[Jpn. J. Parasitol., Vol. 44, No. 5, 365–370, October, 1995]

Morphological Observations of *Paragonimus mexicanus* from Guatemala

YASUMASA TONGU[1], HIDEKAZU HATA[2], YASUHIDE ORIDO[3], MARIO ROBERTO PINTO[4], RAFAEL LAMOTHE-ARGUMEDO[5], MUNEO YOKOGAWA[2] AND MORIYASU TSUJI[6]

[1]*School of Health Sciences, Okayama University, Okayama 700, Japan.*
[2]*Department of Parasitology, School of Medicine, Chiba University, Chiba 260, Japan.*
[3]*Department of Public Health, Kyorin University School of Medicine, Mitaka City, Tokyo 181, Japan.*
[4]*Facultad de Ciencias Medicas, Universidad de San Carlos de Guatemala, Guatemala City, Guatemala, C.A.*
[5]*Laboratorio de Helmintología, Instituto de Biologia, Universidad nacional Autonoma de Mexico, D.F. Mexico, Mexico.*
[6]*Department of Tropical Disease and Parasitology, Kyorin University School of Medicine, Mitaka City, Tokyo 181, Japan.*
(Accepted September 22, 1995)

Abstract

Paragonimus eggs, metacercariae, and adult worms from Guatemala were morphologically examined using a light and scanning electron microscope. On average, the eggs measured 71 μm × 42 μm. The brown egg shell was thin and shallow pits covered the surface. The mean measurements of the fixed metacercariae were 1,156 μm in length. The ventral sucker was larger than the oral one. On average, 25 outer-papillae were counted. The flame cell formula showed 2[(3+3+3+3+3)+(3+3+3+3+3)]=60. The adult worms fixed in 10% formalin under the pressure of a slide glass measured 8.9 mm in length × 4.2 mm in width, and the oral and ventral suckers measured 512 μm × 640 μm and 664 μm × 704 μm, respectively. The ovary was delicately branched. These results showed the features of *Paragonimus mexicanus*.

Key words: *Paragonimus mexicanus*; adult worm; metacercaria; egg; morphology; Guatemala.

Introduction

Since 1968, new *Paragonimus* species have been reported from Central and South America by Little (1968), Miyazaki and Ishii (1968), Miyazaki *et al.* (1969, 1973, 1975), and Voelker and Arzube (1979). Soon after that, *P. peruvianus* and probably *P. ecuadoriensis* proved to be synonymous with *P. mexicanus* (Miyazaki, 1979; Brenes *et al.* 1980). In Guatemala, Caballero (1946, 1957) first reported *P. rudis* (Diesing, 1850) from the opossum and skunk. However, this author simply used the oldest name, *P. rudis* which is regarded as a *species inquirenda* at present, without any taxonomical discussion. Subsequently, Miyazaki and Kifune (1980) described Guatemalan *Paragonimus* as *P. mexicanus*. However, their report lacked the observations of a flame-cell pattern and an electron microscopical view. In the present study, we re-examined the Guatemalan *Paragonimus* in detail using a light and scanning electron microscope, and identified it as *P. mexicanus*.

Correspondence: Yasumasa Tongu
頓宮廉正[1], 畑 英一[2], 織戸康秀[3], Mario Roberto Pinto[4], Rafael Lamothe-Argumedo[5], 横川宗雄[2], 辻 守康[6] ([1]岡山大学医療技術短期大学部, [2]千葉大学医学部寄生虫学教室, [3]杏林大学医学部公衆衛生学教室, [4]サンカルロス大学医学部, [5]メキシコ国立自治大学生物学科, [6]杏林大学医学部熱帯病寄生虫学教室)

This investigation was supported by a Grant-in-Aid for the International Scientific Research Program of the Ministry of Education, Science, Sports and Culture in Japan in 1994 (Grant No. 06041099).

Materials and Methods

The second intermediate hosts, *Pseudothelphusa* spp., were collected at the Quebrada San Jose (a small mountain stream) in Cuilapa and at the Rio San Diego (a small river) in Esquintla, Guatemala in August, 1994. These crabs were brought to the laboratory of San Carlos University and examined for metacercariae. All of the *Paragonimus* metacercariae without cyst walls were obtained

from the liver and muscles of the host crabs. Some metacercariae were brought back to Japan in a crab physiological saline solution in an ice jar, and a cat was infected with the 20 metacercariae. The adult worms were recovered from the host cat at 111 days after infection. At the same time, the eggs were retrieved from the physiological saline solution maintained the adult worms.

