Studies on the Mexican lung flukes, with special reference to a description of *Paragonimus mexicanus* sp. nov. (Trematoda: Troglotrematidae)

ICHIRO MIYAZAKI AND YOICHI ISHII

Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka, Japan

(Received for publication; August 17, 1968)

Introduction

Human paragonimiasis in Mexico had been undetermined until 1961, when Martínez Báez and Jiménez Galán found Paragonimus eggs in the lung tissue excised from a Mexican male patient of 35 years old. Then, Mazzotti & Miyazaki (1965) reported adult lung flukes for the first time in Mexico, which were obtained by the senior author from one of eleven opossums, Didelphis marsupialis L., captured in Colima situated in the mountainous region on the Pacific coast of Mexico. The authors noticed that the Mexican lung fluke was provided with more delicately branched ovary than P. kellicotti Ward, 1908, though both species were morphologically very similar to each other, and they emphasized that the metacercariae of both species should be compared in detail. Miyazaki (1964) investigated a lot of living metacercariae of P. kellicotti in Michigan, USA, and demonstrated their characteristic features, correcting the thickness of the inner cyst wall reported by Ameel (1934). Unfortunately, however, the metacercaria of the Mexican lung fluke has never been investigated as yet. Ishii (1966) studied many adult worms of P. kellicotti in Louisiana, USA, and illustrated the shape of the ovary in detail.

In 1965 Dr. L. Mazzotti in Mexico again

collected many lung flukes from another opossum of the same species at the same locality, Colima, and kindly sent them to the present authors. Recently, Miyazaki and Ishii (unpublished data) compared the specimens from Mexico with P. kellicotti, and revealed that the Mexican lung flukes were divided into two species (A and B) and both species were clearly differentiated from P. kellicotti. Furthermore, they stated that species B was closest to a new lung fluke found in Colombia by Dr. M. D. Little (1968, personal communication), and species A was probably an undescribed fluke, the specific name of which should be discussed later.

In the present paper the authors wish to describe species A as a new species, proposing a specific name, *Paragonimus mexicanus* sp. nov. In addition, species B and specimens from Guatemala and Panama, as well as eggs deposited in the lung tissue of a Mexican male will be briefly discussed.

Materia's and Methods

As mentioned previously, the Mexican lung flukes were collected by Dr. L. Mazzotti from two opossums in Colima and sent to the authors. The flukes amounted to 36 in all, of which 27 were stained with carmine and mounted in balsam, and the remaining nine that were unsuitable for mounting

Supported in part by the Scientific Research Grant from the Ministry of Education, Japan, and in part by the U.S. Army Research and Development Group (Far East), Department of the Army under Contract No. DAJB17-67-C-0044.

were preserved in alcohol and used for observation of the cuticular spines and the eggs collected from the end of the uterus. In some stained specimens the cuticle over and/or under the ovary was removed in order to show the shape of ovary apparently. Forty-seven mature P. kellicotti and 23 mature P. miyazakii Kamo et al.. 1961, which were experimentally obtained in the United States and in Japan, respectively, were used for morphological comparison with P. mexicanus. materials, the arrangement of the cuticular spines, the shape and size of the ovary and the testes, the size of the oral and the ventral sucker, the shape and size of the uterine eggs, and the thickness of the eggshells were carefully investigated. By the courtesy of Drs. E. Caballero y C., M. D. Little and V. E. Thatcher, one adult specimen from Guatemala labeled as P. rudis (Diesing, 1850), two specimens of a new lung fluke from Colombia and ten specimens from Panama labeled as P. rudis were respectively made available for comparative study. Dr. M. Martínez Báez kindly sent to the authors many sections of the human lung tissue containing a lot of Paragonimus eggs that was excised from the abovementioned Mexican male. These eggs were carefully compared with those of P. mexicanus. P. kellicotti and P. westermani.

Results

Among 27 specimens of the Mexican lung flukes, two species (A and B) were clearly distinguished from each other: species A was represented by 26 specimens and species B was by only one. Both species were covered with single spines all over and their eggs were also similar in morphology,

but they were easily distinguished from each other by the shape of the ovary and testes. As shown in Figs. 13 and 14, the ovary and the testes were much more simply branched in species B than in species A. The latter was described as P. mexicanus sp. nov. in the following chapter. Species B, although only one specimen was available, agreed with two specimens of Dr. Little's new species from Colombia in almost all respects. Among ten specimens from Panama, nine appeared to be the same as species A and the remaining one with species B of the Mexican lung flukes. It was likely that the specimen from Guatemala was identical with species A in Mexico. The eggs deposited in the lung tissue of the Mexican male did not agree with those of either P. kellicotti or P. westermani. They appeared to belong to P. mexicanus.

