

## Morphological Features of Larval Stages of Venezuelan *Paragonimus*

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### Abstract

Morphology of eggs, rediae, cercariae, and metacercariae of *Paragonimus* found in Venezuela were studied with the light and scanning electron microscopes.

- 1) Eggs measured  $84 \times 49 \mu\text{m}$  on an average in fixed specimens and  $80 \times 47 \mu\text{m}$  in fresh specimens. The egg shell was thin and had a thickening at abopercular end. Many shallow pits were distributed all over the shell surface.
- 2) Two fixed specimens of rediae measured  $451$  and  $941 \mu\text{m}$  long  $\times$   $144$  and  $169 \mu\text{m}$  wide of the body and  $51$  and  $56 \mu\text{m}$  long  $\times$   $51$  and  $56 \mu\text{m}$  wide of the pharynx respectively. Each redia contained 10 and 13 cercariae.
- 3) Cercarial body measured  $221 \times 67 \mu\text{m}$ , the oral sucker  $49 \times 43 \mu\text{m}$ , the stylet  $27 \times 5 \mu\text{m}$ , and the acetabulum  $27 \times 26 \mu\text{m}$  on an average in fixed specimens. Seven pairs of penetration gland cells and a club-shaped excretory bladder were observed. Two types of spines were seen on the body surface.
- 4) Metacercarial body measured  $822 \times 334 \mu\text{m}$ , the oral sucker  $113 \times 106 \mu\text{m}$ , and the acetabulum  $144 \times 155 \mu\text{m}$  on an average in fixed specimens. In alive specimens, the body measured  $1190 \times 463 \mu\text{m}$ , the oral sucker  $136 \times 122 \mu\text{m}$  and the acetabulum  $186 \times 173 \mu\text{m}$ . These metacercariae had no cyst wall. The flame cell formula was  $2[(3 + 3 + 3 + 3 + 3) + (3 + 3 + 3 + 3 + 3)] = 60$ . Outer papillae were indistinct and varied in number from 10 to 20.

**Key words:** *Paragonimus*, redia, cercaria, metacercaria, ultrastructure, Venezuela

### Introduction

Several species of *Paragonimus* have been described from Central and South America (Little, 1968; Miyazaki and Ishii, 1968; Miyazaki *et al.*, 1969, 1973, 1975a; Voelker and Arzube, 1979). In Venezuela, the reports on *Paragonimus* have been given by Iturbe and Gonzalez (1919),

Iturbe (1942), and Alarcón de Noya *et al.* (1985a,b). An area of the transmission has been described in Sucre State where adult worms and metacercariae were found from opossums and crabs, respectively (Alarcón de Noya *et al.*, 1985a,b; Alarcón de Noya, 1986). They further made additional observations on the eggs, metacercariae and adult worms (Alarcón de Noya, 1986).

We have identified the Central and South American *Paragonimus* collected in Peru, Mexico and Costa Rica as synonymous with *P. mexicanus* (Aji *et al.*, 1984; Ito *et al.*, 1984, 1985; Tongu *et al.*, 1985, 1987). The present study deals with morphological aspects of Venezuelan *Paragonimus* with particular reference to cercariae and metacercariae which differ from those of *P. mexicanus*.

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## Materials and Methods

The intermediate and final hosts of *Paragonimus* were obtained at "El Algarrobo de Buenos Aires", the Cajigal District, Sucre State in February, 1989.

Eggs collected from cystic cavities of the lung of wild opossums, *Didelphis marsupialis*, naturally infected with *Paragonimus* were rinsed with 0.1M cacodylate buffer (pH 7.4), and were fixed in Karnovsky's fixative for 24 hr at 5°C. Their measurements were performed on 30 fixed specimens and on 50 fresh specimens in the stool of the same animal.

Cercariae were collected by crushing from the first intermediate snail host, *Aroapyrgus* sp. Measurements were taken from 5 specimens fixed in 10% hot formalin. When processing for scanning electron microscopy, these larval stages were immediately fixed in Karnovsky's fixative for 24 hr at 5°C. Measurements of rediae were made on 2 specimens fixed in 10% hot formalin without cover glass.

