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Morphology of *Paragonimus* Adult Worms from Venezuela

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Abstract

Adult worms of *Paragonimus* were obtained from wild opossums, *Didelphis marsupialis,* naturally infected in the northeastern area of Venezuela. They were examined morphologically by light and scanning electron microscopy. The oral sucker was located at the extreme anterior end of the body, and was slightly larger than the ventral sucker. The whole body was irregularly covered by single-type spines, most of which were serrated at the tip and split longitudinally. The ovary had a central mass with 6 to 9 broad lobes. The testes were located in the posterior portion of the body and each had 3 to 6 non-bifurcated lobes. Adult *Paragonimus* from Venezuela was similar to that of *P. mexicanus* or *P. caliensis.* The absence of information about redia and cercaria in other species of *Paragonimus* from Latin America did not allow definition of the correct species name of Venezuelan *Paragonimus.*

Key words: *Paragonimus,* adult worm, morphology, Venezuela

Introduction

Three species of *Paragonimus* are well recognized as distinct species in America. They are *Paragonimus kellicotti, P. caliensis,* and *P. mexicanus.* However, other species have been also described as *P. rudis* by Diesing (1850), *P. peruvianus* by Miyazaki *et al.* (1969), *P. amazonicus* by Miyazaki *et al.* (1973), *P. inca* by Miyazaki *et al.* (1975), and *P. ecuadoriensis* by Voelker and Arzube (1979). These have not been widely accepted, since most of the descriptions did not include all the evolutional stages of the parasite. Among these species names, *P. peruvianus* and *P. ecuadoriensis* are synonymous (Miyazaki, 1979; Brenes *et al.*, 1980; Tongu *et al.*, 1985). After the finding of the first indigenous case of human paragonimiasis in Venezuela (Alarcón de Noya *et al.*, 1985a), studies on the morphological features and the life cycle were carried out by Alarcón de Noya *et al.* (1985b). However, some aspects have been studied incompletely. In order to define the species, further characterization of *Paragonimus* in Venezuela is needed. In this paper we describe the morphological characteristics of *Paragonimus* adult worms in Venezuela by light and electron microscopy. Additionally, we compare the morphological features of Venezuelan *Paragonimus* with the most closely resembling species, *P. mexicanus* by Miyazaki and Ishii (1968), and *P. caliensis* by Little (1968).

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Materials and Methods

Wild opossums, *Didelphis marspialis,* were captured at the Cajigal District in Sucre State

(northeastern area of Venezuela) using Sherman live traps. Eleven adult worms of *Paragonimus* were obtained from the lung cyst of naturally infected opossums. The worms were immediately washed in isotonic saline solution at room temperature. For light microscopy, they were fixed in 10% formalin under progressive pressure between two microscope slides during 7 days. The fixed specimens were stained with Semichon Aceto-Carmine and mounted in Canada balsam. For scanning electron microscopy, the worms were incubated in 1% pancreatin solution with 0.1% Na_2HCO_3 for 1 hour at room temperature. Then, they were newly washed 5 times with isotonic saline solution and fixed with 10% hot formalin for 10 minutes. This fixative was changed into cold Karnovsky's fixative, kept for at least 24 hours in an ice box, and postfixed with

Fig. 1 *Paragonimus* sp. from Venezuela.
a. Mature adult worm, ventral view. Scale: 1000 μm.
b. Mature adult worm, ventral view. Scale: 1000 μm.
c. Mature adult worm, dorsal view. Scale: 1000 μm.
d. Immature adult worm, ventral view. Scale: 1000 μm.

1% osmium tetroxide solution buffered at pH7.4 for 1 hour. The worms were dehydrated through a graded series of ethanol solution and placed in isoamyl-acetate. They were critical point-dried and coated with carbon and gold. The specimens were observed with scanning electron microscopes, Hitachi S-450 and JEOL S25-2.

Fig. 2 Oral sucker of adult worms.
a. Subterminal oral sucker. Scale: 500 μm.
b. Typical terminal oral sucker. Scale: 500 μm.

