

## Ultrastructure of Tegumental Surface of the Metacercaria of *Paragonimus peruvianus*

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(Received for publication; September 12, 1983)

**Key words:** *Paragonimus peruvianus*, ultrastructure, metacercaria, papillae

### Introduction

Paragonimiasis in Central and South America were believed to have been caused by *Paragonimus westermani* from Asia, but it was recently shown that local strain of lung flukes existed and that new species had evolved. Example of such new species include; *Paragonimus mexicanus* by Miyazaki and Ishii (1968), *Paragonimus caliensis* by Little (1968), *Paragonimus peruvianus* by Miyazaki *et al.* (1968), *Paragonimus amazonicus* by Miyazaki *et al.* (1973), *Paragonimus inca* by Miyazaki *et al.* (1975) and *Paragonimus ecuadoriensis* by Voelker and Arzube (1979). However, most of these new species were identified morphologically as adults or metacercariae, and the whole life cycle was unclear. Moreover, Brenes *et al.* (1980) and Miyazaki (1979) reported that *P. peruvianus* and *P. ecuadoriensis* were the same as *P. mexicanus*.

We have been conducting epidemiological field surveys in Peru and Ecuador to investigate the life cycle of these flukes for possible

control measures under a grant-in-aid for Japanese Overseas Scientific Research from the Ministry of Education (Yokogawa *et al.* 1975, 1981 a, b). The paper here deals with scanning and transmission electron microscope (SEM, TEM) observation of the body surface of the metacercariae of *P. peruvianus*.

### Materials and Methods

The metacercariae of *P. peruvianus* were obtained from the liver of *Pseudothelphusa chilensis* collected in a river valley of Conga and Rosa Mayo in San Juan, Cajamarca Province, Peru. They were isolated in 0.4% physiological saline solution. The specimens were unencysted and showed active movements. They were washed several times in saline, fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer solution, pH 7.4, and post-fixed with 2% OsO<sub>4</sub> in the same buffer at 4 C.

For scanning electron microscopy, the specimens were further dehydrated through a graded series of ethyl-alcohol and substituted with isoamyl acetate. They were critical-point dried, and carbon-gold-coated specimens were observed with a scanning electron microscope JEOL S25-II. In transmission electron microscopy, the specimens were dehydrated as above, but substituted with n-butyl glycidyl ether, and then embedded with Epon. The sections were double-stained with uranyl acetate and lead hydroxide, and observed with a Hitachi HS-8 type electron microscope.

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This study was supported by Grant-in-Aid for Overseas Scientific Survey (56041013) of Ministry of Education, Japan.

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## Results

The critical-point dried metacercariae were ventrally curved but had various shapes when fixed. The ventral surface of the body was completely covered with single-pointed spines that were 1–3  $\mu\text{m}$  in length (Photo. 1a and 1b). The spines near the ventral sucker were clearly exposed.

The oral sucker had a diameter of approximately 25  $\mu\text{m}$  and was surrounded by some 10 dome-shaped sensory papillae (arrows) that were 2.5 to 5.0  $\mu\text{m}$  in diameter. There were numerous papillae of various sizes randomly distributed around the oral sucker (Photo. 2). Eight to 10 papillae were arranged in two rows on each side of the anterior half of the ventral side (Photo. 3). The ventral sucker had an inner diameter of approximately 50  $\mu\text{m}$ , twice that of the oral sucker. Dome-shaped papillae (diameter 7–8  $\mu\text{m}$ ), similar to those around the oral sucker and protruding spherical-shaped papillae (diameter 5  $\mu\text{m}$ ) were arranged concentrically around the ventral sucker. There were six domed papillae and the about 25 of the protruding type, including those fused together. The wall of the ventral sucker was similar to the superficies around the sucker. The spines could not be observed as they were buried in the tegument. In contrast to the oral sucker, there were few papillae in the body region around the sucker (Photo. 4). The excretory pore (Photo. 5) was surrounded by a double marginal structure, fully distinguishable from the tegument and filled with a rod-like excreta. The spines around the excretory pore were buried in the tegument and could not be observed. However, they appeared at the distal part of the excretory pore. No distinguishing structure was present in the dorsal side, and the spines were single-pointed as those in the ventral side. There were a few papillae, with no regular arrangement (Photo. 6).

The TEM observation indicated that the body surface was covered with the tegument, approximately 7  $\mu\text{m}$  thick, which had a syncytium structure. Numerous, round electron-dense granules about 0.2  $\mu\text{m}$  in diameter

and many mitochondria were scattered in the tegumental layer. The tegument had numerous spines that were about 8  $\mu\text{m}$  in length and 2–3  $\mu\text{m}$  in diameter. Their rootlet extended unevenly to the bottom of the tegumental layer. The basement membrane of the tegumental layer was approximately 0.01  $\mu\text{m}$  thick, and underneath the basement membrane, there was a 1–2  $\mu\text{m}$  thick fibrous layer of very fine fibers. The muscle layer was seen on the inner side of the fibrous layer. These features are common in other flukes (Photo. 7).

The stylet in the oral sucker, found in approximately a half of the metacercariae by light microscope, could not be found under SEM.

## Discussion

Higo and Ishii (1983) have reported on the ultrastructure of the body surface of the metacercariae and adults of *P. westermani* (triploid type), *P. miyazakii*, *P. ohirai* and *P. iloktsuenensis*. They showed that the morphology of the spines of adults was basically identical to that observed by conventional light microscopy. The spines of adults of *P. westermani* and *P. miyazakii* differed from those of *P. ohirai* and *P. iloktsuenensis*. *P. westermani* and *P. miyazakii* had spines that branched off. The papillae of the metacercariae were mostly dome-shaped and were laterally symmetrical, with a relatively larger number around the oral and ventral suckers than in the dorsal and posterior regions.