Metacercariae were obtained from the body cavity and tissues of the second intermediate crab host, *Eudaniela garmani*. For determination of the flame cell pattern, living larvae were placed in 0.4% saline solution under a cover glass sealed with vaseline. For light microscopy, the metacercariae were fixed with 10% formalin under the pressure of cover glass. These specimens were stained with alum carmine. For scanning electron microscopy, the worms were incubated in 1% pancreatin solution with 0.1% NaHCO<sub>3</sub> for 2 hr at room temperature and then washed thoroughly in 0.1M cacodylate buffer solution. The worms were then fixed with 10% hot formalin for 10 min, and quickly transferred into Karnovsky's fixative for 24 hr at 5°C.

One-half of the prefixed specimens was brought to Japan in the buffer solution at ambient temperatures and the other half was left in Venezuela for further processing. For scanning electron microscopy, these specimens were post-fixed with buffered 1% OsO<sub>4</sub> for 2 hr, and dehydrated through a graded series of ethanol. They were critical point-dried, coated with carbon and gold, and examined with scanning electron

microscopes, JEOL S25-II and Hitachi S-450.

## RESULTS

### EGGS

Sizes of the eggs were 70–93 μm (mean 84) in length × 40–63 μm (mean 49) in maximum width in the fixed specimens and 74–91 μm (mean 80) in length × 42–58 μm (mean 47) in width in fresh specimens.

The egg shell was thin. However, almost all the eggs had a thickening of shell at the abopercular end and at the junction between the operculum and egg body (Figs. 1, 2). Many shallow pits were distributed all over the shell surface (Fig. 3).

### REDIA

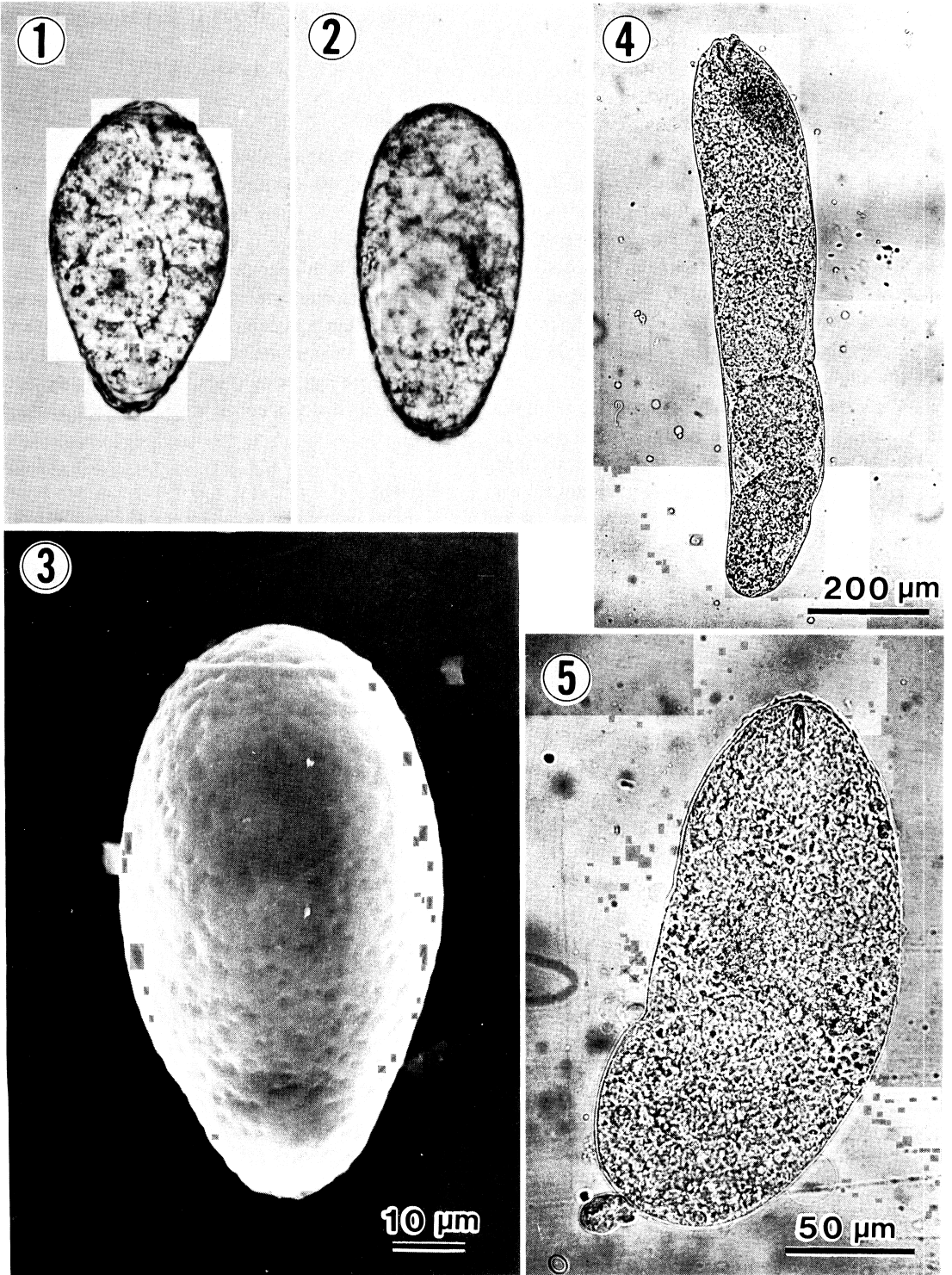
Sizes of rediae (Fig. 4) were 451 and 941 μm in length × 144 and 169 μm in width. The pharynx measured 51 and 56 μm × 51 and 56 μm. Each redia contained 10 or 13 cercariae.

