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Surface Ultrastructure of *Paragonimus mexicanus* Miyazaki et Ishii, 1968

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Introduction

In Central and South America the causative agent of human paragonimiasis has long been regarded as *Paragonimus westermani* which had been imported by immigrants from the Orient.

Since 1968, however, many new species of *Paragonimus* have been reported in Mexico, Colombia, Peru, and Ecuador as follows; *Paragonimus mexicanus* (Miyazaki and Ishii, 1968) in Mexico, *P. caliensis* (Little, 1968) in Colombia, *P. peruvianus* (Miyazaki, *et al.*, 1969), *P. amazonicus* (Miyazaki *et al*, 1973) and *P. inca* (Miyazaki *et al.*, 1975) in Peru, and *P. ecuadoriensis* (Voelker and Arzube, 1979) in Ecuador.

Recently, Brenes *et al.* (1980) and Miyazaki (1979) reported that *P. peruvianus* and probably *P. ecuadoriensis* would be synonymous of *P. mexicanus*.

These opinions, however, were mainly based on the morphological features of the adult worms and/or the metacercariae by light microscope.

In the present study the ultrastructural features of the eggs, metacercariae, and adult worms of *P. mexicanus* are compared with those of *P. peruvianus* described by Yokogawa (1983) and Aji *et al.* (1984).

Materials and Methods

Adult worms of *P. mexicanus* were obtained from a naturally infected Mexcan opossum, *Didelphis virginiana californica*, in Colima, Mexico and from experimental cats three months after infection. Nonencysted metacercariae were collected from the second intermediate host, *Pseudothelphusa dilatata*, in Colima, Mexico. Eggs were removed from the uterus of the adult worms.

The specimens were fixed with 2.5 % phosphate buffered glutaraldehyde (pH 7.4) for 12 hr, and postfixed with buffered 1 % osmium tetroxide solution for 1 hr. In some specimens of the metacercariae and adult worms prepared for scanning electron microscopy (SEM), the tegument was destroyed by an exposure to ultrasonic waves for 5 min before postfixation to obtain a full size image of the spines. For scaning electron microscopy, the specimens were dehydrated through a graded series of alcohol and placed in isoamyl acetate. They were critical-point dried, and carbon gold-coated specimens were observed with a scanning electron microscope JEOL S 25-II. For transmission electron microscopy (TEM), the specimens were dehydrated, then placed in n-butylglycidyl ether and finally embedded in epon. The sections were

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double-stained with uranyl acetate and lead hydroxide, and observed with a Hitachi HS-8 type electron microscope.

Results

Egg :

The size of the eggs of *P. mexicanus* was 63 to 73 μm×37 to 42 μm. Many shallow pits (Figs. 1, 2), 1 to 2 μm in diameter, were observed on the surface of the egg shell. The egg was provided with an operculum and an asteroid projection (Fig. 2, arrow) at the aboperculated end. The junction between the operculum and egg body was thick and raised.

Metacercariae :

The orifice of the oral sucker (Fig. 4) had a diameter of approximately 37 μm and was surrounded by numerous papillae. These papillae were especially prominent and grouped on the dorsal lip of the oral sucker (Fig. 4). The orifice of the ventral sucker (Fig. 5), approximately 50 μm in diameter, was larger than that of the oral sucker. Two circles of papillae consisting of inner (Fig. 5, black arrow) and outer papillae (Fig. 5, white arrow head) were situated around the ventral sucker. The number of inner papillae (referred to as 6 dome-shaped papillae by Aji *et al.*, 1984) was consistently 6 in each metacercaria and that of the outer papillae (referred to as the spherical shaped papillae by Aji *et al.*, 1984) was between 30 to 35.

The papillae could be seen all over the surface of the entire body even on the inner surface of the sucker (Fig. 5, white double arrow) and at the part around the excretory pore (Fig. 6, arrow). These, however, were especially prominent on the oral sucker, the ventral sucker, and on the ventrolateral surface between the oral and ventral suckers (Fig. 3).

Some of these papillae bore a cilium (Fig. 7, arrow head).