For light microscopy, the living metacercariae were observed without staining using an ordinary light microscope or a phase-contrast microscope. The adult worms were fixed using 10% formalin under the pressure of a slide glass and stained with alum carmine.

For scanning electron microscopy, the eggs were fixed in 10% formalin and postfixed in 1% osmium tetroxide solution. The specimens were placed in 2-methyl-2-propanol after dehydration through a graded series of ethanol. They were dried with a Hitachi ES-2030 freeze dryer and observed with a Hitachi S-2300 scanning electron microscope after carbon and gold coatings.

The metacercariae were fixed in 10% hot formalin for 10 min and quickly transferred into 2.5% glutaraldehyde solution at pH 7.4. The specimens were postfixed in 1% osmium tetroxide solution at a pH 7.4 for 2 h in an ice box. After dehydration through a graded series of ethanol, they were placed in isoamyl acetate and critical-point dried with liquid carbon dioxide. The dried specimens were coated with carbon and gold and observed with a Hitachi S-2300 scanning electron microscope.

Results

Egg

The eggs varied in shape and sizes. They measured 66 to 76 μm (mean 71 from 20 eggs) × 38 to 46 μm (mean 42 from 20 eggs) in fixed specimens. The brown egg shell was thin and had a thickening at the abopercular end and at the junction between the operculum and egg body (Fig. 1). Shallow pits were distributed all over the shell surface (Fig. 2).

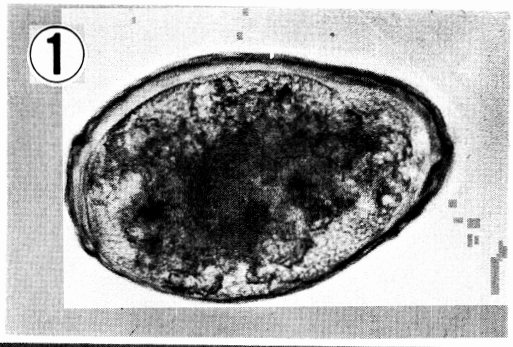
Metacercaria

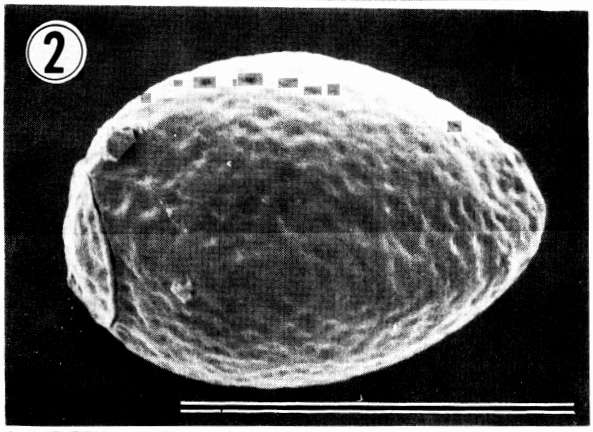
The mean measurements of the 10 metacercariae fixed in 10% hot formalin, without a cover slip, were as follows:

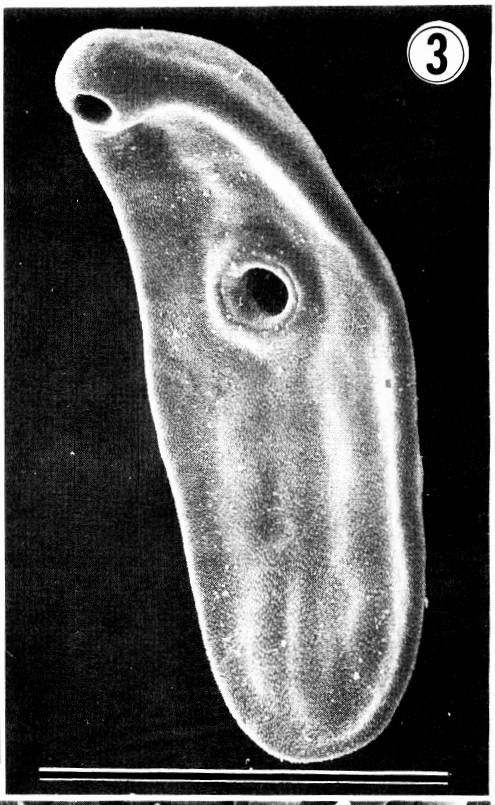
body length	1,156 μm
oral sucker	122 μm × 122 μm
ventral sucker	192 μm × 189 μm