### CERCARIA

Sizes of these cercariae were as follows:

body	198–248 μm (mean 221) × 59–75 μm (mean 67)
oral sucker	49 × 43 μm
stylet	27 × 5 μm
acetabulum	27 × 26 μm

Cercariae had a stylet (Fig. 5) in the oral sucker and sensory papillae (Figs. 6, 7), particularly, around the oral sucker. In the orifice of the oral sucker, two peculiar sensory papillae (Fig. 7, arrow heads) were seen. There were two types of spines on the body surface. Firstly, small spines were all over the body surface and secondly, long ones, grew densely on the ventral surface just behind the oral sucker, on the front margin of the acetabulum, on the posteroventral surface, and the tip of the tail (Figs. 6, 7). Seven pairs of penetration gland cells were observed in front of acetabulum (Fig. 13). A pharynx was situated behind the oral sucker. No intestine was observed. In the posterior part of the body, a club-shaped excretory bladder was located between the acetabulum and tail end. A conical short tail was attached at the posterior end.



## METACERCARIAE

Measurements of 10 metacercariae fixed in 10% hot formalin, without cover glass, were as follows:

body	703–990 $\mu\text{m}$ (mean 822) $\times$ 248–355 $\mu\text{m}$ (mean 334)
oral sucker	89–129 $\mu\text{m}$ (mean 113) $\times$ 89–129 $\mu\text{m}$ (mean 106)
acetabulum	109–168 $\mu\text{m}$ (mean 144) $\times$ 129–178 $\mu\text{m}$ (mean 155)

Measurements of 16 alive metacercariae under light pressure of coverslip were as follows:

body	967–1432 $\mu\text{m}$ (mean 1190) $\times$ 395–510 $\mu\text{m}$ (mean 463)
oral sucker	119–154 $\mu\text{m}$ (mean 136) $\times$ 95–142 $\mu\text{m}$ (mean 122)
acetabulum	158–205 $\mu\text{m}$ (mean 186) $\times$ 142–208 $\mu\text{m}$ (mean 173)

The metacercariae had no cyst wall, and crept vigorously and freely within the body cavity of the crab host. Most worms were characterized by an oral sucker with a stylet. The entire body surface was covered by single-pointed spines (Figs. 10, 11, 12). Numerous papillae surrounded the oral sucker. Some of these papillae grouped, in particular, on the dorsal lip of the oral sucker (Fig. 10). A circle consisting of papillae (inner papillae) (Fig. 11, arrows) was situated distinctly on the lip margin of acetabulum. However, the papillae, located around acetabulum (outer papillae) (Fig. 11, arrow heads), were smaller and less distinct than the inner papillae. The number of inner papillae was consistently 6, while the outer ones did not have a uniform number varying in number from 10 to 20.