Results

Light microscopy

Adult worms were lanceolate when they were flattened. Six worms had a mean size of 10.7mm long (ranging from 8.5 to 15.0mm) by 4.4mm wide (ranging from 3.2 to 7.0mm) (Fig. 1). The oral sucker was situated at the anterior end of the body and was slightly larger than the ventral sucker measuring 595 μm in length (ranging from 450 to 757 μm) and 802 μm in width (ranging from 573 to 961 μm) (Figs. 1, 2). The ventral sucker was most of oval and measured 740 μm in length (ranging from 409 to 859 μm) and 771 μm in width (ranging from 389 to 1104 μm). The average ratio of oral sucker/ventral sucker was estimated at 1.04. The digestive tract consisted of a spherical pharynx (Fig. 2), a short esophagus, and two broad ceca that extended in a zigzag manner to the posterior end of the body (Fig. 1).

The ovary lay to the right of the midline and to the posterolateral to the ventral sucker in 5 out of 6 worms examined (Figs. 1 and 3). This organ was divided into 6 to 9 short broad lobes without short branches at tip (Figs. 3 and 4). The size of the ovary was 997 μm in length (ranging from 675 to 1288 μm) and 653 μm in width (ranging from 348 to 1023 μm). The uterus formed a series of coils behind the ventral sucker on the side opposite the ovary (Figs. 1 and 3).

Testes were located in the posterior portion of the body and each had 3 to 6 non-bifurcated lobes (Figs. 1, 3, and 5). Only one testis showed a long efferent duct (Fig. 5a). The right testis had 3 to 5 lobes (average size, 1636×818 μm) (Figs. 1 and 5). A long excretory bladder extended to the bifurcation of the ceca (Fig. 1a).

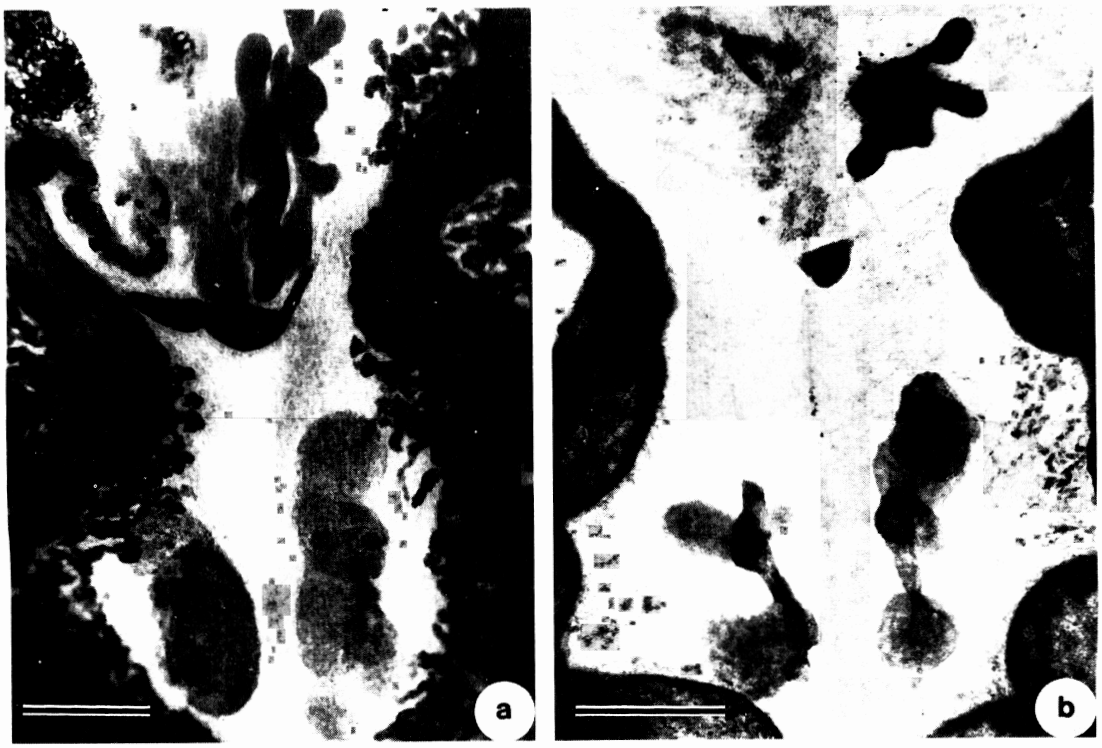

Fig. 3 Reproductive system.
a. Mature worm with eggs in uterus. Vitelline duct, ovary and testis are also evident. Dorsal view. Scale: 500μm.
b. Immature worm. Uterus is not well developed as well as vitelline gland, ovary, and testis. Scale: 500 μm.