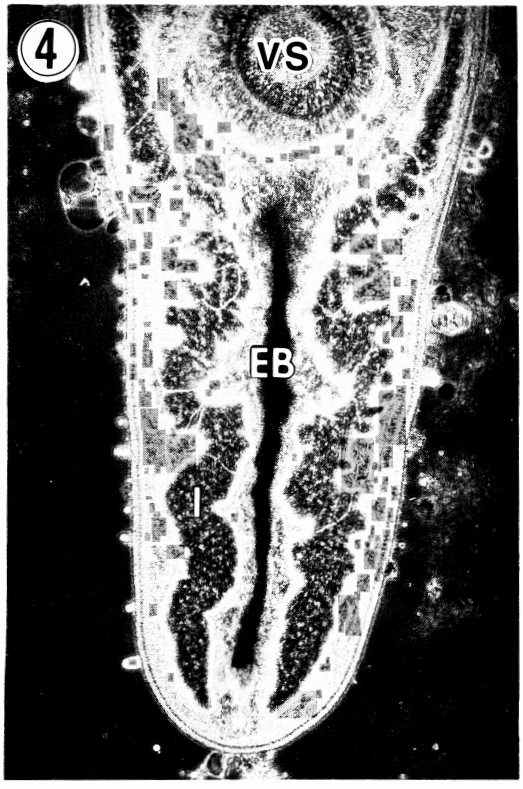
The metacercariae (Fig. 3) had no cysts and vigorously and freely crept within the body cavity of the crab host. The entire body surface was covered with single-pointed spines (Fig. 5). Red granules were widely distributed throughout the body. Numerous papillae were grouped around the oral sucker. Two circles consisting of the inner-papillae and outer-papillae were distinctly situated on the lip and around the ventral sucker (Fig. 7). The number of inner ones (Fig. 7, IP) was consistently 6 and that of the outer ones (Fig. 7, OP) was varied ranging from 20 to 30 (mean 25 from 14 specimens). The outer ones were smaller than the inner ones. The oral sucker (Fig. 6) was smaller than the ventral one in all specimens and was armed with a small stylet that was 23 μm in length. The mouth was followed by a pharynx and a short esophagus. After a short distance from the pharynx, the intestine (Fig. 4, I) was divided into 2 branches with 3 folds and almost reached the posterior end of the body. The excretory bladder (Fig. 4, EB) was black and was extended from the posterior end to the bifurcation of the intestine. From each lateral side of the middle part of the excretory bladder, a main collecting duct arose laterally and then divided into an anterior and a posterior secondary excretory duct. The anterior segment ran forward receiving branches at 5 points. The posterior segment branched off at 5 points as in the case of the anterior duct. Each capillary excretory duct provided with a flame cell at its tip. The flame cell formula was 2[(3+3+3+3+3)+(3+3+

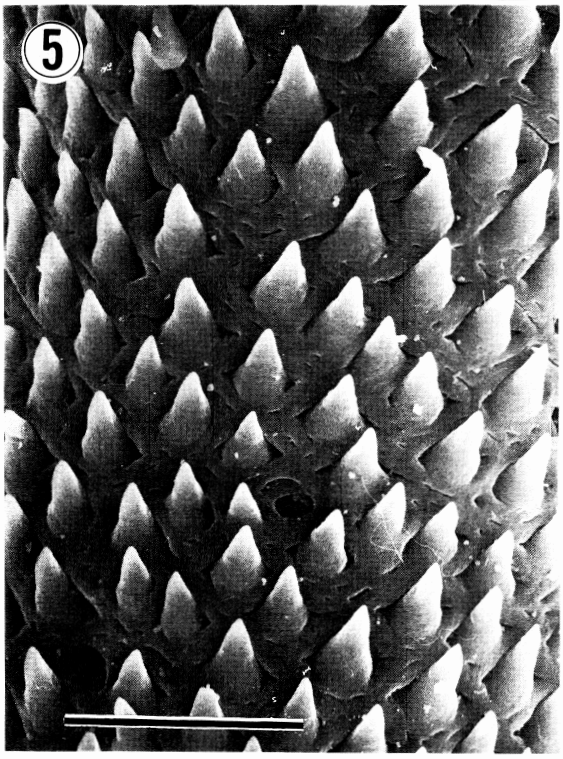
Fig. 1 Light microscopic observation of an egg laid in physiological saline solution.
Fig. 2 Scanning electron micrograph of an egg (bar=50 μm).
Fig. 3 Scanning electron micrograph of a metacercaria (bar=500 μm).
Fig. 4 Posterior half of a living metacercaria under the phase contrast microscope. I, intestine; EB, excretory bladder; VS, ventral sucker.
Fig. 5 Scanning electron micrograph of surface spines (bar=10 μm).

