

Scanning Electron Microscopy of the Newly Excysted Juveniles  
*Paragonimus westermani* (Kerbert, 1878) Braun, 1899  
(Parthenogenetic Type) and *P. miyazakii* Kamo,  
Nishida, Hatsushika and Tomimura, 1961

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(Received for publication; April 4, 1984)

**Key words:** *Paragonimus westermani*, *P. miyazakii*, metacercariae, sensory papillae, scanning electron microscopy

### Introduction

There have been many reports on the morphology of the metacercariae of *Paragonimus westermani* and *P. miyazakii* based on light microscopy (Nakagawa, 1920; Yamaguti, 1943; Kamo *et al.*, 1961; Komiya and Tomimura, 1964). Hatsushika (1967) observed the metacercariae of *P. westermani* and *P. miyazakii* in detail and noticed several morphological differences between the two species. However, it is difficult to distinguish the metacercariae of *P. westermani* from those of *P. miyazakii* by light microscopy.

The present study was carried out to describe and compare the surface ultrastructure of the excysted metacercariae of *P. westermani* with that of *P. miyazakii* by using scanning electron microscopy (SEM). Special attention was given to the shape and arrangement of the sensory papillae.

### Materials and Methods

Metacercariae of *Paragonimus westermani* (parthenogenetic type) were obtained from the crab, *Eriocheir japonicus*, in the Tsushima Islands, Nagasaki Prefecture. Metacercariae of *P. miyazakii* were taken from *Potamon dehaani* in Rokuroshi, Iwakuni City, Yamaguchi Prefecture. These metacercariae

were excysted by incubating them for 2 to 3 hours in the solution of 1% pancreatin and 0.1% NaHCO<sub>3</sub> at 37°C. After washing in physiological saline the juveniles were fixed for 2 hours in phosphate buffered (pH 7.4) 3% glutaraldehyde, then postfixed for 2 hours in buffered 1% OsO<sub>4</sub>, dehydrated through a graded series of ethanol, and dried in a Hitachi critical point drying apparatus. These specimens were coated with gold by using a JEOL JFC-1100 ion sputtering apparatus, and examined with a JEOL JSM-U3 scanning electron microscope.

