Dr. Little kindly sent to the author some heat-killed metacercariae of *P. caliensis*. They were stained with carmine and mounted in balsam by the author, and eight of them measured 0.88 to 1.06 (av. 0.95) by 0.41 to 0.48 (av. 0.45) mm in body size and two stylets which were horizontally fixed measured 23 and 25 micra in length. As compared with *P. peruvianus* metacercariae, those of *P. caliensis* have evidently smaller body and

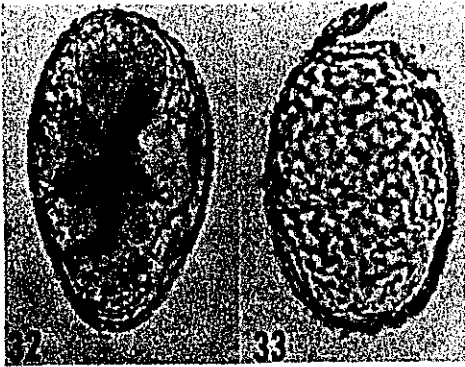


Fig. 12. Eggs of *P. caliensis*. 32. Formalin-fixed egg. 33. Surface view of shell of hatched egg. (Little, 1968)

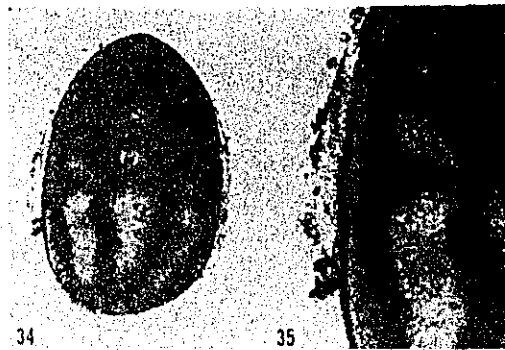


Fig. 13. Metacercaria of *P. caliensis*. 34. Encysted larva; ventral view. 35. Lateral portion of the same larva, showing thin cyst membrane. (Little, 1968)

a little longer stylet.

Paragonimus mexicanus Miyazaki et Ishii, 1968

This specific name was proposed by Miyazaki and Ishii (1968 b) for the lung flukes collected by the late Dr. Luis Mazzotti from common opossums, *Didelphis marsupialis* in Colima, Mexico. The same authors suggested the occurrence of this fluke in Guatemala, Costa Rica and Panama, as well as the possibility of human infection with this fluke. The snail and the crustacean host have never been determined as yet; larval stage of this fluke is unknown.

Adults (Figs. 14 and 15): Cuticular spines are originally singly spaced. Oral sucker is mostly larger than the ventral. Ovary is delicately branched and testes are moderately. From three worms 100 uterine eggs were each measured, and they averaged 74.1 by 44.5, 73.7 by 42.3, and 66.2 by 44.5 micra, respectively. Their eggshells are thin and slightly undulated, but not so remarkably as *P. peruvianus* eggs (Fig. 4). Thickness of them averaged at lateral side 1.17, 1.13 and 1.19 micra, respectively. The character of eggs is the best criterion for separating this fluke from *P. kellicotti*, which were formerly confused with each other.

Paragonimus peruvianus Miyazaki, Ibáñez et Miranda, 1969

About the beginning of this century some indigenous cases of human paragonimiasis were reported in Peru by Barton (1910), Arce (1915) and Corvetto (1921), and the causative agent of the disease was presumed to be *P. westermani*, which had been imported by immigrants from the Orient. But, little medical