We observed numerous papillae around the oral and ventral suckers of the metacercariae of *P. peruvianus*. Six inner dome-shaped and 22–25 outer spherical-shaped papillae were arranged concentrically around the ventral sucker. The six inner papillae were identical with those described by Higo and Ishii (1983), are probably characteristics of the genus *Paragonimus*. Higo and Ishii (1983) observed approximately 10 outer spherical-shaped papillae in *P. westermani* and 18–20 in *P. miyazakii*, but none in *P. ohirai* and *P. iloktsuenensis*. If the morphology and number of the outer spherical-shaped papillae varies between species, then it might be helpful in classification.

It is, therefore, important to make a comparative study of the metacercariae of *P. mexicanus* and *P. ecuadoriensis*.

Our description here is the first report of the regular arrangement of papillae, in two rows, in the anterior part of *Paragonimus*.

It is quite interesting that papillae may differ among species. However, the papillae around the oral and ventral suckers and the two rows in the anterior part of the ventral side were not observed in adults. Most of the spines 1–3  $\mu\text{m}$  in length which covered the whole ventral and dorsal surface of the body of metacercariae were branched off in adults. Such papillae changes following growth can be observed in liver flukes. Other studies have also shown that the spines of metacercariae disappear in adulthood (unpublished our data).

### Summary

The body surface of the metacercariae of *P. peruvianus* of Central and South America was examined under the electron microscope.

The oral sucker was surrounded by some 10 dome-shaped papillae of various sizes (2.5 to 5.0  $\mu\text{m}$  in diameter). The ventral sucker showed papillae similar to those of the oral sucker (inner papillae), and concentrically arranged protruding spherical-shaped papillae (outer papillae). There were six papillae in the former but 25 varying 2 or 3 in the latter. The presence of six inner papillae was considered to be characteristics of the genus *Paragonimus*. If the number of outer papillae varies in the *Paragonimus* species, it could be important in classification. Thus, a comparative study on the metacercariae of *P. mexicanus* and *P. ecuadoriensis* may be of taxonomical value.

Eight to 10 pairs of papillae were arranged in two rows on each side of the anterior half of the ventral side. Their function is still unknown. These papillae and those around the oral and the ventral sucker disappear during growth to adulthood.

Spines that cover the body surface of metacercariae were single-pointed spines. Most of these spines also branched off in

adulthood. Our observations might be useful in the identification of many *Paragonimus* species of Central and South America.

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## ペルー肺吸虫 (*Paragonimus peruvianus*) metacercaria 体表の微細形態的観察

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ペルー肺吸虫 metacercaria の形態的特徴として、体長1.45~1.68 mm、体幅0.55~0.72 mm で排泄嚢が大きく体中央部の全んどを占め、腹吸盤の上方、腸管の分歧部にまで達している。SEM による観察では、体表は全面単生の小棘で被われており、特に腹吸盤の前後部は大きく露出し明瞭に観察された。口吸盤の大きさは内径25 μm でその周囲に半円状に隆起した大小の十数個の papillae がみられた。腹吸盤の内径は50 μm と口吸盤の倍である。その周囲は口吸盤でみられたと同様の papillae と、明らかに突き出た球状の papillae が同心円状に配列している。前者は6ヶで内周を形成し、後者は外周に融合したものを含め25ヶ存在しておりその機能に興味もたれる。又外周の papillae の数は肺吸虫属の種により差を示し、肺吸虫属 metacercaria 同定の鍵と

して今後検討する必要がある。

これらの papillae は成虫になると消失した。腹吸盤の形態も成虫と比べ明らかに差異が認められ、tegument の連続的な陥入の様相を呈していた。

Metacercaria の背側には特に目立つ構造は観察されなかつたが、腹側体前半部には8~10対の papillae の二列の配列構造がみられた。その機能については不明である。排泄孔の周囲は特異な構造がみられ、二重の唇構造を持ち、棒状、針状の排泄物で満たされていた。

透過電顕による tegument の構造は、成虫における構造と大差なく、外皮は syncytium を形成しその下層には基底膜、fibrous layer、二層の筋肉層など吸虫類の tegument 構造と同様であった。

### Explanations of Photographs

Photo. 1a Ventral view of a metacercaria of *Paragonimus peruvianus*. The body surface is covered by single-pointed spines. Bar indicates 100  $\mu\text{m}$ . os: oral sucker, vs: ventral sucker.

1b Enlarged picture of spines on a surface. Bar indicates 10  $\mu\text{m}$ .

Photo. 2 The oral sucker is surrounded by some 10 dome-shaped sensory papillae (arrows) and numerous papillae of various sizes distributed between the spines in the body around the oral sucker. Bar indicates 10  $\mu\text{m}$ . os: oral sucker.

Photo. 3 Eight to 10 pairs of papillae are arranged in two rows on each side of the anterior half of the ventral side. Bar indicates 10  $\mu\text{m}$ . os: oral sucker.

Photo. 4 Enlargement of a ventral sucker. Similar papillae to those of the oral sucker and protruding spherical-shaped papillae are arranged concentrically around the ventral sucker. Bar indicates 10  $\mu\text{m}$ . ip: inner papillae, op: outer papillae.

Photo. 5 The excretory pore is surrounded by a double marginal structure, fully distinguishable from the tegument, and filled with rod-like excreta. Bar indicates 10  $\mu\text{m}$ . ep: excretory pore.

Photo. 6 Dorsal view of a metacercaria of *P. peruvianus*. Some papillae are found but there is no regular arrangement. Bar indicates 100  $\mu\text{m}$ .

Photo. 7 A longitudinal section of the spine in the tegument. Bar indicates 1  $\mu\text{m}$ . s: spine, f: fibrous layer, m: muscle layer.



