

Morphological features of *Paragonimus metacercariae* from Costa Rica

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Abstract

Paragonimus metacercariae from fresh water crab (*Pseudothelphusa tristani*) in Costa Rica were examined morphologically with a light and an electron microscope.

1) Metacercariae contained red granules in their body cavity. The body size was similar to *P. mexicanus*. However, the diameter of ventral sucker was larger than that of the metacercariae from Mexico and Peru.

2) The collecting tube of the excretory system gave off branches at ten points. These characteristic branches were in agreement with the flame-cell formula of *Paragonimus mexicanus*.

3) The papillae of metacercariae were situated especially on both suckers. Two circles of papillae consisting of inner and outer papillae were arranged characteristically around the ventral sucker. The number of inner papillae was consistently 6 among individual metacercariae. The number of outer papillae varied 28 to 38 (mean 32).

4) From our present study, the metacercariae from Costa Rica belonged to *Paragonimus mexicanus*.

Key words: *Paragonimus mexicanus*, metacercaria, Ultrastructure, Costa Rica

Introduction

Paragonimus in Central America was first mentioned by Caballero (1956) and Caballero and Montero (1961) as *P. rudis* from a grey fox and a four-eyed opossum in Alajuela Province, Costa Rica. The metacercariae in this country were reported by Sogandares-Bernal and Smalley (1965) from the fresh water crab, *Pseudothelphusa tristani*. They only referred

briefly to the genus name and morphology. Sogandares-Bernal and Smalley (1967), and Brenes et al. (1968) followed these findings. In 1974, The metacercariae in Costa Rica were identified as *P. peruvianus* by Miyazaki. But Brenes et al. (1980) claimed that *P. peruvianus* was synonymous with *P. mexicanus* and so the Costa Rican lung fluke should have the latter name. Miyazaki (1979) revised his opinion, and suggested that not only *P. peruvianus* but *P. ecuadoriensis* by Voelker and Arzube (1979) are synonyms of *P. mexicanus*.

Ito et al. (1985) and Yokogawa (1985) observed the cercaria and metacercaria of Mexican *Paragonimus*, and confirmed that *P. mexicanus* and *P. peruvianus* were the same by their light microscopy. Tongu et al. (1985) made an electron microscopical observation on the same metacercaria, and they also got similarities between these two species. The present study describes the morphological features of Costa Rican *Paragonimus*.

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Materials and Methods

Metacercariae were collected from the intermediate host, *Pseudothelphusa dilatata*, at San Mateo and Tabarcia Province in Costa Rica from July to August in 1985. For the observation of living specimens, the worms were immediately washed with 1.2% saline solution and enclosed in 0.4% saline solution with a cover glass sealed with vaseline on its margin. The measurement of metacercariae was taken with coverglass after fixation with 10% hot formalin and staining with carmine.

For scanning electron microscopy, metacercariae were well washed in 1.2% saline solution, then fixed in 10% hot formaline for 5 min. These were immediately transferred into 2.5% glutaraldehyde at pH 7.4 for about 1 month either in ice box or in ambient temperature. The specimens rinsed with the buffer solution were postfixed in 1% osmium tetroxide solution at pH 7.4 for 2 h in 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 scanning electron microscope JEOL S25-II.

Results

1) Light microscopic observations

The metacercaria (Fig. 2) was characterized by its naked body without cyst wall. Therefore, they crept vigorously and freely with a strong extension or contraction of their body in the body cavity of the crab host. Red granules were scattered in the body cavity. However, one out of 17 specimens from Quebrada Zuniga in Tabarcia had yellow granules instead of red granules. The worm measured 1170 long by 600 μm wide (average from 2 specimens), the oral sucker 160 by 120 μm , and the ventral sucker 270 by 220 μm . A round oral sucker (Figs. 1, 4; OS) was located at the anterior end of the body. The mouth opening was followed by a pear-shaped pharynx and a short esophagus