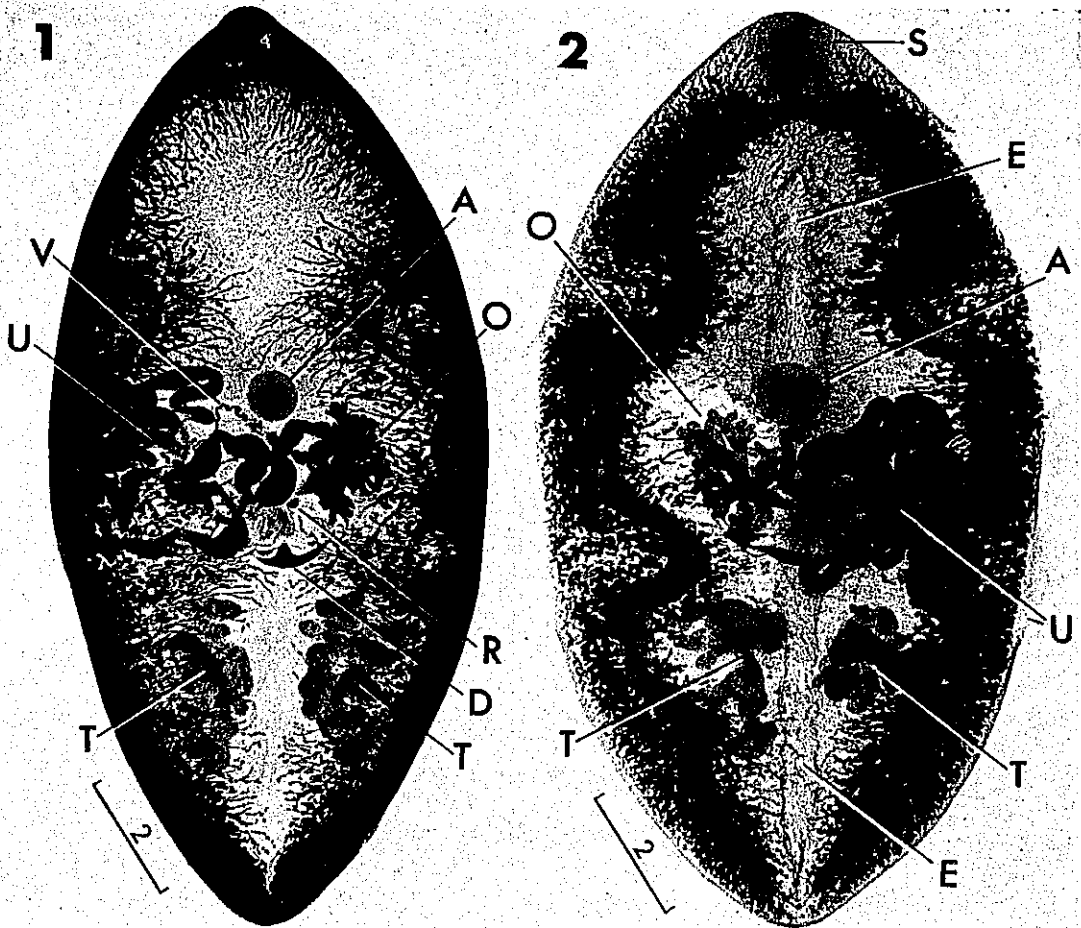


Fig. 14. Holotype (1) and paratype (2) of *P. mexicanus* from common opossum; dorsal view. Scales in mm. A: ventral sucker, D: vitelline duct, E: excretory bladder, O: ovary, R: seminal receptacle, S: oral sucker, T: testis, U: uterus, V: seminal vesicle

attention had been paid to the disease until recent years, when Grados et al. (1962), Morales (1963) and Miranda et al. (1967) reported human cases in Department of Cajamarca, northern part of Peru. Ibáñez and Miranda (1967, 68) first found adult lung flukes different from *P. westermani* in a domestic cat and then in an opossum, *Didelphis azarae pernigra* from Cajamarca, where human paragonimiasis is known to be present, and in 1968 they sent to the author some flukes from the cat for taxonomical study. In the next year, Miyazaki, Ibáñez and Miranda (1969) proposed the specific name, *Paragonimus peruvianus*,



Fig. 14b. Adult *P. mexicanus*. 3. Cuticular spines on dorso-anterior part of body. 4. Uterine egg; operculum on the left. Note slight undulation of shell (see Fig. 4).

also moderately branched, occasionally subdividing more highly than ovary. Eggs measured 75 to 86 by 44 to 53, averaging 79 by 48 micra, when free from pressure. Eggshell is thin (about 1.5 micra) and uniform in thickness, but shows irregular undulation, as demonstrated in Fig. 4. Therefore, eggs of this fluke are readily separated from those of *P. kellicotti*, but are hardly distinguished

for the new lung fluke, and they thought that the disease in Peru was not introduced from the Orient, but it has been caused by the autochthonous lung fluke from ancient times. Although this lung fluke has never been demonstrated from human body, it is doubtless that the fluke is infecting man in Cajamarca area.