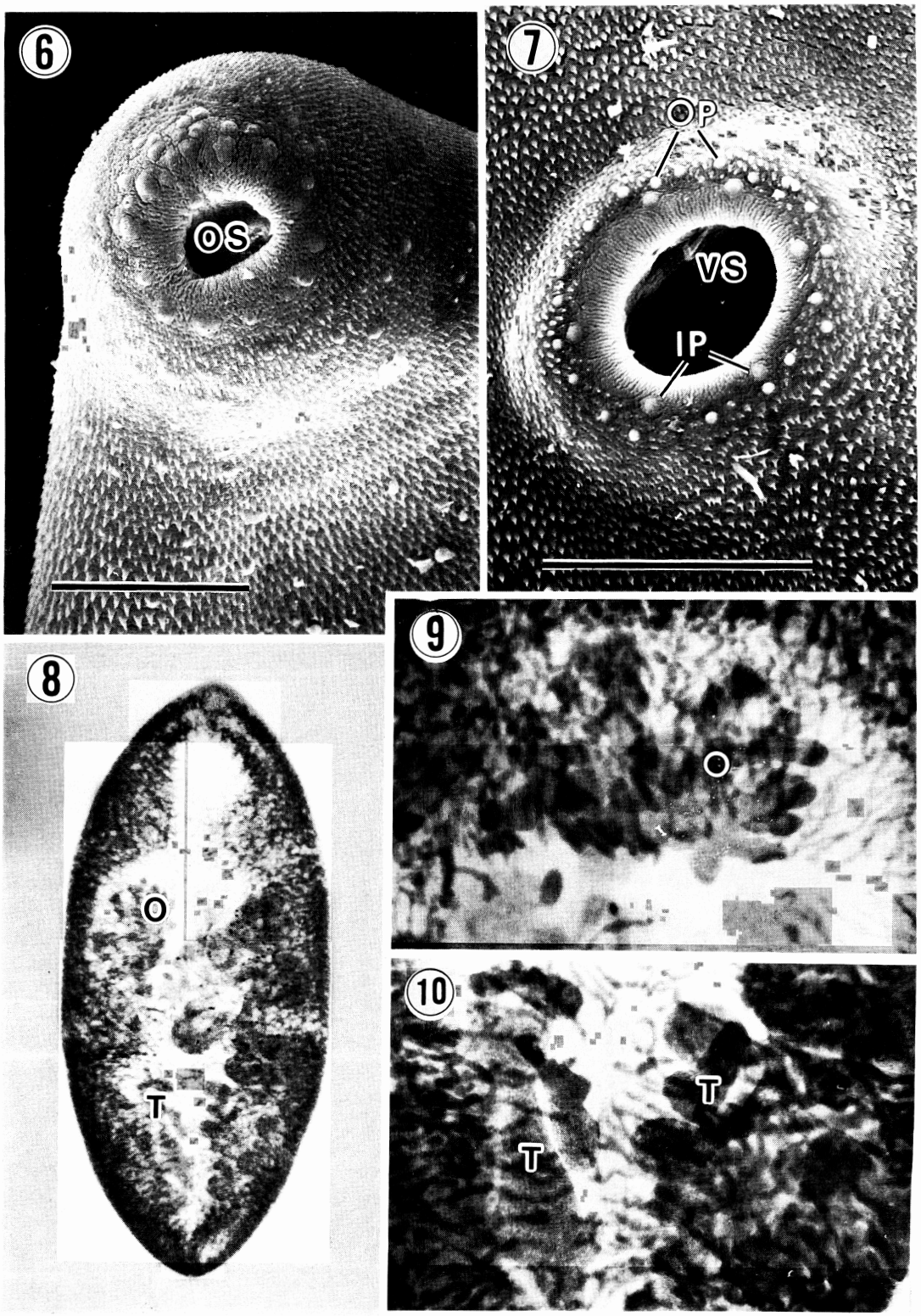

1


2


3


4
VS
EB
I


5


6
OS
7
P
VS
IP
8
O
T
9
O
10
T
T

3+3+3)]=60. However, we were unable to observe all of these flame cells in each specimen.