Description of Paragonimus mexicanus sp. nov.

Holotype (Figs. 1 and 13)

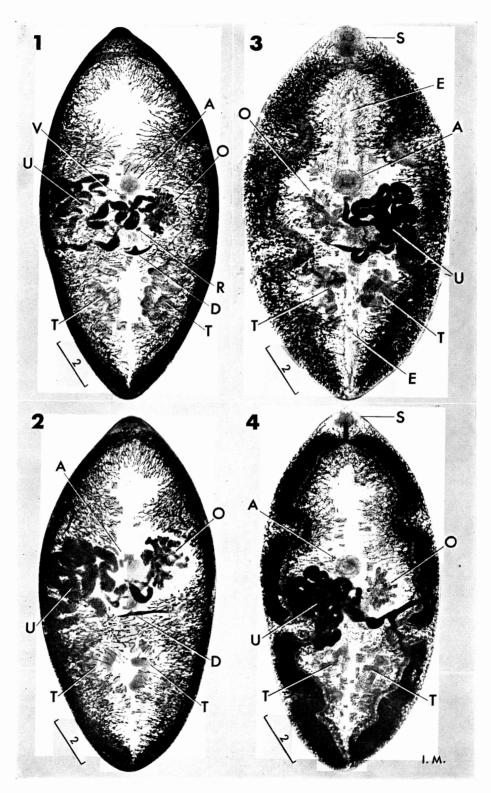
Body spindle-shaped, 14.6 mm long by 7.0 mm wide. Whole body covered with singly spaced cuticular spines, the tip of which is pointed or serrated (Fig. 11). Some spines split into two or more. Oral sucker 1.12 by 0.56 mm, followed by a small pharynx (0.48 by 0.34 mm) and a short esophagus (0.51 mm). Ventral sucker 0.83 by 0.78 mm, situated slightly anterior to the center of the body. Ovary delicately branched and located on the right side of the body, measuring 1.90 by 1.70 mm in outline. Seminal receptacle recognized, which measured 0.24 by 0.17 mm. Uterus coils on opposite side to the ovary, containing numerous eggs. Vitelline gland widely distributed on both

⁽All scales in mm)

Fig. 1. Holotype of P. mexicanus sp. nov. from an opossum. Dorsal view.

Fig. 2. Paratype No. 1. Dorsal view. Cuticle and vitelline glands were removed over and under the ovary.

^{Figs. 3 and 4. Paratypes No. 2 and No. 3. Ventral view. The oral sucker is a little larger than the ventral one in both specimens. (A: ventral sucker, D: vitelline duct, E: excretory bladder, O: ovary, R: seminal receptacle, S: oral sucker, T: testis, U: uterus, V: seminal vesicle)}



other is *P. kellicotti* Ward, 1908 found from the cat in the United States. Thereafter, the latter species had frequently been studied by many investigators and its validity was definitely verified. But, *P. rudis* was so briefly described that its morphological features were quite unknown, and the re-description of this species has never

appeared in literature, though it has passed over 100 years since the original description. Ultimately, *P. rudis* must be regarded as *nomen nudum*.

Caballero (1946, 1956) reported *P. rudis* from the opossum, *Didelphis mesamericana mesamericana* and the skunk, *Mephitis macroura macroura* in Guatemala, and from

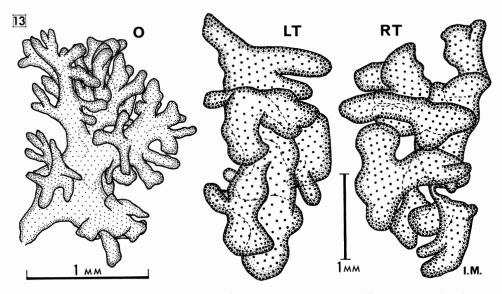


Fig. 13. Ovary and testes of the holotype of *P. mexicanus* under different magnification. Dorsal view. (O: ovary, LT: left testis, RT: right testis)

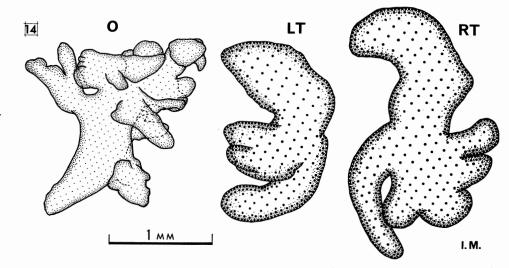


Fig. 14. Ovary and testes of species B of the Mexican lung fluke under the same magnification.