This fluke seems to be widely distributed in tropical area, because it was recently found in Panama by Miyazaki (1972) and it appears to occur in Ecuador judging from the paper of Rodriguez (1963) and by a personal communication from Dr. M. Yokogawa of Chiba University, who investigated two metacercariae in that country. Two species of fresh water crabs, *Pseudothelphusa chilensis* in Peru and *P. richmondi* in Panama, were determined as the host of this lung fluke, but no snails have been decided as its first intermediate host. The former crab, *P. chilensis* (Fig. 16), is regarded as the most important vector of human paragonimiasis in Peru.

Adults (Figs. 17 and 18) : Cuticular spines are originally singly spaced. Oral sucker is mostly larger than the ventral. Ovary is moderately branched and smaller than testes, which are

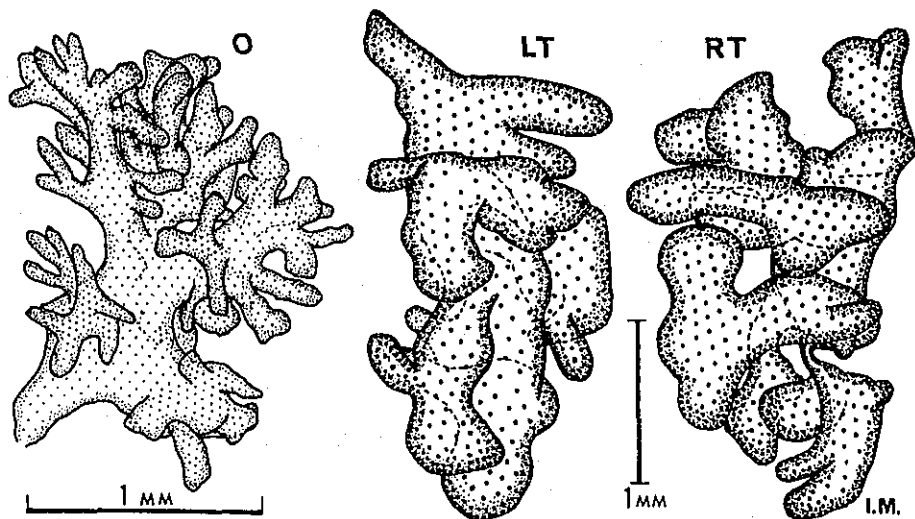


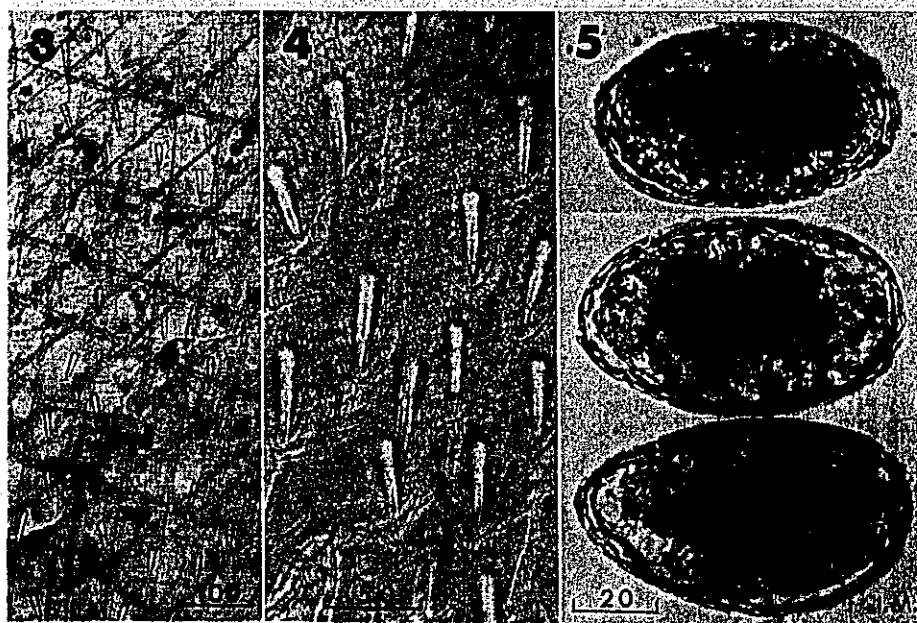
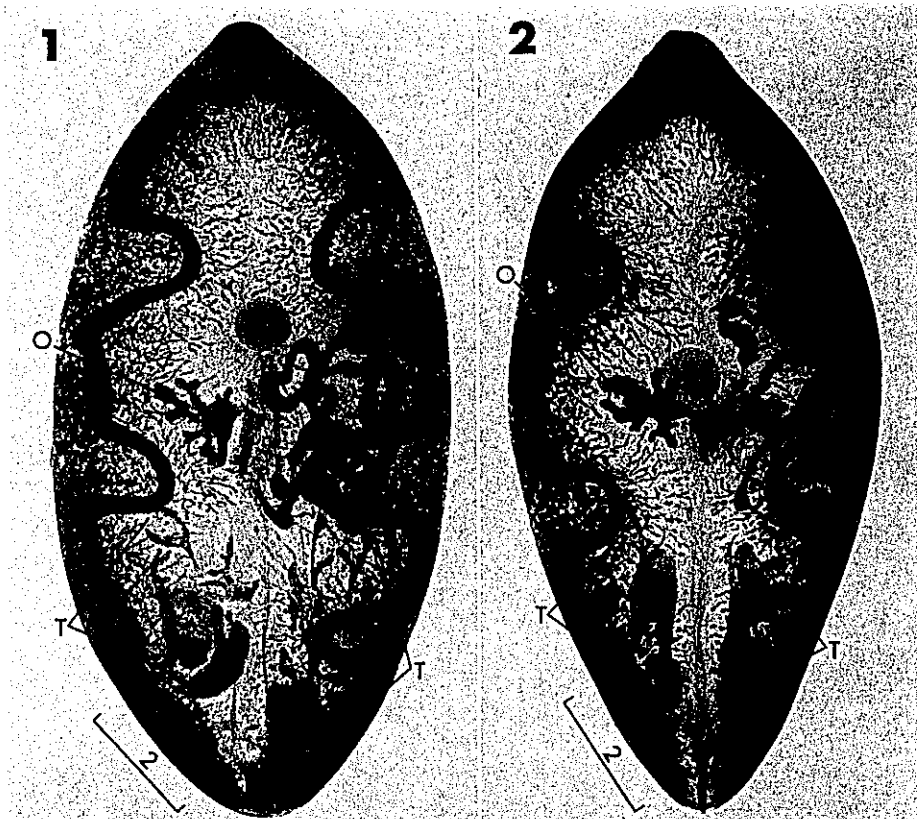
Fig. 15. Ovary and testes of the holotype of *P. mexicanus*, Dorsal view.
O : ovary, LT : left testis, RT : right testis

from those of *P. caliensis*.

Metacercariae (Fig. 19): Forty-nine crabs, *P. chilensis*, were examined in Cajamarca, and 34 or 69 % were positive for the larva, its number per crab varying from one to 73 (Miyazaki, Ibáñez and Miranda, 1971). Metacercariae crawl freely in the liver of crabs without encystation, and many folds are visible on the wall of intestines and excretory bladder, but they become less conspicuous after fixation, as shown in Nos. 1 and 2. Intestines are yellow in color, and red granules are recognized exclusively in the ven-



Fig. 16. *Pseudothelphusa chilensis* in Cajamarca, hanging down from a pencil. The most important vector of human paragonimiasis in Peru.



tral side of body. Excretory bladder reaches to the bifurcation of intestines. Thirty mounted metacercariae measured 1.09 to 1.50 by 0.53 to 0.61 (av. 1.30 by 0.56) mm, showing the biggest body size among all known species. Oral sucker averaged 0.14 by 0.12, and the ventral 0.23 by 0.22 mm, being apparently larger than the oral. Length of six stylets that were fixed horizontally measured 13 to 18 (av. 16.5) micra.