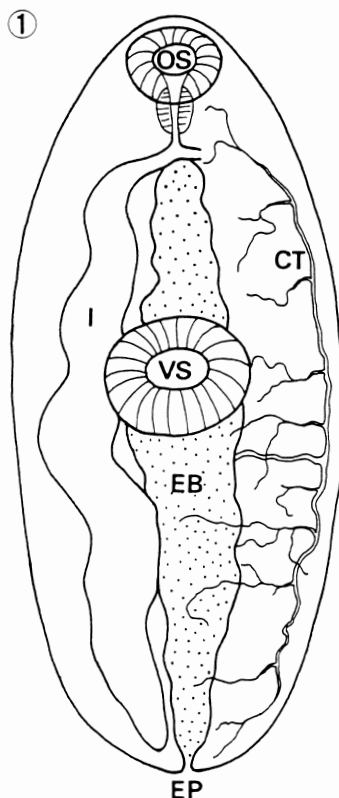
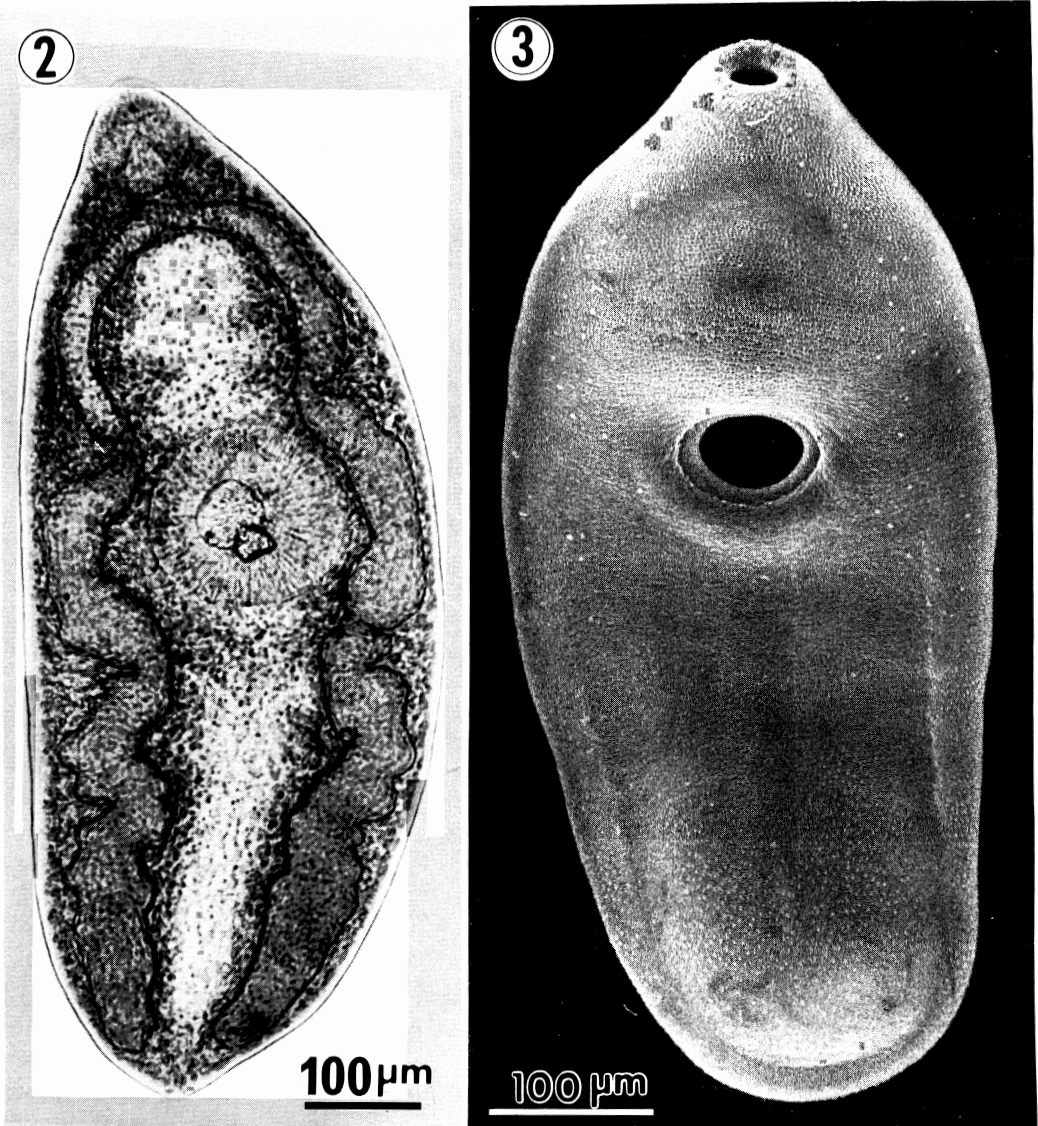


Fig. 1 Schematic drawing of a metacercaria.