Scanning electron microscopy

Examination of 5 adult worms confirmed some characteristics observed by light microscopy. The oral sucker was clearly terminal in 4 out of 5 samples and larger than the ventral sucker (Fig. 6). It was not covered by spines, but, indistinct papillae were sometimes seen, although rarely. The ventral sucker was wide and oval. Its

Fig. 4 Ovaries, ventral view.
a. Ovary with a central mass and 7 short and broad lobes. Scale: 500 μm.
b. Ovary with a central mass and 9 short and broad lobes. The vitelline duct is also noticeable. Scale: 500 μm.

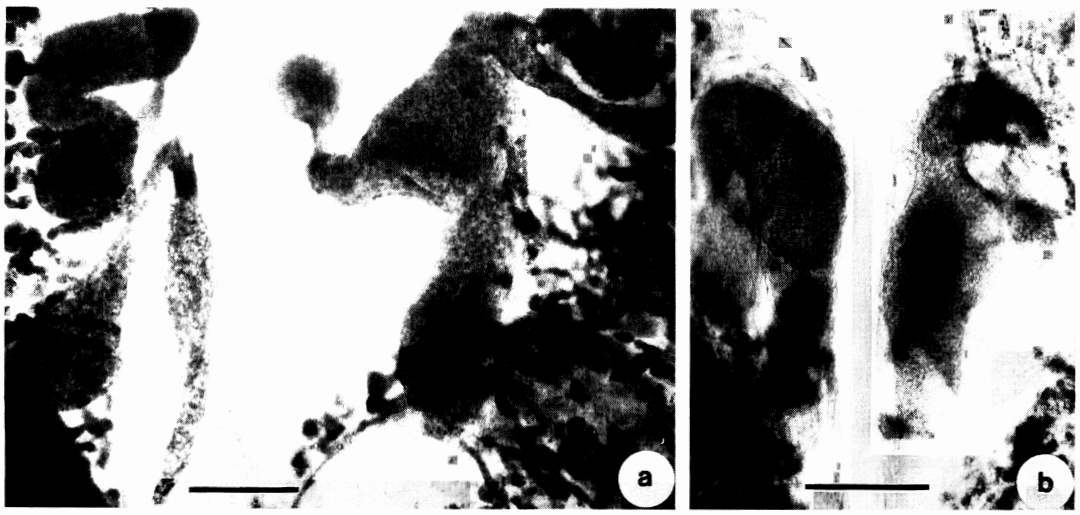

Fig. 5 Testes, ventral view.
a. Testis poorly branched and with a long efferent duct. Scale: 500 μm.
b. Testes with typical broad and short lobes. Scale: 500 μm.

surface was smooth without spines.

The body was irregularly covered with single-type spines. These were predominant in the anterior portion of the body around the oral sucker as a wide collar (Fig. 6b). The spines showed two different shapes, pointed and forked (Figs. 7b–d). The forked spines were predominant and many split longitudinally into 4 to 6 pieces with a common root (Fig. 7b). The excretory pore was not surrounded by these spines and papillae (Fig. 7a).

Discussion

In the pathological and parasitological study of the first human case of paragonimiasis in Venezuela, it was suggested that Venezuelan *Paragonimus* may represent a new species or, less probably, *P. caliensis* or *P. mexicanus* based on a very particular pattern of spines found in a fragment of the adult worm (Alarcón de Noya *et al.*, 1985b). Subsequent studies on adult worms and metacercariae recovered from wild animals in the endemic area of paragonimiasis did not show the same pattern of spines (Alarcón de Noya *et al.*, 1985b). These observations pointed out the need for further studies of all parasitic stages and of the complete life cycle in the laboratory as proposed by Brenes *et al.* (1980). The classification of Venezuelan *Paragonimus* has not thus far been accomplished.

In the morphological classification of adult *Paragonimus,* characteristic features of ovary and testis are of the most important criteria. Although Venezuelan *Paragonimus* and *P. caliensis* had ovaries with short broad lobes (Little, 1968), *P. mexicanus* had dendritic or coral shaped ovaries (Miyazaki and Ishii, 1968). The shape of testis of Venezuelan *Paragonimus* was elongated, diverticular, and occasionally antler-like lobes, closely similar to *P. caliensis* and in contrast to *P. mexicanus.* The terminal position of the oral sucker was the feature of Venezuelan *Paragonimus* that differed from the other two closely related species. However, these characteristics were not constant in each individual specimen. The shape and single-spaced arrangement of tegumental spines of Venezuelan *Paragonimus* were similar to those found in *P. mexicanus* and *P. caliensis.*