The single-pointed spines (Figs. 7, 8), 6 μm in length, were rarely bifurcate (Fig. 8, white arrow), and covered the entire surface of the metacercariae except around the excretory pore (Fig. 6, arrow head) and the lumen of the suckers. They showed a spoon-like shape(Fig.8), when the tegument was removed by ultrasonic waves. The base of the spines embedded in the tegument reached the basal membrane of the tegument (Fig. 9, S).

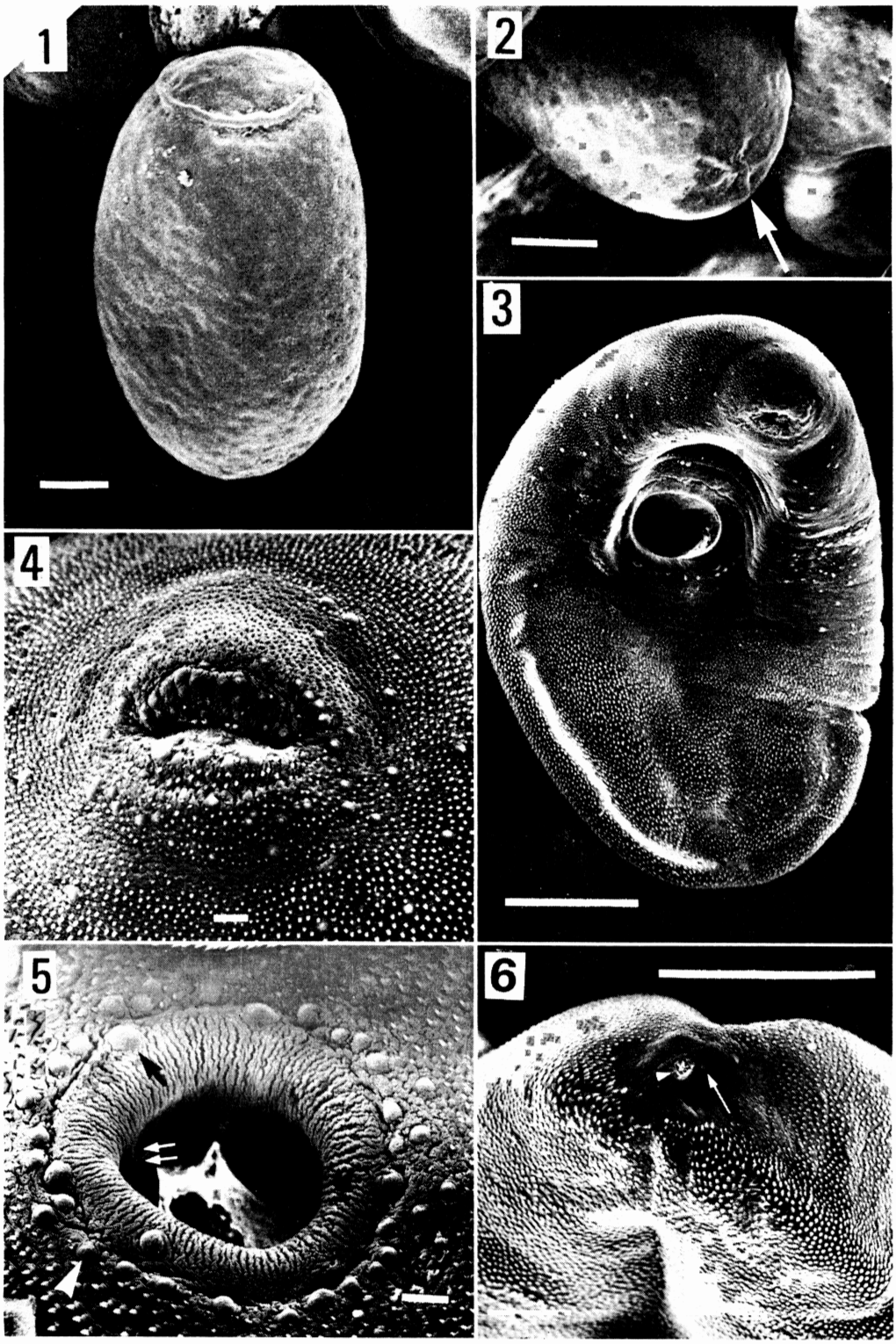
Adult worms :

The orifice of the ventral sucker, approximately 180 μm in diameter, was larger than that of the oral sucker which was approximately 150 μm (Fig. 10). The 6 regular papillae (inner papillae seen in the metacercarial stage) were still present on the ventral sucker of adult worm, but the outer papillae were no longer evident.