The mouth was followed by a pharynx and a short esophagus. After a short distance from the pharynx, the thick intestine was divided into 2 branches with several folds and almost reached the posterior end of the body. The excretory bladder was black in color, and was extended from the posterior end to the bifurcation of the

intestine (Figs. 8, 14). From each lateral side of the middle part of the excretory bladder, a main collecting duct arose laterally, and then was divided into an anterior and a posterior secondary excretory duct. The anterior duct ran forward receiving branches at 5 points. The posterior one, branched off at 5 points intervals as in the case of the anterior duct. Each capillary excretory duct provided with a flame cell at its tip. The flame cell formula (Fig. 14) was  $2[(3 + 3 + 3 + 3 + 3) + (3 + 3 + 3 + 3 + 3)] = 60$ .

## Discussion

Eggs used in the present study were regarded as completely mature. The sizes of the eggs overlapped with those of other species of *Paragonimus* reported from Central and South America. The longest one (93  $\mu\text{m}$ ) was almost equal to the average length (90  $\mu\text{m}$ ) of *Paragonimus amazonicus* egg (reported by Miyazaki *et al.*, 1973), which is the longest one in American *Paragonimus*. New World *Paragonimus* eggs except those of *P. kellicotti* show many small pits on the egg surface. These pits are evident, in particular, under the scanning electron microscope (Ishii and Miyazaki, 1970; Miyazaki *et al.*, 1973, 1975; Voelker and Arzube, 1979; Tongu *et al.*, 1985). Eggs of Venezuelan *Paragonimus* presented almost the same morphological characteristics as those of other Latin American *Paragonimus*.