The morphological features of larval stages (rediae, cercariae and metacercariae) and eggs of Venezuelan *Paragonimus* were reported by Tongu *et al.* (1990). In the present study we describe the morphological characteristics of the adult worms of Venezuelan *Paragonimus* and

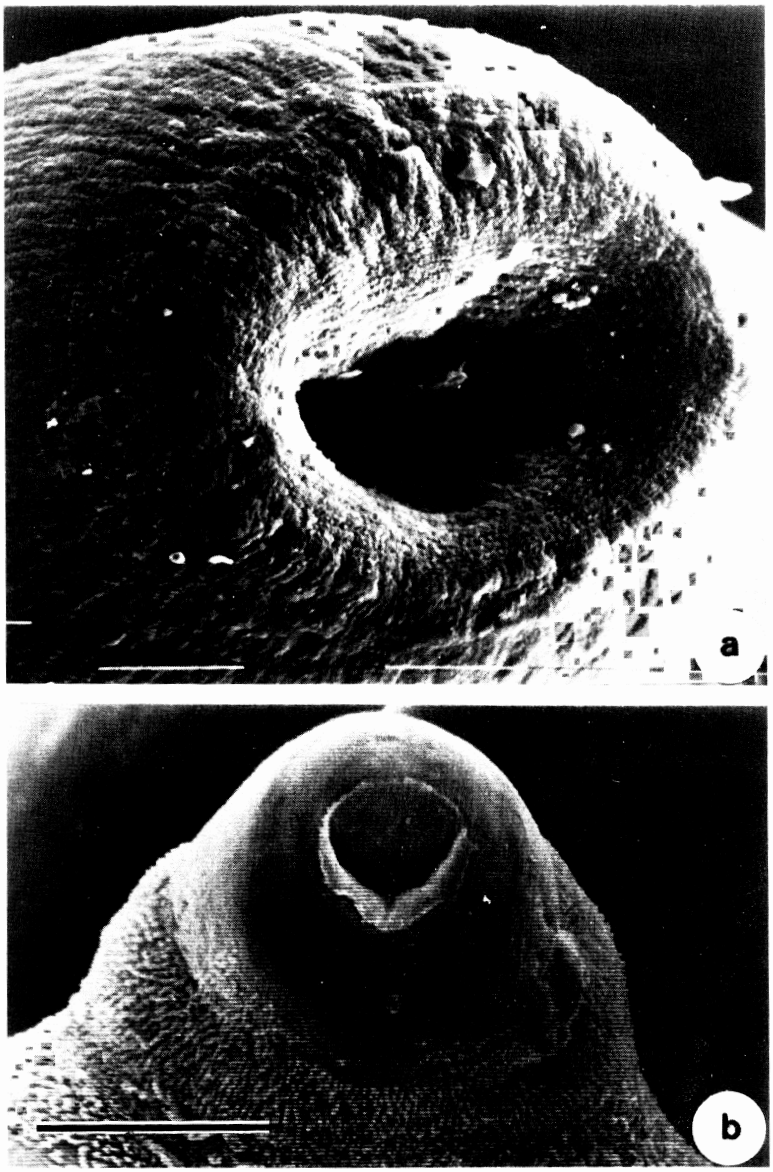

Fig. 6 Scanning electron micrographs of oral sucker.
a. Typical terminal oral sucker. Scale: 125 μm.
b. Subterminal oral sucker. Collar of spines surround it. Scale: 500 μm.

summarize the main differences of this parasite with *P. caliensis* and *P. mexicanus,* which are the most closely resembling species.

Eggs of the Venezuelan *Paragonimus* shared almost the same features as the other Latin-American *Paragonimus* (Tongu *et al.*, 1990). In the metacercarial stage, Venezuelan *Paragonimus* and *P. mexicanus* were very similar in body size and pattern of flame cells (Tongu *et al.*, 1990). But Venezuelan *Paragonimus* differed from *P. caliensis* in having 96 flame cells and encystment in the metacercarial stage. In the cercarial stage, however, the electron microscopy of Venezuelan *Paragonimus* did not reveal the presence of a

