

SCANNING ELECTRON MICROSCOPY OF NEWLY EXCYSTED METACERCARIAE OF *PARAGONIMUS WESTERMANI* - LIKE, NAKHON NAYOK, THAILAND

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Abstract. Scanning electron microscope (SEM) observation of surface structure was done on newly excysted metacercariae of *Paragonimus westermani* - like (Nakhon Nayok, Thailand). The surface of the body was covered with numerous single-pointed tegumentary spines, large dome-shaped papillae, small one with a pit, and small one with a smooth surface were situated around the suckers. There were 27 to 30 of the small dome-shaped papillae with a pit around the oral sucker and 10 to 13 of the small ones with a smooth surface around the ventral sucker. The present report is the new record of excysted metacercariae of *P. westermani* - like (Nakhon Nayok, Thailand) by SEM.

INTRODUCTION

Paragonimus species is principally a parasite of the lung causing pulmonary paragonimiasis in the final host, including humans beings in many parts of the world. So far, there are six species of *Paragonimus* in Thailand; two belonging to human hosts are *P. westermani* and *P. heterotremus*, and four belonging to animal hosts are *P. siamensis*, *P. bangkokensis*, *P. harinasutai* and *P. marcrochis* (Vajrasthira, 1986). The morphological character of adults and metacercariae of *P. heterotremus* from Thailand have been described by a light microscopy (Miyazaki and Vajrasthira, 1967). In addition, Sugiyama *et al* (1990) has studied the surface ultrastructure of newly excysted metacercariae of *P. heterotremus*, but no study has been done on *P. westermani* from Thailand. In the present study, we examined the ultrastructure of the excysted metacercariae of *P. westermani* - like, Nakhon-Nayok, Thailand.

MATERIALS AND METHODS

The metacercariae of *P. westermani* - like (Fig 1) were harvested from naturally infected mountaineous and stream crabs in Nakhon Nayok

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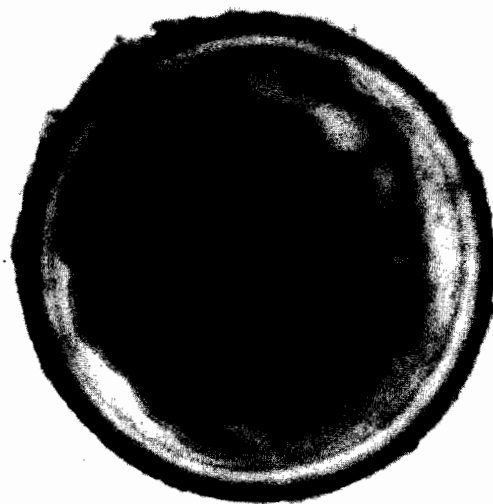


Fig 1—Live metacercariae of *Paragonimus westermani* - like. Bar = 0.1 mm.

Province, Thailand. The soft tissues of the crabs, first were crushed in a blender, then emulsified in artificial gastric solution (0.25 % pepsin in 0.25 % conc HCl at 37°C, 1 hour), which made it easy to isolate metacercariae. Intact metacercariae without redundant crab tissue on the surface were washed several times in physiological saline and used in the excystment. The excysted larvae were obtained by incubation in Hank's balanced salt solution, pH 7.4

at 37°C. They were washed several times in 0.1 M phosphate buffered saline, pH 7.4, fixed in 2.5 % phosphate buffered glutaraldehyde and postfixed in 1 % phosphate-buffered osmium tetroxide. The worms were dehydrated in a graded series of ethanol, transferred to amyl acetate, dried in a critical point dryer (Hitachi HCP-2), mounted on a stub and coated with gold in ion coater (EIKO IB-3). The specimens were then examined under SEM (Hitachi S-450) at an accelerating voltage of 15 KV.

RESULTS

The body of the excysted metacercariae was fattened dorsoventrally (Fig 2a). The body surface was covered with numerous single pointed cuticular spines (Fig 2b). Dome shaped papillae were regularly distributed over the whole body.