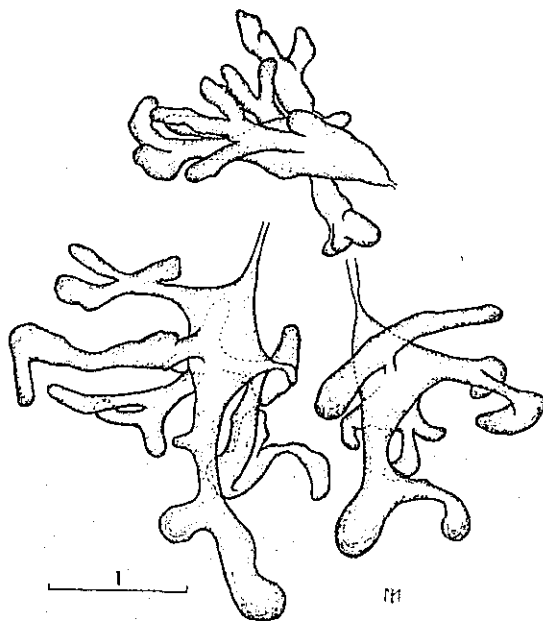
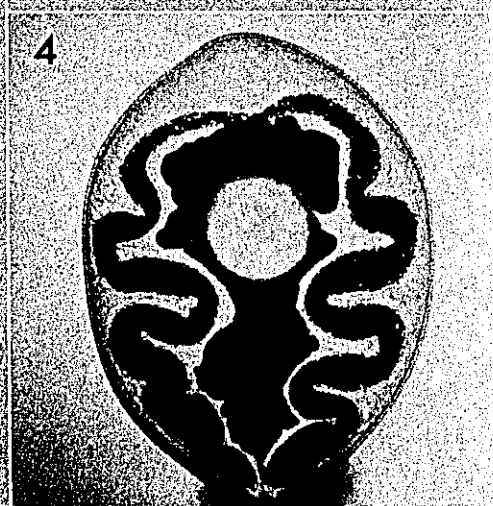
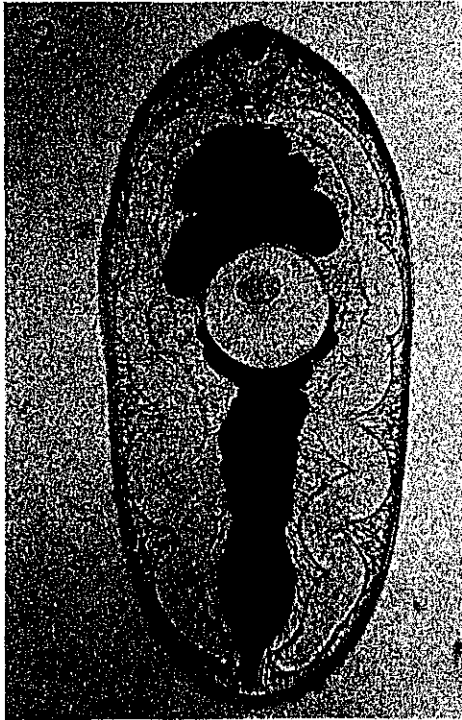
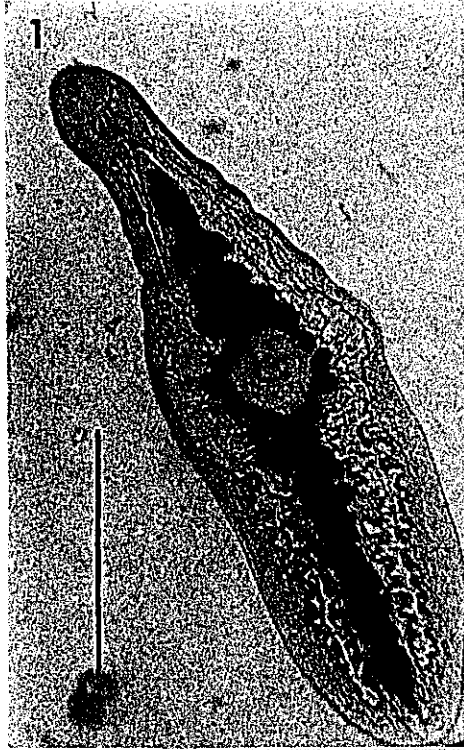


Fig. 18. Details of ovary (upper) and testes (lower) in another paratype of *P. peruvianus*. Ventral view. Scale in mm.

←Fig. 17. Adult *P. peruvianus*. 1 and 2. Ventral view of the holotype and a paratype from a cat. Scales in mm. (O: ovary, T: testis) 3 and 4. Cuticular spines on ventro-posterior part of body. Scales in micra. 5. Three uterine eggs; operculum on the right. Note irregular undulation of eggshell. Scale in micra.