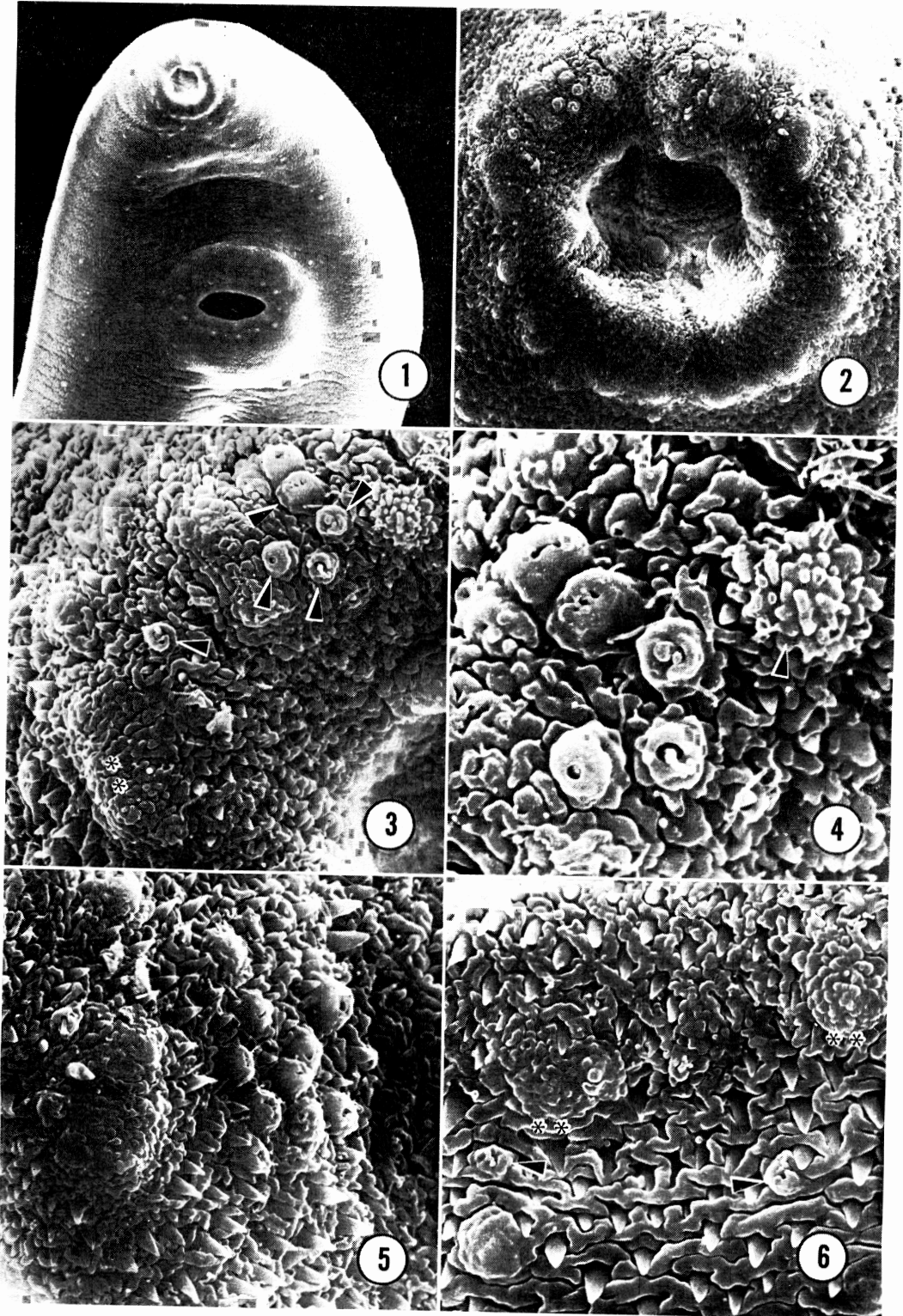
### Results

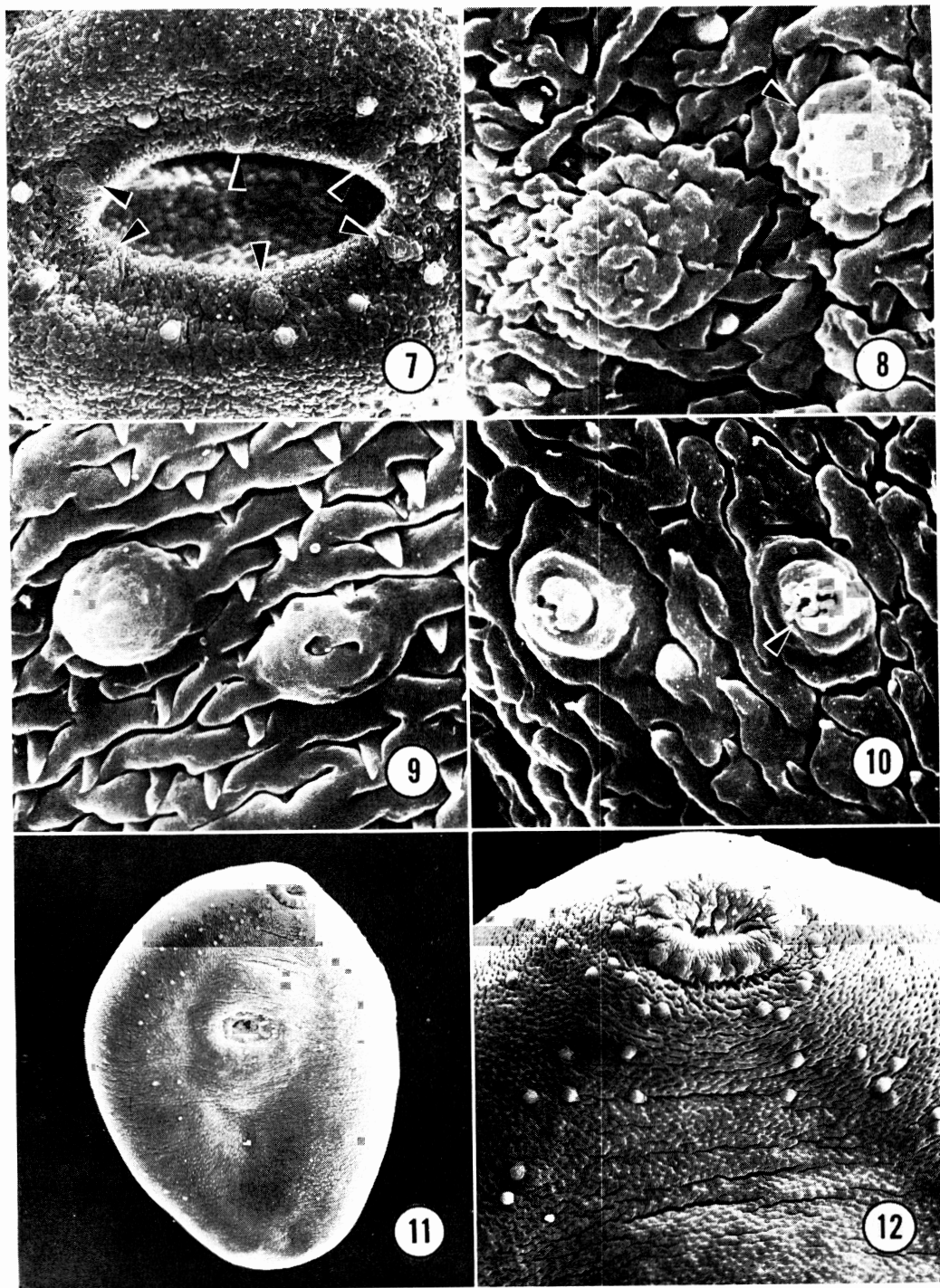
#### (1) *Paragonimus westermani*

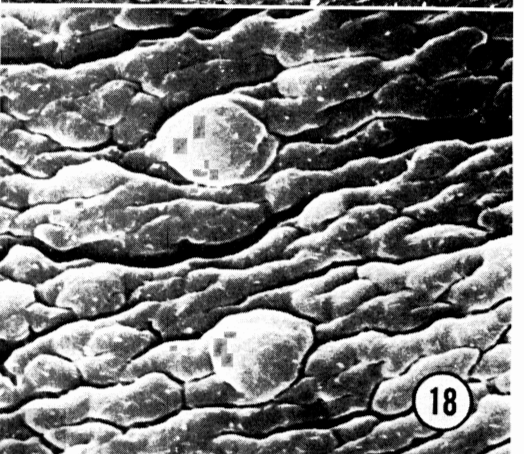
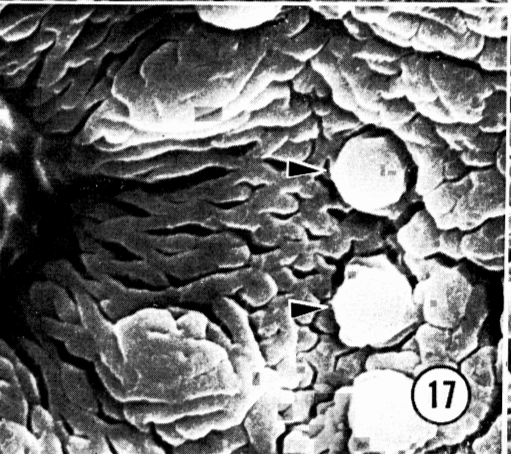
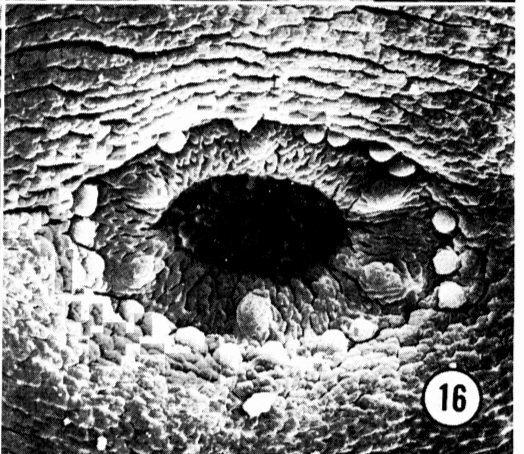
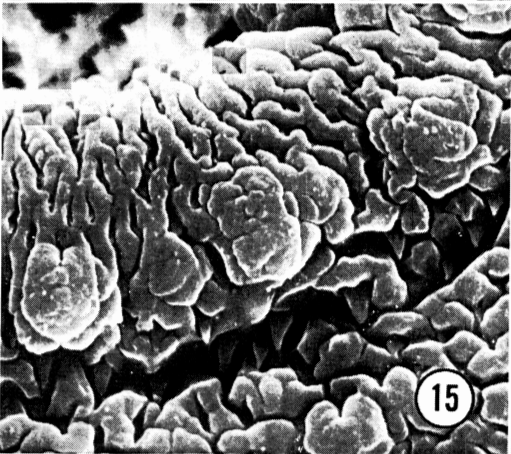
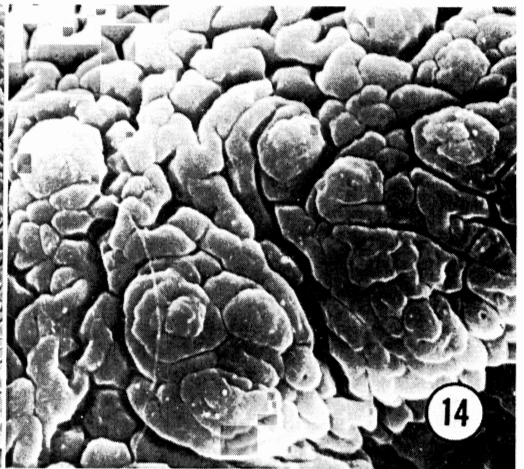
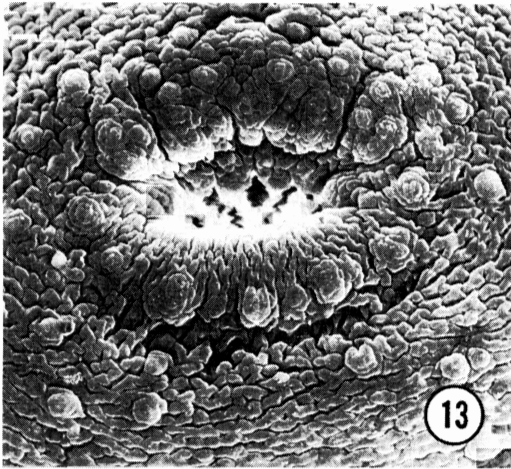
The body was flattened dorsoventrally. The ventral sucker, larger than the oral sucker, was situated in the middle of the body. The body was encircled by rows of spines arranged in a quincuncial pattern. Spines were single-pointed and thorn-like in shape, and larger anteriorly (1.4 to 2.2  $\mu\text{m}$  long and 0.6 to 1.0  $\mu\text{m}$  wide at the base) than posteriorly (0.2 to 0.8  $\mu\text{m}$  long and 0.2 to 0.4  $\mu\text{m}$  wide at the base).