I: intestine, EB: excretory bladder, EP: excretory pore, OS: oral sucker, VS: ventral sucker, CT: collecting tube

(Figs. 1, 2). The esophagus was divided into two branches of the thick intestine (Figs. 1, 2; I). These intestines (Figs. 1, 2), after several undulations, almost reached to the posterior end of the body. A well-developed ventral sucker (Figs. 1, 2, 3, 7; VS) was situated a little anteriorly from the middle part of the ventral surface, and was much bigger than the oral sucker (Figs. 1, 3, 4; OS).

The excretory bladder (Fig. 1; EB) was black in color because of excretory granules, and was extended from the posterior end to just behind the bifurcation of the intestine. From each lateral side of the middle part of the bladder, a pair of main collecting tubes (Fig. 1, CT) arose laterally. This main tube was divided into an anterior and a posterior collecting tube.

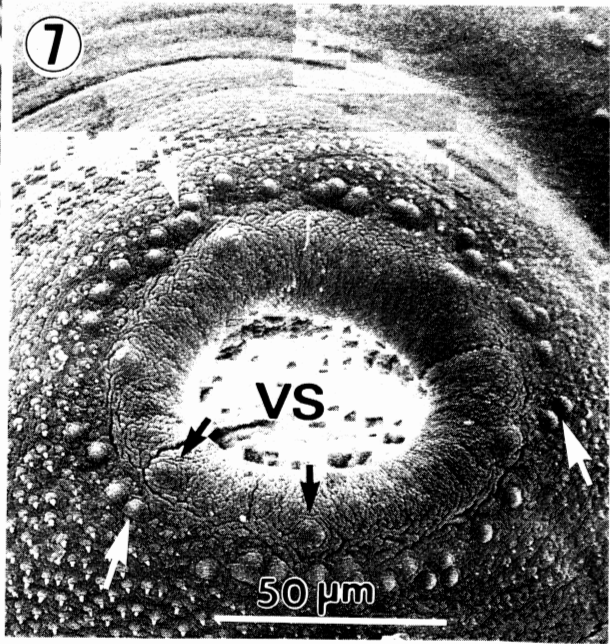
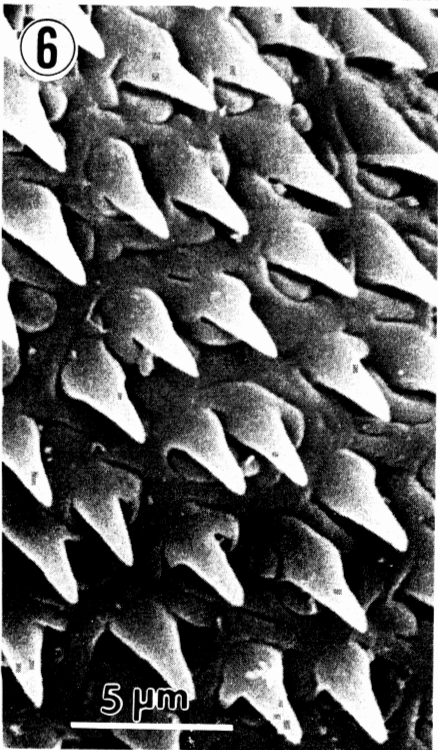


- Fig. 2 Metacercaria fixed with 10% hot formalin and stained with carmine
 Fig. 3 Ventral view of a metacercaria with a scanning electron microscope.
 Fig. 4 The lip of oral sucker (OS) is surrounded by several papillae.
 Fig. 5 Posterior end of the body. Spines are not seen around the excretory pore (EP).
 Fig. 6 Single pointed spines on the dorsal surface between oral and ventral sucker.
 Fig. 7 Ventral sucker (VS) has 6 inner papillae (black arrows) and outer papillae (white arrows)

The anterior one ran forward receiving branches at five points. The posterior one branched off at five points at intervals as in the case of the anterior tube. The capillary tube and the flame cell were not identified completely. Therefore,

the flame cell formula was unknown in the present study. These characteristics are shown in a schematic drawing of (Fig. 1, CT).