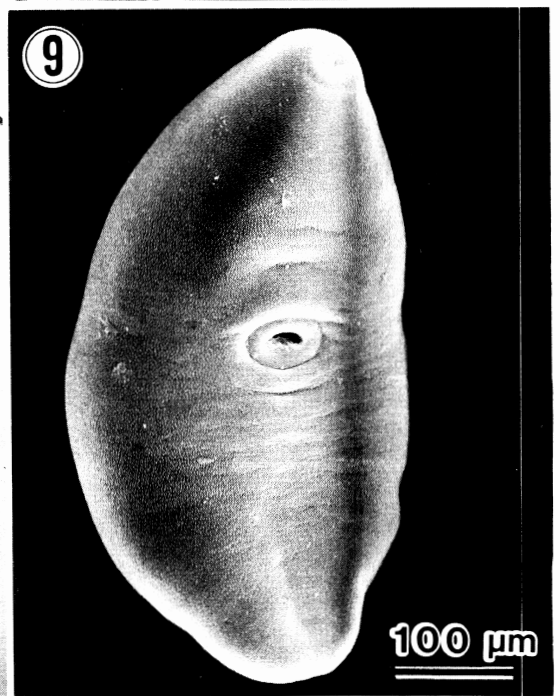
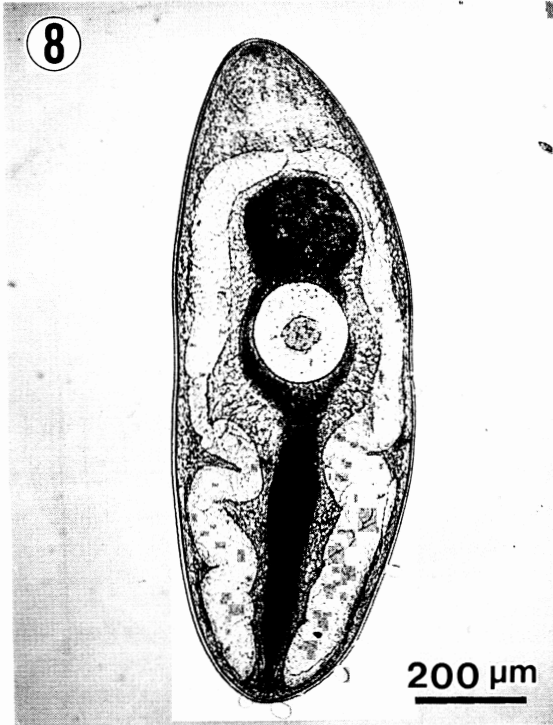
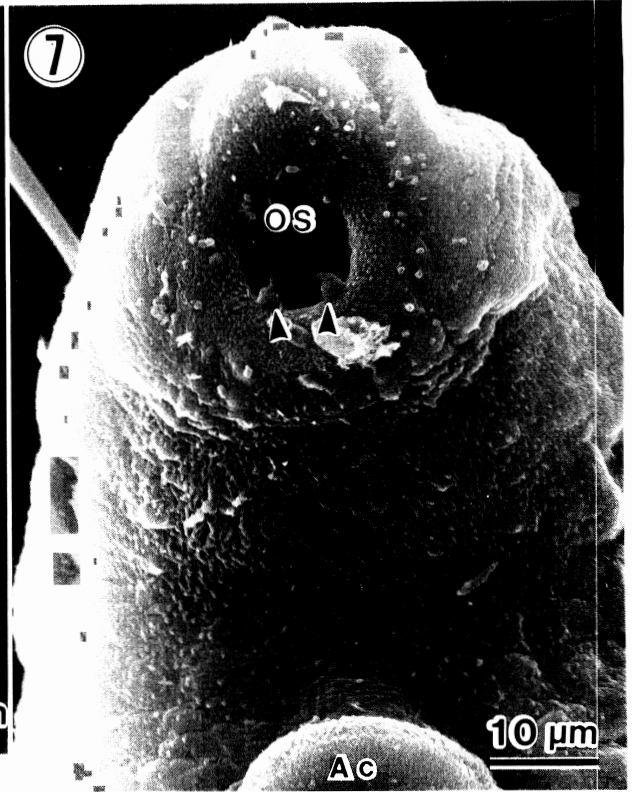
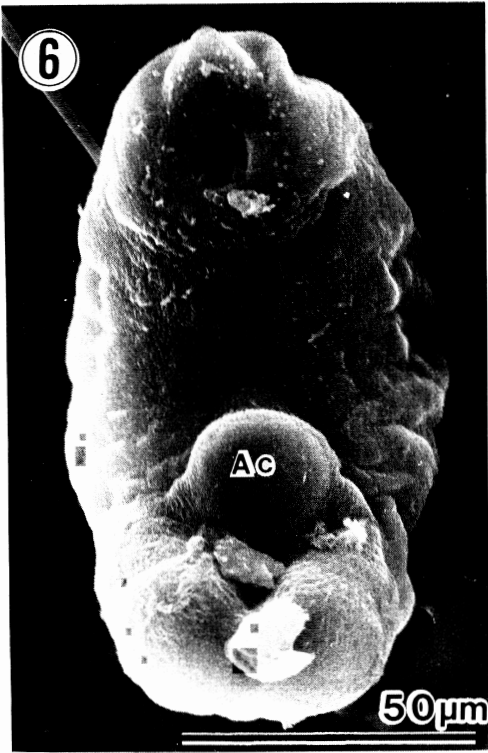
Ito *et al.* (1985), studying the morphological features of cercaria of *Paragonimus mexicanus* from Colima, Mexico, noted that this cercaria was characterized by a pseudo-sucker on the ventral posterior half of the body. Although we were unable to find the pseudo-sucker on Venezuelan cercariae, these cercariae had 7 pairs of penetration gland cells and a club-shaped excretory bladder. This characteristic constitutes a common feature of *Paragonimus* cercariae. In

Figs. 1, 2 Eggs laid in cystic cavities of the lung of a wild opossum. (same magnification)

Fig. 3 Scanning electron micrograph of a formalin fixed egg.

Fig. 4 Living redia under coverslip pressure.

Fig. 5 Cercaria fixed in 10% hot formalin.