Papillae presumed to be sensory were arranged symmetrically, most numerous around the oral sucker (Fig. 1). Three types of papillae were recognized: a large-domed type with a ragged or knobbed surface (Figs. 4, 6, 8), a small-domed type with a smooth surface (Figs. 8, 9), and a pit type bearing

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irregular shape, with a pit at the top occasionally having some short cilium-like projections (Figs. 4, 5, 9, 10). These papillae were 2.6 to 3.6  $\mu\text{m}$  in diameter in the large-domed type, 2.2 to 3.0  $\mu\text{m}$  in the small-domed type, and 1.2 to 2.4  $\mu\text{m}$  in the pit type. The large-domed type papillae were confined to areas around the suckers: 7 to 10 pairs on the lip of the oral sucker (Fig. 2), and 6 regularly spaced on the lip of the ventral sucker (Fig. 7). The small-domed type papillae were distributed over the entire body: about 10 pairs around the oral sucker, 7 to 11 surrounding the large-domed type papillae of the ventral sucker (Fig. 7), about 20 pairs on the ventrolateral surface and 25 pairs on the dorsolateral surface. Pit type papillae were numerous around the oral sucker: 6 pairs on the dorsal (Figs. 3, 4), 9 pairs on the dorsolateral (Fig. 5), and 3 to 5 pairs on the ventral side (Fig. 6), each bearing a cilium-like projection or none. Additionally, several pairs

of the pit type were located on the ventrolateral and dorsolateral surface (Fig. 9), and 2 pairs on the posteroventral area sometimes had several cilium-like projections (Fig. 10).