Adult worm

The mean measurements of the 5 specimens fixed in 10% formalin under the pressure of slide glass were as follows:

body	8.9 mm in length × 4.2 mm in width
oral sucker	512 μm × 640 μm
ventral sucker	664 μm × 704 μm

The digestive tract consisted of a spherical pharynx, a short esophagus, and 2 thick intestines with 3 folds which reached the posterior end of the body. Cuticular spines covered the entire body surface. These spines were split at their tips. However, they had a common root (A single type group). The ovary was delicately branched as shown in the Fig. 9. The uterus contained many eggs which formed a series of coils behind the ventral sucker. A pair of testis (Fig. 10, T) were located in the posterior portion of the body and each had several lobes. An excretory bladder extended to the bifurcation of the intestine.

Discussion

Guatemalan *Paragonimus* was initially reported by Caballero (1946, 1957) as *P. rudis*. However, his description lacked a taxonomical view. Miyazaki and Kifune (1980) reported *P. mexicanus* from Guatemala after an observation of the metacercariae and adult worms using light microscopy in an unknown report. And furthermore, they lacked the observations of a flame-cell pattern, the pits of the egg shell surface and number of outer-papillae. In the present study, we completed the morphological observations of Guatemalan *Paragonimus* with a scanning electron microscopy in particular.

The sizes of the eggs overlapped with those of *P. mexicanus* (Miyazaki and Ishii, 1968; Tongu *et al.*, 1985). However, the longest one (76 μm) was smaller than that of Venezuelan *Paragonimus* (Alarcón de Noya *et al.*, 1985; Tongu *et al.*, 1990) or other Latin American *Paragonimus* (Little, 1968; Miyazaki *et al.*, 1973, 1975; Voelker and Arzube R., 1979). These results might be due to the difference between the donor hosts, opossum or cat. The pitted surface characteristics were similar to those of *P. mexicanus*.

The light microscopical observation of *Paragonimus* metacercariae from Latin America has been completed (Little, 1968; Miyazaki *et al.*, 1969, 1971, 1974, 1975; Voelker and Arzube R., 1979; Lamothe A., 1979; Aji *et al.*, 1984; Tongu *et al.*, 1985, 1987, 1990). These metacercariae except *P. caliensis* cannot be morphologically classified according to their species using a light microscope. Only *P. caliensis* has a cyst wall and 96 flame cells. In the present study, Guatemalan *Paragonimus* had a flame cell formula as well as *P. mexicanus* metacercaria (Ito *et al.*, 1984; Tongu *et al.*, 1987). Aji *et al.* (1983) and Tongu *et al.* (1985, 1987, 1990) described the external morphology with a scanning electron microscope and have reported the different numbers of outer-papillae surrounding the ventral sucker in the different Latin American metacercarial strains. The average number of 25 outer-papillae in the present study was similar to those of *P. mexicanus* from Peru (Aji *et al.*, 1984). However, the present *Paragonimus* metacercaria distinctly differed from the Venezuelan one, which has more indistinct and a fewer number of outer-papillae.

In the present study, we obtained the adult worms from a cat at 111 days after metacercariae infection. The ovary branches and testes are the most important criteria for distinguishing the species of adult worms. The Guatemalan *Paragonimus* had a delicately branched-ovary and testes divided into several lobes, and single-type spines. These morphological features were almost the same as *P. mexicanus*. In conclusion, we identified the Guatemalan *Paragonimus* as *P. mexicanus*.

Fig. 6 Scanning electron micrograph of anterior portion of a metacercaria. OS, oral sucker (bar=50 μm).

Fig. 7 Scanning electron micrograph of the ventral sucker of a metacercaria provided with 6 inner-papillae (IP) and 20 outer-papillae (OP). VS, ventral sucker (bar=100 μm).

Fig. 8 Adult worm fixed in 10% formalin under the pressure and stained with alum carmine. O, ovary; T, testis.

Fig. 9 Ovary stained with alum carmine. O, ovary.

Fig. 10 Testes stained with alum carmine. T, testis.