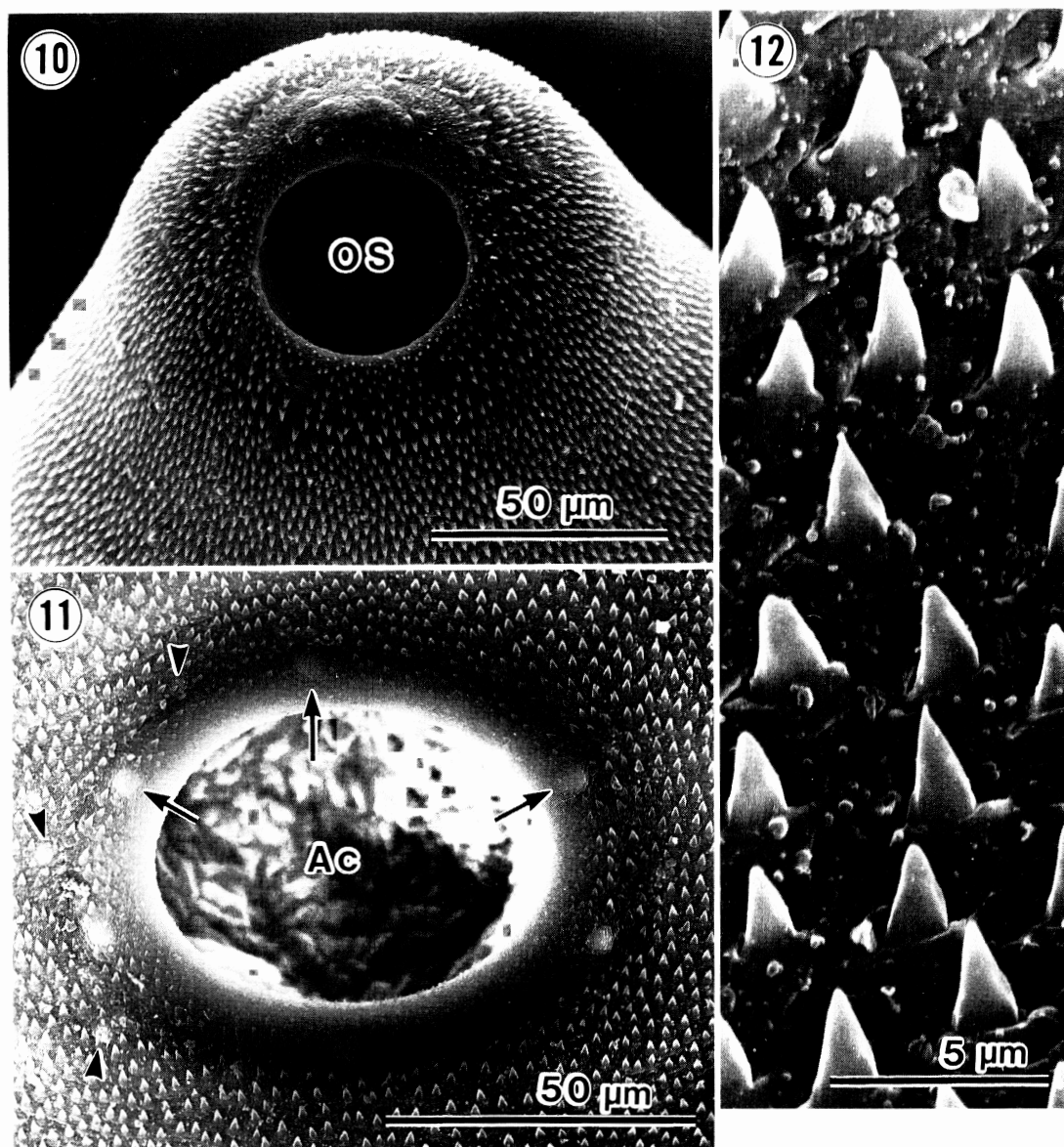


Fig. 10 Oral sucker of metacercaria under a scanning electron microscope.

Fig. 11 Scanning electron micrograph of a ventral sucker of metacercaria. Six inner papillae (arrows) are on the sucker lip and outer papillae (arrow heads) around the sucker.

Fig. 12 Scanning electron micrograph of a metacercaria showing single-pointed spines on the ventral surface just behind the acetabulum.

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- Fig. 6 Ventral view of a cercaria under a scanning electron microscopy.  
 Fig. 7 Higher magnification of Fig. 6. Arrow heads show sensory papillae.  
 Fig. 8 Living metacercaria under coverslip pressure.  
 Fig. 9 Scanning electron micrograph of a metacercaria, ventral view.