(2) *P. miyazakii*

No differences between *P. westermani* and *P. miyazakii* were evident in the shape and distribution of spines, but those of *P. miyazakii* were generally buried in the tegument (Fig. 12).

Sensory papillae were similar to those of *P. westermani* in the distribution and shape except that the pit type papillae were not seen (Figs. 11, 12). The large-domed and small-domed type papillae were larger (4.4 to 7.1  $\mu\text{m}$  and 2.5 to 4.8  $\mu\text{m}$  in diameter, respectively) than those of *P. westermani*. Of the large-domed type papillae 6 to 8 pairs were situated on the lip of the oral sucker (Fig. 13), and those on the dorsal lip appeared to be fused and ill-defined (Fig. 14). Six papillae were distributed in regular

Figs. 1-10 Excysted metacercariae of *P. westermani*

Fig. 1 Ventral surface of the body.  $\times 288$ .

Fig. 2 Whole view of the oral sucker.  $\times 1,440$ .

Fig. 3 The pit type (arrows) and the large-domed type papillae (two stars) on the dorsolateral lip of the oral sucker.  $\times 3,850$ .

Fig. 4 Enlargement of the pit type and the large-domed type (arrow) papillae in Fig. 3.  $\times 8,100$ .

Fig. 5 The pit type papillae on the lateral side of the oral sucker.  $\times 3,850$ .

Fig. 6 The large-domed type (two stars) and the pit type (arrows) papillae on the ventral side of the oral sucker.  $\times 4,260$ .

Fig. 7 Whole view of the ventral sucker. Six large-domed type papillae (arrows) are on the lip of the ventral sucker, and outside them 10 small-domed type ones.  $\times 1,140$ .

Fig. 8 Enlargement of the large-domed type and the small-domed type (arrow) papillae around the ventral sucker.  $\times 7,200$ .

Fig. 9 The small-domed type (left) and the pit type (right) papillae on the midventral surface of lateral side.  $\times 7,200$ .

Fig. 10 The pit type papillae on the posteroventral end. One of them (arrow) has fine and short projections.  $\times 5,600$ .

Figs. 11-18 Excysted metacercariae of *P. miyazakii*

Fig. 11 Ventral view of the whole body.  $\times 158$ .

Fig. 12 Anterior region of the ventral surface.  $\times 576$ .

Fig. 13 Whole view of the oral sucker.  $\times 1,210$ .

Fig. 14 Fused and irregular papillae of the large-domed type on the dorsal lip of the oral sucker.  $\times 3,550$ .

Fig. 15 The large-domed type papillae on the ventral lip of the oral sucker.  $\times 3,200$ .

Fig. 16 Whole view of the ventral sucker. The six large-domed type papillae are on the lip of the ventral sucker, and about 20 small-domed type ones outside them.  $\times 1,010$ .

Fig. 17 Enlargement of Fig. 16. Arrows show the small-domed type papillae.  $\times 3,200$ .

Fig. 18 The small-domed type papillae on the midventral surface of lateral side.  $\times 3,550$ .

space on the lip of the ventral sucker (Fig. 16). Many small-domed type papillae were distributed over the whole body: about 10 pairs around the oral sucker (Fig. 12), 15 to 25 individual papillae surrounding the 6 large-domed type around the ventral sucker (Figs. 16, 17), about 30 pairs on the ventrolateral surface, and about 25 pairs on the dorso-lateral surface (Fig. 11).