Lung Flukes first removed from Man in Peru

As mentioned previously, it is most probable that human paragonimiasis in Department of Cajamarca is caused chiefly by *P. peruvianus* and occasionally by *P. callensis*. But, the lung fluke itself of any kind had never been demonstrated from man in Peru. In 1970, Dr. C. Arellano of "Hipólito Unanue" Hospital in Lima surgically obtained two adult lung flukes from a single worm cyst in the inferior lobe of the right lung of a 36-year-old Peruvian male, who had been treated for pulmonary tuberculosis for several years. The patient was suspected to have been infected in the jungle of Aguaytia, Department of Loreto, which is located in the central part of Peru. Both flukes (Fig. 20) were sent to the author for identification, but he had hesitation in identifying them definitely, because they did not show their normal morphology due to bithionol administered before the operation. Finally, these valuable flukes were reported as an unknown species of *Paragonimus* by Miyazaki, Arellano and Grados (1972).

A new Lung Fluke recently found in Peru

In 1971, the author visited Tingo Maria, Department of Huánuco, and found a single *Paragonimus* metacercaria of unknown species from a crab, *Pseudohelphusa chilensis*. Therefore, he again visited the same locality in September 1972 in order to make a joint research with Dr. O. Grados and Miss N. Uyema of National Institute of Public Health in Lima. Twenty-five crabs of the same species were examined for *Paragonimus* metacercariae with negative result. Fortunately, however, five adult lung flukes were found from two kinds of mammals belonging to Didelphidae: i. e. two flukes from a four-eyed opossum, *Philander opossum*, and three from a water opossum, *Chironectes minimus*.

Body of the new fluke (Fig. 21) is big and extremely elongated, and is very easily distinguished from known species of American *Paragonimus*. Cuticular spines are originally single; ventral sucker, which is almost the same size with the oral, is situated remarkably anteriorly. Ovary is moderately branched and testes simply, both gonads being small as compared with body size. Uterus is extended somewhat bilaterally. Sixty eggs from a worm cyst,

Fig. 19. Metacercariae of *P. peruvianus*. 1. Crawling larva. Note remarkable folds on the wall of intestines and excretory bladder. Scale: 0.5 mm. 2. Same larva fixed under pressure. Scale same as no. 1. 3. Mounted larva; stained with carmine. Contour of excretory bladder clear. Scale: 0.2 mm. 4. Living larva under pressure. Presumably young form of the same species. Note blackish intestines. Scale same as no. 1.