The spines were distributed on the dody surface of the adult worms. Most of them were split at their tips (Figs. 13, 14, 15). SEM micrographs after exposure to ultrasonic waves showed that the spines split at the middle part of the spine (Fig. 15). However, TEM micrographs showed that these split spines had a common root (Fig. 11, S). On the surface of the oral sucker, the single-pointed spines were arranged in groups (Fig. 12).

Discussion

The report by Mazzotti and Miyazaki (1965) is the first reference to an adult lung fluke in Mexico. They suggested that this fluke might be a new species and subsequently Miyazaki and Ishii (1968) described this fluke as *P. mexicanus* as a new species. Miyazaki *et al.* (1969) reported for the first time *P. peruvianus* from Peru, and they noted that *P. peruvianus* could be distinguished by the characteristic branching of the ovary and testes as well as by the character of the eggs. Human cases of *Paragonimiasis* in Peru were also recorded by Miyazaki *et al.* (1972). They suggested that the worms might belong to an unidentified species. In Panama Miyazaki (1972) reported that the metacercariae obtained from *Pseudothelphusa richmondi* agreed in all details with *P. peruvianus* metacercariae. However, Miyazaki (1979) suggested that *P. peruvianus* was the same as *P. mexicanus*. Ishii and Miyazaki (1970) sludied on the surface ultrastructure of the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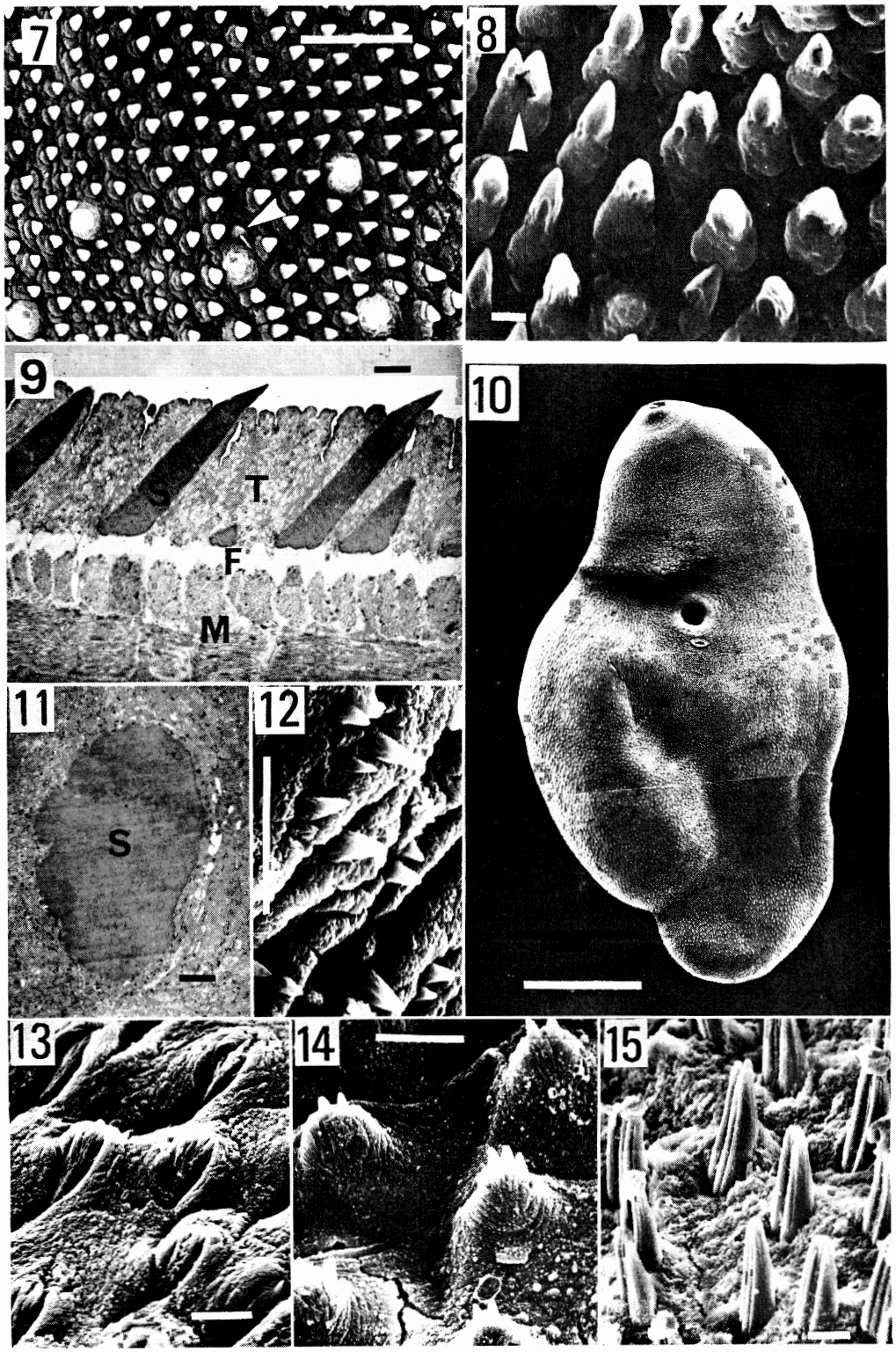
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egg-shells of *P. kellicotti*, *P. mexicanus*, and *P. peruvianus*. They showed that the surface of the egg shell of *P. mexicanus* slightly undulated, but not as remarkably as that of *P. peruvianus*. Yokogawa (1983) showed by SEM that the egg shell of *P. peruvianus* had many small pits.