Dorsal view. (O: ovary, LT: left testis, RT: right testis)

the fox, Urocyon cinereoargenteus costaricensis in Costa Rica. But, the present authors are very skeptical of his identification, because he was of opinion that P. rudis was the only species of the genus and others such as P. westermani, P. compactus (Cobbold, 1859), P. ringeri (Cobbold, 1880), P. kellicotti, P. edwardsi Gulati, 1926, P. ohirai Miyazaki, 1939, P. iloktsuenensis Chen, 1940, and P. macacae Sandosham, 1953 were all synonymous with P. rudis. Later, Caballero and Montero (1961) obtained Paragonimus from an opossum, Philander opossum fuscogriseus in Costa Rica which they called P. rudis, and in the catalogue of trematodes they considered that the lung flukes other than P. rudis were all synonyms of this oldest species. Thatcher (1967), on the other hand, found P. rudis in some wild and domestic animals of Panama such as the common opossum, Didelphis marsupialis (in 2 of 17 examined), the four-eyed opossum, Philander opossum (8 of 15), the coati, Nasua narica (1 of 1), the jaguar, Felis onca (2 of 2), the cat, Felis catus (1 of 1), and the dog, Canis familiaris (1 of 3, by eggs in feces). Besides, the author described "P. rudis (=P. kellicotti)". Thus, the lung fluke called "P. rudis" seems to occur widely in Central America, but the present authors cannot agree with their opinion about the taxonomy of *Paragonimus* for the reasons mentioned previously.

P. mexicanus sp. nov. is similar to P. kellicotti in morphology, but they are distinguished by the shape of the ovary, the size of the oral and the ventral sucker, the size of uterine eggs, and the thickness of eggshells. Of these criteria for differentiation, eggs are most reliable for separating the two species, although it is generally difficult to identify lung fluke by the character of the egg. As shown in Table 2 and Fig. 12, the eggs of P. mexicanus are significantly smaller than those of P. kellicotti and the eggshells are thinner in the former than in the latter. The ovary is usually more delicately branched in P.

mexicanus than in the other, as already indicated by Mazzotti & Miyazaki (1965). In P. mexicanus the oral sucker was larger than the ventral one in 17 of 23 specimens examined, while in P. kellicotti only eight of 28 specimens examined had a larger oral than ventral sucker. The longest diameter of the oral and the ventral sucker was compared in each specimen, and the ratio of the former to the latter was 1.078 + 0.101 (mean standard deviation) in P. mexicanus, ranging from 0.93 to 1.35, while that of P. kellicotti was 0.978±0.092, ranging from 0.84 to 1.25. The difference of the ratio between the two species was statistically significant (P < 0.001). On the other hand, P. mexicanus is more similar to P. miyazakii than to P. kellicotti, and either ovary or uterine eggs cannot be used for differentiation of the two species, as shown in Table 2. But, they are distinguished by the size of two suckers. For this pupose the longest diameter of the oral and the ventral sucker was compared in each of 23 mature P. miyazakii, and it was revealed that only one specimen had a larger oral than ventral sucker. Besides, the ratio of the oral to the ventral sucker in this species averaged 0.840 ± 0.076, ranging from 0.70 to 1.06, and was significantly smaller than that of P. mexicanus (P<0.001). Furthermore, the dissimilarity of geographical distribution between the two species can support the authors' opinion that P. mexicanus is quite different from P. miyazakii, which has not yet been found outside Japan. Incidentally, P. mexicanus seems to be separated from an unidentified lung fluke in Colombia by the distribution of the uterus, which was reported by Dr. Little (1968, pers. comm.) to be close to *P. uterobilateralis* Voelker et Vogel, 1965.