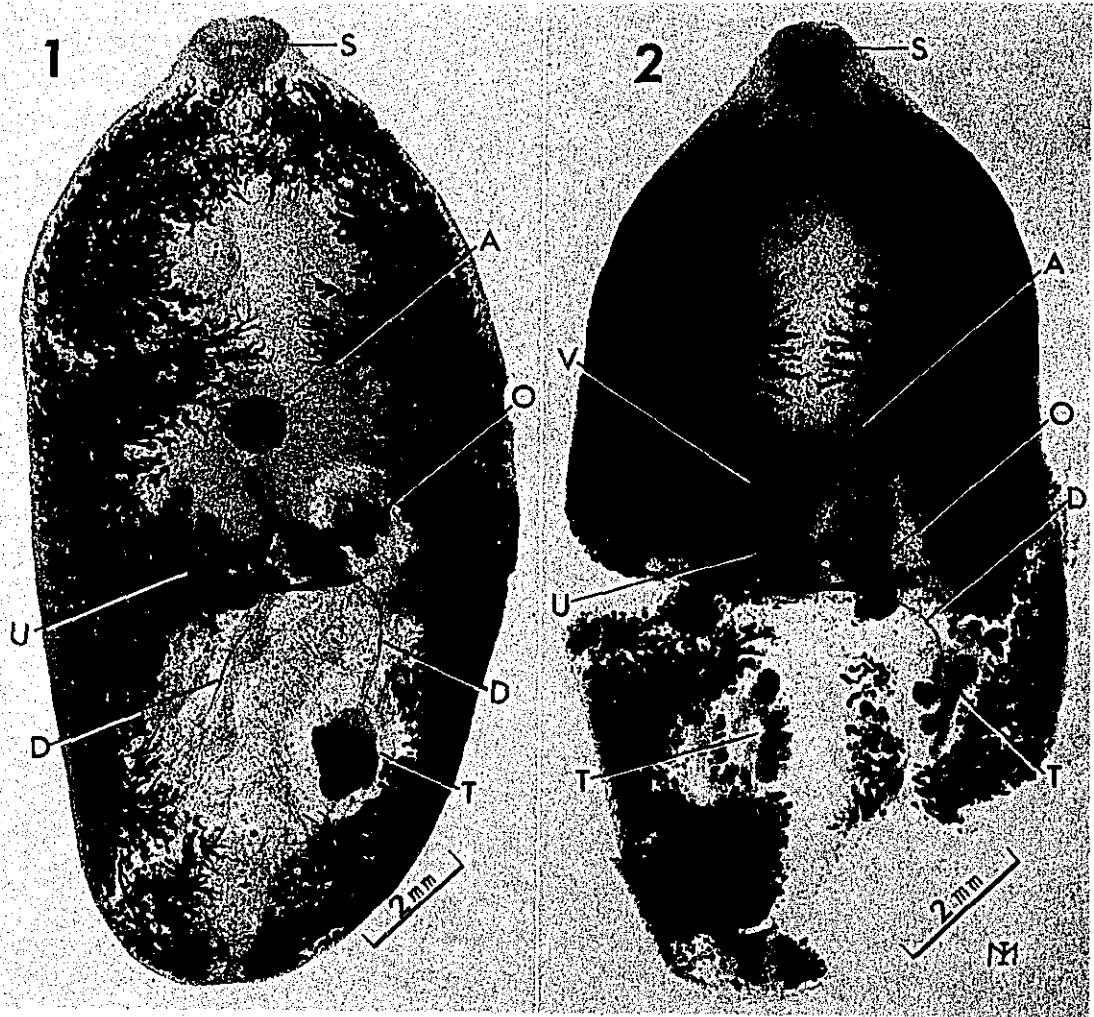


Fig. 20. Two adults of *Paragonimus* sp. from man in Peru. 1. Dorsal view. 2. Ventral view. A: ventral sucker, D: vas deferens, O: ovary, S: oral sucker, T: testis, U: uterus, V: seminal vesicle.

which was preserved in formalin, measured 81 to 102 by 43 to 55, averaging 90 by 48 micra, when free from pressure. Eggshell is thin and irregularly undulated just like that of *P. peruvianus* or *P. callensis*. Accordingly, eggs of these three species are not easily separated, but it may be somewhat useful for differentiation that eggs of the new fluke are also elongated as compared with those of the other two species. The new specific name, *Paragonimus amazonicus*, will be proposed by Miyazaki, Grados and Uyema in the near future.

The crab host of this fluke is most probably *Pseudothelphusa chilensis* (Fig. 16). Because this crab is eaten by some people in Tingo Maria, it is likely that there is possibility of human infection with the new lung fluke.

Acknowledgement

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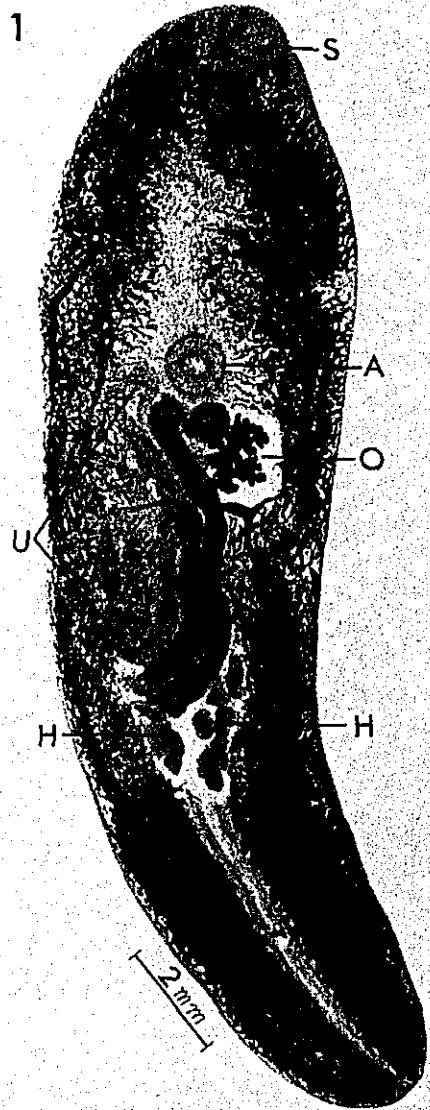


Fig. 21. *P. amazonicus*. 1. Adult from a four-eyed opossum. Dorsal view. S: oral sucker, A: ventral sucker, O: ovary, U: uterus, H: testis. 2. Formalin-fixed egg from a worm cyst of the lung. Note irregular undulation of eggshell.

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