We have observed numerous pits on the egg shell of *P. mexicanus*. These are similar to those of *P. peruvianus* in number and shape.

According to Miyazaki (1974), cuticular spines of lung flukes can be divided into two groups: single spines and grouped spines. *P. westermani*, *P. peruvianus*, and *P. mexicanus* belong to the former group. However, spines are inclined to split longitudinally in older flukes, and therefore, some spines which were originally single look like grouped spines. Yokogawa (1983) observed single-pointed and forked spines on adult *P. peruvianus* by electron microscopy.

Our present observations on *P. mexicanus* adults by electron microscopy demonstrated single-pointed and forked spines. Furthermore, most spines which seem to be single-pointed are split longitudinally except for the root. Metacercariae had single-pointed spines exclusively. These features were almost the same as those of *P. peruvianus*.

The papillae on the metacercariae were situated primarily on both of oral and ventral suckers. Higo and Ishii (1984) classified the metacercarial papillae of *P. westermani* and *P. miyazakii* into three types, and the number of the outer papillae of the ventral sucker were different between these two species: 7 to 11 in *P. westermani* and 14 to 21 in *P. miyazakii*. Aji *et al.* (1984) observed six inner papillae and 22 to 25 outer papillae arranged concentrically around the ventral sucker of the metacercariae of *P. peruvianus*. They suggested that these papillae were important to make a comparative study of the metacercariae of the lung flukes.

Concerning the inner papillae of the ventral sucker, Higo and Ishii (1983) reported that the cercariae of *P. ohirai*, *P. iloktsuenensis*, and *P. miyazakii* had 6 inner papillae on their ventral sucker, and these numbers were constant among three species. Furthermore, Bennet (1975) and Fujino *et al.* (1979) observed 6 inner papillae in the metacercariae of other trematodes, *Fasciola hepa-*