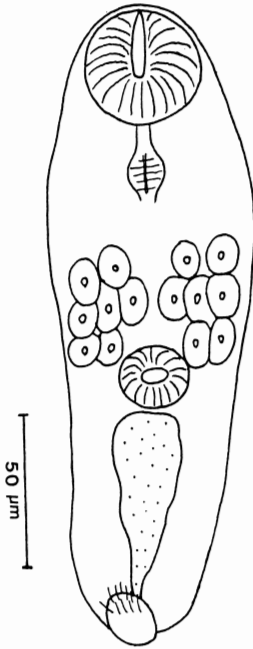


Fig. 13 Schematic drawing of a cercaria of *Paragonimus* sp.

the present species the orifice of the oral sucker bore two papillae. The similar ones were noticed in *P. westermani* as an “another type papillae with some tubercles at the tip” by Higo *et al.* (1980) under a scanning electron microscope. Furthermore the present cercariae were characterized by long spines as in the case of *P. westermani* (Higo *et al.*, 1980). The flame cell formula, however, was unknown. For a better classification of Latin American *Paragonimus*, it is necessary to study the cercariae of the various species reported previously.

The light microscopy of *Paragonimus* metacercariae from Latin America has been carried out (Voelker and Arzube, 1979; Little, 1968; Miyazaki, 1969, 1971, 1974, 1975b; Lamothe-Argumedo, 1979; Tongu, 1987). At this occasion, five species of *Paragonimus* are of common knowledge. Among them, *P. amazonicus* and *P. inca* metacercariae have not been reported, and their flame cell formula was confirmed only in *P. mexicanus* (Ito *et al.*, 1984) of 60 cells and in *P. caliensis* (Little, 1968) of 96 cells. The flame

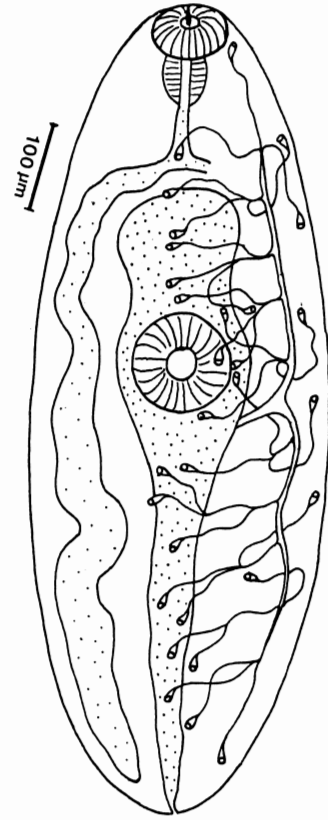


Fig. 14 Schematic drawing of a metacercaria of *Paragonimus* sp.

cell formula consisting of 60 cells in the present metacercariae from Venezuela coincided with that of *P. mexicanus*.

Miyazaki (1975b) classified Latin American *Paragonimus* metacercariae into A, B, and C forms. He identified the A-form as *P. peruvianus* (at the present time considered as synonymous of *P. mexicanus*) and the B-form as *P. caliensis*. He suggested that “these three forms are easily differentiated from each other by their characteristic features”. According to his classification, the metacercaria obtained in Venezuela belonged to *P. mexicanus* group based on the shape of the excretory bladder.

Aji *et al.* (1983) and Tongu *et al.* (1985, 1987) who studied morphological features of tegumental surface of *P. mexicanus*, reported that the number of outer papillae around the acetabulum was different between metacercariae from Peru,

Mexico, and Costa Rica. Ten to twenty outer papillae and the ventral body papillae of Venezuelan metacercariae were fewer in number and more indistinct in shape than those of Peruvian, Mexican, and Costa Rican metacercariae.

Alarcón de Noya *et al.* (1985b) suggested that the *Paragonimus* found in a Venezuelan patient might represent a new species or, less probably, *P. caliensis* or *P. mexicanus* based mainly on the presence of paired pointed spines. A lack of pseudo-sucker in the cercaria was a unique difference between the present *Paragonimus* and *P. mexicanus*. In the metacercariae, the flame cell number of the present species differed from that of *P. caliensis*. The present *Paragonimus* seems a new species.

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