For taxonomy of the genus *Paragonimus* it is very important to investigate larval forms, particularly the metacercaria. Unfortunately, however, any larvae have not yet been found in Mexico; while in Costa Rica a kind of *Paragonimus* metacercaria

was reported by Sogandares & Smalley (1965, 1967) from two fresh-water crabs, *Ptychophallus tristani* and *Potamocarcinus magnus*. But the metacercaria was not identified because of a failure in getting adults by experimental infection to a domestic cat. The present authors suppose that the metacercaria of *P. mexicanus* will show more clear features than its adult worms, by which it is easily distinguished from *P. kellicotti* and *P. miyazakii*.

From geographical point of view, it seems that *P. mexicanus* is occurring not only in Mexico but also in other countries of Central America such as Guatemala, Costa Rica and Panama. In addition, by the character of eggs found in the human lungs in Mexico, it is probable that *P. mexicanus* causes human paragonimiasis in Mexico and in other parts of Central America. The second species in Mexico, which is less prevalent in this country, seems to be occurring also in Panama and Colombia. Further studies are quite necessary to clarify this lung fluke.

Summary

Paragonimus mexicanus sp. nov. was described from the opossum, Didelphis marsupialis L., captured in Colima situated on the Pacific coast of Mexico. The new species was similar to P. kellicotti Ward, 1908 and P. miyazakii Kamo et al., 1961 in morphology, but it was separated from the former by the size of the oral and the ventral sucker, the shape of the ovary and the character of uterine eggs, and from the latter by the size of two suckers and the geographical distribution. It is probable that P. mexicanus occurs also in Guatemala, Costa Rica and Panama. The patient of paragonimiasis in Mexico seems to have been caused by the new species. Another lung fluke was found in Mexico, which was easily distinguished from P. mexicanus by much more simple branching of the ovary and testes. This species closely resembles the new species found by Little in Colombia.

Acknowledgement

The authors express their sincere thanks to Drs. Luis Mazzotti, Eduardo Caballero y C. and Manuel Martínez Báez in Mexico, Dr. Vernon E. Thatcher in Panama, and Dr. Maurice D. Little in the United States for their kind support with valuable specimens and communications.

References

- Ameel, D. J. (1934): Paragonimus, its life history and distribution in North America and its taxonomy. Amer. J. Hyg., 19, 279-317.
- 2) Caballero y C., E. (1946): Estudios helmintológicos de la región oncocercosa de México y de la República de Guatemala. Trematoda II. Presencia de *Paragonimus* en reservorios naturales y descripción de un nuevo género. An. Inst. Biol., 17, 137-165.
- Caballero y C., E. (1956): Presencia de *Paragonimus rudis* (Diesing, 1850) Braun, 1899 en mamíferos silvestres en Centroamérica. *ibid.*, 27, 397-401.
- 4) Caballero y C., E. and Montero Gei, F. (1961): Descripción de dos tremátodos de un marsupial de la República de Costa Rica y un catálogo de los tremátodos que parasitan a Marsupialia Illiger, 1811. An. Esc. Nac. Cien. Biol., 10, 45-86.
- Diesing, K. M. (1850): Systema Helminthum, 1, 360-361.
- Diesing, K. M. (1855): Neunzehn Arten von Trematoden. Denkschr. d. k. Akad. d. Wissensch. (Wien), Mathem.-Naturw. Cl., 10, 59-70
- Ishii, Y. (1966): Differential morphology of Paragonimus kellictti in North America. J. Parasit., 52, 920-925.
- 8) Kamo, H., Nishida, H., Hatsushika, R. and Tomimura, T. (1961): On the occurrence of a new lung fluke, *Paragnimus miyazakii* n. sp. in Japan. Yonago Acta Med., 5, 43-52.
- 9) Little, M. D.: (1968, Personal communication)
- 10) Martínez Báez, M. and Jiménez Galán, A. (1961): Un caso de trematodiasis pulmonar registrado en Mexico. Rev. Inst. Salubr. Enferm. trop. (Mex.), 21, 101-114.
- Mazzotti, L. and Miyazaki, I. (1965): The first record of adult lung flukes *Paragonimus* in Mexico. Jap. J. Parasit., 14, 34-36.
- 12) Miyazaki, I. (1964): Notes on the metacercaria of *Paragonimus kellicotti* Ward, 1908 in North America. (Jap. text with Eng. abstr.) Jap. J