- 2) Scanning electron microscopic observations



The oral sucker had an orifice of approximately $23\ \mu\text{m}$ in diameter and was surrounded by numerous papillae on the lip even in the inner surface of the sucker (Fig. 4, OS). These papillae were prominent and bigger than others on the body surface. Some of papillae grouped especially on the dorsal lip of the oral sucker (Fig. 4).

The orifice of the ventral sucker (Fig. 7, VS), approximately $56\ \mu\text{m}$ in diameter, was larger than that of the oral sucker. Two circles of papillae consisting of inner (Fig. 7, black arrows) and outer papillae (Fig. 7, white arrows) were situated characteristically around the ventral sucker. The inner papillae were located at the margin of the sucker lip. These numbers were consistently 6 among all individuals studied. The outer papillae (Fig. 7, white arrows) were distributed around the sucker, and were smaller than inner papillae. Their number varied between 28 to 38 (average 32, from 5 metacercariae). The papillae could be seen all over the surface of the entire body, especially on the ventrolateral surface between the oral and ventral sucker.

The single pointed spines (Fig. 6) of $8\ \mu\text{m}$ in length covered the entire surface of the metacercariae except around the excretory pore (Fig. 5) and the inner surface of the sucker. Most of the spine base were embedded into the tegument.

Discussion

Costa Rican *Paragonimus* has been investigated on cercariae (Brenes et al., 1980), metacercariae (Sogandares-Bernal and Smalley, 1965, 1967; Miyazaki, 1974; Brenes et al., 1968, 1980), and adults (Caballero, 1956; Caballero and Montero, 1961; Morera, 1968; Brenes et al., 1968, 1980). Among these, the morphological features of metacercariae were observed in detail by Miyazaki (1974) (referred to as *P. peruvianus*) and Brenes et al. (1980).

The metacercariae obtained in Costa Rica in the present study closely resembled those by Miyazaki (1974) and Brenes (1980) in the ex-

ternal morphology and in the measurements of oral sucker, ventral sucker, and body length by body width. In detail, the diameter of ventral sucker of $270 \times 220\ \mu\text{m}$ was shorter than that by Miyazaki (1974) and Brenes (1980). Our data are rather similar to *P. peruvianus* (synonym of *P. mexicanus*) from Peru by Miyazaki et al. (1971) and Ito et al. (1985).

Ito et al. (1985) and Yokogawa (1985) described the morphology of cercaria and metacercaria of *P. mexicanus* from Mexico. Yokogawa (1985) noted the flame cell formula of metacercariae as $2((3 \times 5) + (3 \times 5)) = 60$. In the present study, we were unable to determine the flame cell number. However, the characteristic branches of collecting tubes were in agreement with the formula of *P. mexicanus* shown by Yokogawa (1985).

Electron microscopical examination of Central American *Paragonimus* was done only by Ishii and Miyazaki (1970) on the egg of *P. mexicanus* and *P. peruvianus*, Aji et al. (1984) on the metacercaria of *P. peruvianus*, and Tongu et al. (1985) on *P. mexicanus*. The surface ultrastructure on the metacercariae from Costa Rica had no fundamental difference compared with the metacercariae from Peru (Aji et al., 1984) and from Mexico (Tongu et al., 1985). Six inner papillae are constantly observed on the ventral sucker of metacercariae of *Paragonimus* (Higo and Ishii, 1984; Aji et al., 1984; Tongu et al., 1985). This fixed number was also seen in Costa Rican metacercariae. Therefore, the 6 inner papillae seems to be a characteristic feature of *Paragonimus* in general. The number of outer papillae of metacercariae was the only ultrastructural difference between *P. peruvianus* and *P. mexicanus*. This, however, may not be of taxonomic value (Tongu et al., 1985). In Costa Rican metacercariae, the average number of outer papillae of 32 (28–38) differed from that of Peruvian metacercariae. These numbers were rather similar to those of Mexican metacercaria. This difference is, however, considered to be a small variation not beyond the limit of species criterion.

From our light and electron microscopic

observations, we can support that Costa Rican *Paragonimus* belongs to *P. mexicanus* as stated by Miyazaki (1979) and Brenes et al. (1980).

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