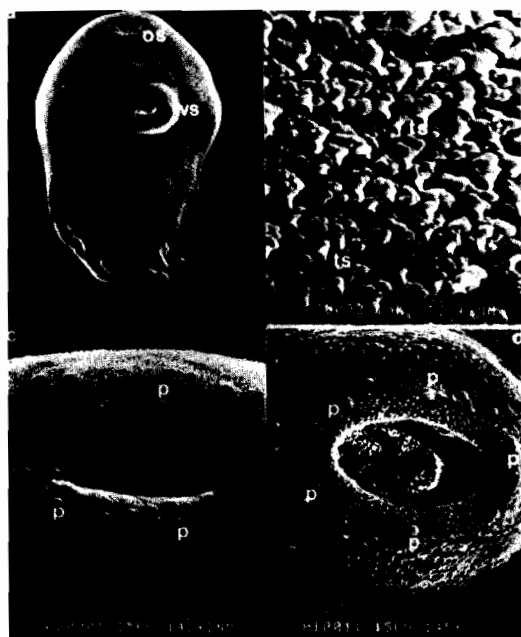


Fig 2—Scanning electron microscopy of newly excysted metacercariae of *Paragonimus westermani*-like. (a) Whole body in ventral view. (b) Tegumentary spines covered all surface topography of the worm. (c) Oral sucker showing the papillae. (d) Ventral sucker showing the papillae. os, oral sucker; p, papillae; ts, tegumentary spine; vs, ventral sucker

The oral sucker was situated on the anterior end of the worm's body. Around the oral sucker, three types of papillae were shown; large dome-shaped papillae with a ragged surface, small ones with a smooth surface and small ones with an irregular shaped pit (Fig 2c). There were 27-30 dome-shaped papillae with a pit distributed mainly on the dorsal or dorsolateral side of the oral sucker. The small dome-shaped papillae with a smooth surface situated around the oral sucker numbered approximately 19-20.

The ventral sucker was situated somewhat anteriorly from the center of the body. Around the ventral sucker, the inner large and outer small dome-shaped papillae, both with a smooth surface, were distributed circularly (Fig 2d). The number of the large papillae was constantly 6 and that of the small ones was approximately 10-13.

DISCUSSION

The observation on the type and the difference in type of papillae readily provided the criterion to distinguish *Paragonimus* species in the metacercarial stage (Higo and Ishii, 1987; Sugiyama *et al*, 1990). By SEM, the body surface of newly excysted metacercariae of five species of Japanese lung flukes, showed that the number of the small dome-shaped papillae around the ventral sucker varied with the species *ie.* 0 to 2 in *P. iloksuenensis*, 2 to 6 in *P. ohirai*, 5 to 11 in *P. pulmonalis* (triploid type of *P. westermani*), 5 to 13 in *P. westermani* (diploid type) and 13 to 19 in *P. miyazakii* (Higo and Ishii, 1987). On excysted metacercariae of *P. mexicanus*, the number of the small dome-shaped papillae around the ventral sucker was 22 to 38 (Aji *et al*, 1984; Tongu *et al*, 1985, 1987). Sugiyama *et al* (1990) examined the excysted metacercariae of *P. heterotremus* from Thailand and revealed that the small smooth surface papillae around the ventral sucker, were 9 to 13.

The arrangement of the small dome-shaped papillae with a pit around the oral sucker varied also among the five Japanese lung flukes (Higo and Ishii, 1987). Many small papillae with a pit were seen around the oral sucker of *P. westermani*, *P. pulmonalis* and *P. ohirai*, but in *P. miyazakii* and *P. iloksuenensis*, there were only a few and none, respectively. In addition, Sugiyama *et al* (1990) revealed that approximately 30 small dome-shaped papillae with a pit were around the oral sucker of the excysted metacercariae of *P. heterotremus*.

In this study, the excysted metacercariae of *P. westermani* - like from Thailand revealed that approximately 27 to 30 small dome-shaped papillae with a pit and 10 to 13 small ones with a smooth surface were seen around the oral and ventral suckers, respectively. The arrangements of the papillae described above, by SEM, *P. westermani* - like from Thailand was similar to *P. westermani*, *P. pulmonalis* and *P. heterotremus*, but *P. heterotremus* can easily be distinguished from *P. westermani*-like in the present study even by the shape of the metacercarial cyst with light microscopy (Vajrasthira, 1986). On the basis of the present observation, it seems reasonable to conclude that the feature of *P. westermani* - like metacercariae from Thailand might be identical with *P. westermani*.

The surface ultrastructure from the present study would be used as a taxonomic aid, and in parasite identification. Further studies are needed to elucidate the ultrastructure of other *Paragonimus* spp from Thailand.

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