References

1) Aji, T., Oh, H., Tongu, Y., Inatomi, S., Hata, H., Kobayashi, M., Yokogawa, M., Miranda, H. and Ibáñes, N. (1984): Ultrastructure of tegumental surface of the metacercaria of *Paragonimus peruvianus*. Jpn. J. Parasitol., 33, 15–21.
2) Alarcón de Noya, B., Abreu, G. and Noya, G. O. (1985): Pathological and parasitological aspects of the first autochthonous case of human paragonimiasis in Venezuela. Am. J. Trop. Med. Hyg., 34, 761–765.
3) Brenes, R. R., Zeledón, R. and Rojas, G. (1980): Biological cycle and taxonomic position of a Costa Rican *Paragonimus* and the present status of *Paragonimus* from the New World. Brenesia, 18, 353–366.
4) Caballero, 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. Méx., 17, 137–165.
5) Caballero, E. (1957): Presencia de *Paragonimus rudis* (Diesing, 1850) Braun, 1899 en mamiferos silvestres en Centroamérica. An. Inst. Biol. Méx., 27, 397–401.
6) Ito, J., Yokogawa, M., Kobayashi, M., Hata, H., Tsuji, M., Kojima, S., Tongu, Y., Lamothe-A., R., Pineda, R. and Osorio, D. (1984): On the morphology of cercaria and metacercaria of *Paragonimus mexicanus* Miyazaki et Ishii, 1968 (in Japanese). Jpn. J. Parasitol., 33 (Suppl.), 7.
7) Lamothe-Argumedo, R., Caballero-Deloya, J. and Mancilla, E. L.-C. (1979): Descripcion de la metacercaria de *Paragonimus mexicanus* Miyazaki e Ishii, 1968 (Trematota: Troglotrematidae). Neumol. Cir. Tórax., Méx., 40, 179–187.
8) Little, M. D. (1968): *Paragonimus caliensis* sp.n. and paragonimiasis in Colombia. J. Parasitol., 54, 738–746.
9) Miyazaki, I. (1974): Occurrence of the lung fluke, *Paragonimus peruvianus* in Costa Rica. Jpn. J. Parasitol., 23, 280–284.
10) Miyazaki, I., Grados, O. and Uyema, N. (1973): A new lung fluke found in Peru, *Paragonimus amazonicus* sp. n. (Trematoda: Troglotrematidae). Jpn. J. Parasitol., 22, 48–54.
11) Miyazaki, I., Ibáñez, N. and Miranda, H. (1969): On a new lung fluke found in Peru, *Paragonimus peruvianus* sp. n. (Trematoda: Troglotrematidae). Jpn. J. Parasitol., 18, 123–130.
12) Miyazaki, I., Ibáñez, N. and Miranda, H. (1971): Studies on the metacercaria of *Paragonimus peruvianus* (Trematoda: Troglotrematidae). Jpn. J. Parasitol., 20, 425–430.
13) Miyazaki, I. and Ishii, Y. (1968): Studies on the Mexican lung flukes, with special reference to a description of *Paragonimus mexicanus* sp. nov. (Trematoda: Troglotrematidae). Jpn. J. Parasitol., 17, 445–453.
14) Miyazaki, I. and Kifune, T. (1980): Taxonomical and biological studies on the lung flukes of Central America. Part 2. General accounts and results of the survey in Guatemala. Report of the overseas scientific survey of Ministry of Education, Science and Culture in Japan in 1978 and 1979, p. 12–28.
15) Miyazaki, I., Mazabel, C., Grados, O. and Uyema, N. (1975): Studies on the lung fluke in Tingo Maria, Peru, with special reference to the description of *Paragonimus inca* sp. n. (Trematoda: Troglotrematidae). Med. Bull. Fukuoka Univ., 2, 303–311.
16) Tongu, Y., Aji, T., Oh, H., Ishii, A., Yokogawa, M., Hata, H., Ito, J. and Lamothe-Argumedo, R. (1985): Surface ultrastructure of *paragonimus mexicanus* Miyazaki et Ishii, 1968. Jpn. J. Parasitol., 34, 441–447.
17) Tongu, Y., Iwanaga, Y., Hata, H., Tsuji, M., Yokogawa, M., Morera, P. and Conejo, M. (1987): Morphological features of *Paragonimus* metacercariae from Costa Rica. Jpn. J. Parasitol., 36, 236–241.
18) Tongu, Y., Noya G., O., Iwanaga, Y., Hata, H., Alarcon de Noya, B., Botto, C., Alvarez, M. and Tsuji, M. (1990): Morphological features of larval stages of Venezuelan *Paragonimus*. Jpn. J. Parasitol., 39, 356–364.
19) Voelker, J. and Arzube R., M. (1979): Ein neuer Lungenegel aus der Küstenkordillere von Ecuador: *Paragonimus ecuadoriensis n. sp.* (Paragonimidae; Trematoda). Tropenmed. Parasit., 30, 249–263.