- Parasit., 13, 453-457.
- 13) Miyazaki, I. and Ishii, Y.: Comparative study of the Mexican lung flukes with *Paragonimus* kellicotti Ward, 1908. J. Parasit. (in press)
- 14) Sogandares-Bernal, F. and Smalley, A. E. (1965): Paragonimus metacercariae in Pseudothelphusa tristani Rathbun from Costa Rica. J. Parasit., 51, 304.
- 15) Sogandares-Bernal, F. and Smalley, A. E. (1967): Studies on American paragonimiasis. V. Further observations on the presence of *Paragonimus* in fresh-water crabs from Costa Rica,

- with notes on susceptibility to cercariae of *P. kellicotti*. Tulane Stud. Zool., 13, 125-128.
- 16) Thatcher, V. E. (1967): Paragonimus in some wild and domestic animals of Panama. Trans. Amer. Microsc. Soc., 86, 335-336.
- 17) Voelker, J. und Vogel, H. (1965): Zwei neue Paragonimus-Arten aus West-Afrika: Paragonimus africanus und P. uterobilateralis. Z. Tropenmed. Parasit., 16, 125-148.
- 18) Ward, H. B. (1908): Data for the determination of human entozoa. 2. Trans. Amer. Microsc. Soc., 28, 177-201.

メキシコ産肺吸虫の研究,とくに新種 Paragonimus mexicanus sp. nov.

(メキシコハイキュウチュウ,新称)の記載 [特別掲載]

宮崎一郎 石井洋一

(九州大学医学部寄生虫学教室)

アメリカ大陸には,これまでに P. rudis (Diesing, 1850) と P. kellicotti Ward, 1908 の2種がしられて いる。ところが、最古の肺吸虫である前者は、記載がか んたんで、その特長がわからない。その後100年以上も たっているのに、まだ1度も再記載がなく、いまだに、 ナゾである. ただし, 中米で Caballero (1946, 1956), Caballero & Montero (1961), および Thatcher (1967) が, グァテマラ, コスタリカ, ならびにパナマから P. rudis を報告したが、彼らは最古の種名を用いたにすぎ ない. Caballero らは、P. rudis 以外のものは全部こ れのシノニムと考えており、 Thatcher も P. rudis(=P. kellicotti) とかいている. したがって、彼らの同 定をそのまま認めるわけにはいかない. Mazzotti & Miyazaki (1965) は初めてメキシコから肺吸虫成虫を報 告し、Miyazaki & Ishii (未発表) はこれと P. kellicotti との間では、卵の大きさと卵殻の厚さが、最も よい区別点になるとのべた、材料は Dr. Luis Mazzotti がメキシコの Colima で、2頭のフクロネズミ Didelphis marsupialis からえたものである. これを米国産

の P. kellicotti, 日本産の P. miyazakii, グァテマラ とパナマ産の P. rudis, ならびにメキシコ人の肺切片 標本中の卵と比較して、つぎの成績をえた. 1)メキシ コ産の肺吸虫に 2 種を区別した (AとB). Bは 27 中わ ずか1個体であったが、南米コロンビアで Little (未発 表)がみつけた新種に一致するようである. Aは27中26 をかぞえ,他の新種として上記の学名と和名を提唱した. 2) この新種は P. kellicotti に似てはいるが, 口吸盤が 腹吸盤より大きい個体の多いこと, 卵巣の分枝がより複 雑なことの他に, 最良の区別点として, 卵が明らかに小 さく,卵殻のうすいことを重視した. 3) P. miyazakiiとは、一層似ているが、両吸盤のちがいと地理的分布の 差から、別種と考えた. 4) グァテマラからの1個体と パナマ産10中 9 個体は P. mexicanus, のこりの 1 個体 は Little による新種と思われた. 5) メキシコ人(6) の肺切片中の卵は P. westermani にも P. kellicotti に も一致せず, P. mexicanus に属するようである. 人体 寄生の可能性が考えられるので、中米では注目すべき肺 吸虫になりそうである.

Addendum (October 28, 1968)

- Little, M. D. (1968): Paragonimus caliensis sp. n. and paragonimiasis in Colombia. J. Parasit., 54, 738-746.
- Miyazaki, I. and Ishii, Y. Comparative study of the Mexican lung flukes with *Paragonimus* kellicotti Ward, 1908. J. Parasit., 54, 845-846.