Fig. 1 Egg with many shallow pits on the shell surface. Bar=10 μm.
Fig. 2 Egg showing aboperculated end with an asteroid projection (white arrow). Bar=10 μm.
Fig. 3 Ventral surface of a metacercaria. Bar=100 μm.
Fig. 4 Oral sucker of metacercaria with many papillae visible around the sucker. Bar=10μm.
Fig. 5 Ventral sucker of metacercaria showing 6 inner papillae (black arrow), 30 to 35 outer papillae (White arrow head), and some papillae on the inner surface of the sucker (white double arrow). Bar=10 μm.
Fig. 6 Excretory pore of metacercaria. Spines are not seen around the pore but some papillae (white arrow)are present. Bar=100 μm.
Fig. 7 Ventrolateral surface between oral and ventral suckers of metacercaria. The arrow head shows a papilla wtih a cilium. Bar=10μm.
Fig. 8 Ventrolateral surface at the level of ventral sucker. Spines taken off the tegument show a spoon-like shape. The bifurcated spine (arrow head) is rarely seen. Bar=1 μm.
Fig. 9 Longitudinal section of the spines through TEM. Bar=1 μm.
T: tegument, S: spine, F: filamentous layer, M: Muscle
Fig. 10 Ventral surface of an adult worm. Bar=1.000 μm.
Fig. 11 Cross-section of a spine in the adult worm. Bar=1 μm. S: spine.
Fig. 12 Single pointed spines on the inner surface of adult oral sucker. Bar=10 μm.
Fig. 13 Branched spines on the ventral surface near the oral sucker of adult worm. Bar=10 μm.
Fig. 14 Branched spines on the adult ventral surface between oral and ventral sucker. Bar=10 μm.
Fig. 15 Branched spines near the ventral sucker of adult worm after removing the tegument with ultrasonic waves. Bar=10 μm.

tica and *Clonorchis sinensis*.

In our description on *P. mexicanus*, the metacercariae constantly have 6 inner papillae and 30 to 35 outer papillae. The fixed number of the inner papillae seems to be a characteristic feature of trematodes in general. The number of the outer papillae of metacercariae was the only ultrastructural difference between *P. peruvianus* and *P. mexicanus*. This difference, however, may not be of taxonomic value. From our ultrastructural observations on the eggs, metacercariae, and adults of *P. mexicanus* we also support that *P. peruvianus* is a synonym of *P. mexicanus*.

Summary

The surface ultrastructure of the eggs, metacercariae, and adult worms of *P. mexicanus* has been examined. The egg shell has many shallow pits which are 1 to 2 μm in diameter. The ventral sucker of the metacercariae is surrounded by two circles of papillae: 6 inner papillae on the margin of the sucker and 30 to 35 outer papillae around the sucker. The 6 inner papillae are a constant feature among all individuals studied. The number of outer papillae varied in a narrow range from one metacercaria to the next. The number of these was the only ultrastructural difference between *P. peruvianus* and *P. mexicanus*. Spines covered the entire surface of metaecercariae and adults. In the metacercariae, these were all single-pointed but most of the spines in adults were split longitudinally at their tip. On the basis of the present observations on *P. mexicanus*, it seems reasonable to conclude that *P. peruvianus* is identical with *P. mexicanus*.

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メキシコ肺吸虫 *Paragonimus mexicanus* の微細形態学的観察

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メキシコ肺吸虫の虫卵，メタセルカリア，成虫の体表構造を電子顕微鏡を用いて観察した．虫卵の表面には多数の浅い小孔 (1～2μm) がみられた．メタセルカリアの体表にはいくつかの乳頭突起があるが特に両吸盤間の腹部側面及び口吸盤，腹吸盤上またはその周囲に多い．なかでも腹吸盤上の6個の inner papillae は個体間で一定であるが，腹吸盤周囲の outer papillae は個体間で相違があるものの30～35個みられた．6個の inner papillae は成虫の腹吸盤上でもかすかに認められた．皮棘はメタセルカリアでは総て単棘でスプーン状を呈している．成虫になると大部分はフォーク状の分岐をしていた．又一見単棘状のものでも周囲の外皮を取除くと長軸にそって数本に分岐していることがわかった．これら微細形態学的特徴をペルー肺吸虫と比較するとメタセルカリアの outer papillae の数が唯一の相違点であったが，全体的にみてペルー肺吸虫はメキシコ肺吸虫のシノニムと考えられる．