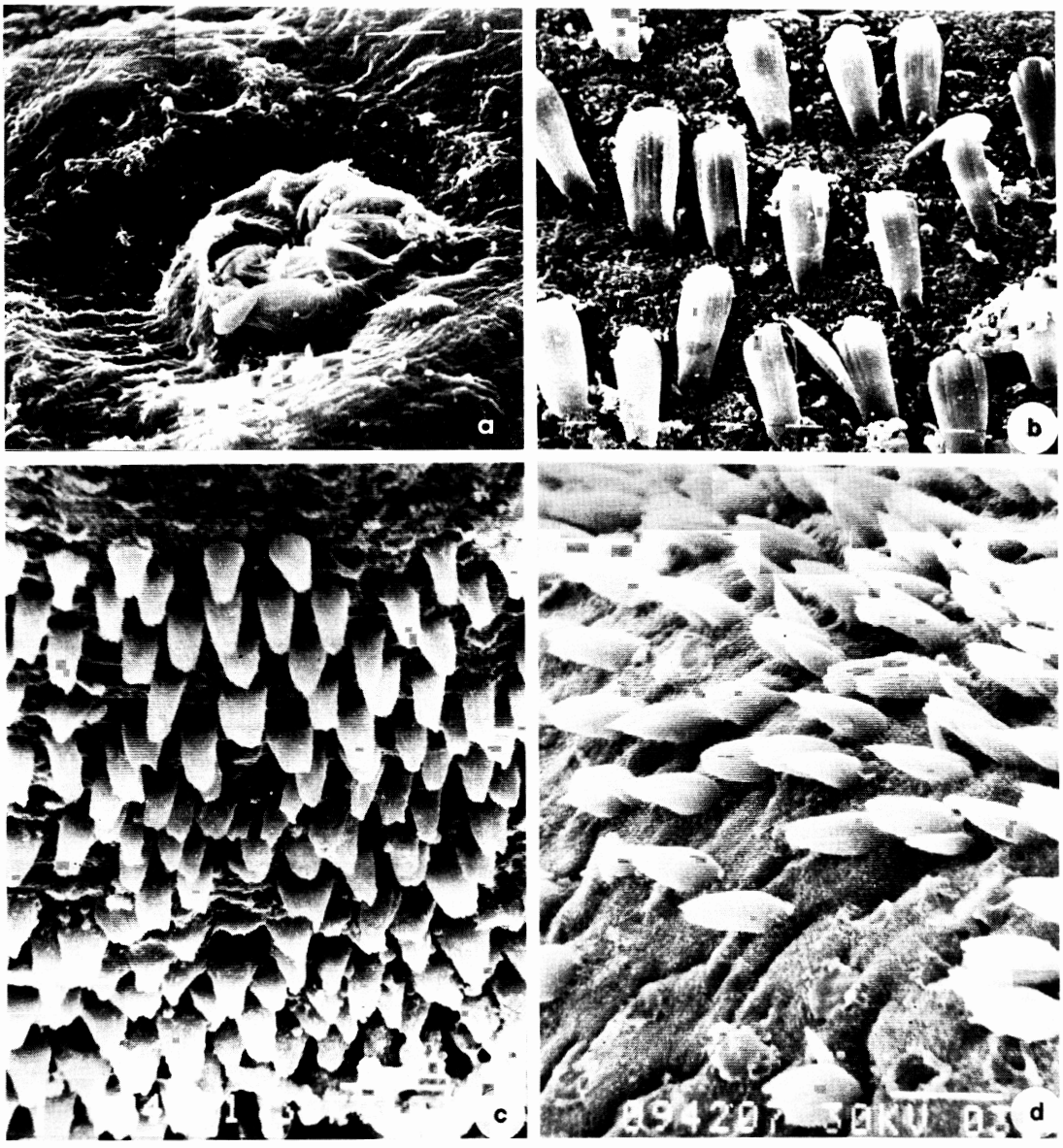

Fig. 7 Spines with scanning electron microscope.
a. Excretory pore. No prominent papillae or spines surround it. Scale: 5 μm.
b. Detail of forked spines. They split longitudinally into 4 to 6 pieces. Scale: 10 μm.
c and d. Detail of pointed and forked spines. Scale: 30 μm.

pseudo-sucker as described in *P. mexicanus* by Ito *et al.* (1985). Description of this stage for *P. caliensis* has not been reported in the literature.

Noticeable differences exist between the Venezuelan *Paragonimus* and the other two species. At the present time, we can only conclude that Venezuelan *Paragonimus* is very similar to *P. caliensis* with the exception of the metacercaria having 96 flame cells. Lack of information on the cercaria of *P. caliensis* limits the comparison. Further experimental studies, in particular, on its life cycle, will allow a determination of the proper

taxonomic position, if Venezuelan *Paragonimus* belongs to a new species or represents intraspecies variation.

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