### Discussion

No remarkable morphological differences between *Paragonimus westermani* and *P. miyazakii* were evident except for the size and arrangement of sensory papillae. The small-domed type papillae around the ventral sucker varied in number between these two species: 7 to 11 in *P. westermani* and 14 to 21 in *P. miyazakii*. The large-domed and small-domed types in *P. miyazakii* were larger than those in *P. westermani*. The pit type papillae were present around the oral sucker and on the ventrolateral and dorsolateral surface in *P. westermani*, while papillae of this type were not seen in *P. miyazakii*. However, further observations by scanning and transmission electron microscopy are required for the confirmation of this finding. These differences in the sensory papillae were remarkable and can serve effectively to distinguish the juveniles of *P. westermani* from those of *P. miyazakii*. Aji *et al.* (1984) observed the metacercariae of *Paragonimus peruvianus* with SEM and noticed many domed shaped papillae around the oral sucker and 6 around the ventral sucker, and 25 protruding spherical-shaped papillae surrounding the domed type on the lip of the ventral sucker. *P. peruvianus* is similar to *P. miyazakii* in the distribution of papillae.

It appears that the large-domed and small-domed type papillae of *P. westermani* and *P. miyazakii* correspond to the slightly domed, platelike swellings and the larger, rounded swellings in the juveniles of *Clonorchis sinensis* described by Fujino *et al.* (1979), and the former type were confined to the areas around the suckers. Six large-domed type papillae which were regularly spaced

around the ventral sucker were also observed in the metacercariae of *Fasciola hepatica* (Bennett, 1975), *C. sinensis* (Fujino *et al.*, 1979) and *P. peruvianus* (Aji *et al.*, 1984). These 6 papillae may generally exist in metacercariae of digenetic trematodes. It seems that the large-domed type function as tango-receptor involved in the contraction of suckers, and the small-domed type also function as tango-receptor for the body movement.

The pit type papillae in *P. westermani* were irregular in shape, some around the oral sucker having a presumed cilium or none, and sometimes 2 pairs on the posteroventral end had several such projections. The unciliated papillae around the oral sucker were similar to those that have been described in many species of digenetic trematodes (Bennett, 1975; Nadakavukaren and Nollen, 1975; Kjøie, 1976; Edward *et al.*, 1977; Fujino *et al.*, 1979). It seems likely that the pit type papillae, especially ones around the oral sucker, have chemoreceptive function for the migration and food ingestion. This type papillae with several projections were rarely found in metacercariae and resembled the ones with multiple short cilia of *Ribeiroia marini* cercaria reported by Page *et al.* (1980).

### Summary

The newly excysted juveniles of *Paragonimus westermani* (parthenogenetic type) and *P. miyazakii* were examined with SEM and special attention was given to the sensory papillae.

In *P. westermani* three types were observed: large-domed papillae with knobbed surface (large-domed type), hemispherical and smooth papillae (small-domed type), and papillae with irregularly shaped pits (pit type), occasionally bearing one or more short cilium-like projections. In *P. miyazakii* the pit type papillae were not seen. The small-domed type papillae surrounding the ventral sucker varied in number between *P. westermani* and *P. miyazakii*, and the large-domed and small-domed type papillae also differed

in size between the two species. These differences may be useful to distinguish between the metacercariae of *P. westermanni* and *P. miyazakii*.

#### Acknowledgements

We would like to express our sincere gratitude to Dr. Paul C. Beaver, Tulane University for reviewing the manuscript. We also express our appreciation to Dr. T. Fujino, Kyushu University for valuable suggestions.

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### ウエステルマン肺吸虫 *Paragonimus westermanni* (Kerbert, 1878) Braun, 1899 (単為生殖型) と宮崎肺吸虫 *P. miyazakii* Kamo, Nishida, Hatsushika and Tomimura, 1961 の脱囊幼虫における走査電子顕微鏡的観察

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単為生殖型ウエステルマン肺吸虫と宮崎肺吸虫の脱囊幼虫について走査電顕を用いて、主に感覚乳頭における比較研究を行なった。

ウエステルマン肺吸虫には3種類の感覚乳頭がみられた。すなわち、表面に多数の襞を持つ大型の乳頭、表面が滑らかで半球形型の乳頭、それに不定形の pit を持つ

乳頭である。pit を持つ乳頭には細く短かい線毛を持つものもある。宮崎肺吸虫にはこの pit を持つ乳頭がみられなかつた。乳頭の配列および大きさは両肺吸虫間で異なり、これらの差異は SEM レベルにおいて両種を区別する有効な手段になりうると思われる。