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# Chaetotaxy of Newly Excysted Metacercariae among Five Species of Thai *Paragonimus*

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## Abstract

Scanning electron microscopy was used to study the papillae distribution (chaetotaxy) among five species of newly excysted *Paragonimus* metacercariae (*P. bangkokensis, P. harinasutai, P. heterotremus, P. siamensis,* and *P. westermani*). Comparative data on the distribution of papillae in these species indicate considerable differences in the arrangement of papillae on the surfaces of the oral and ventral suckers. The numbers of papillae in the mouth of the oral sucker should be used to differentiate these species. A key to the species of Thai *Paragonimus* excysted metacercariae is given.

**Keywords:** *Paragonimus*, metacercariae, papillae, chaetotaxy, scanning electron microscopy, Thailand

## Introduction

Six species of *Paragonimus* lung flukes (*P. bangkokensis, P. harinasutai, P. heterotremus, P. macrorchis, P. siamensis,* and *P. westermani*), have been reported in Thailand [1]. Among these species, *P. heterotremus* and *P. westermani* are known to infect humans in Southeast Asia, while *P. heterotremus* is the only confirmed causative agent of human paragonimiasis in Thailand [2-5]. Thai *Paragonimus* have been extensively studied in various parasitic stages. Species identification reports of these parasites are mainly confined to the adult stage, whereas little information is found of other stages. *Paragonimus* is a species for which identification is complicated, usually based on observation of some external and internal morphological characteristics of the adult worm under a light microscope. However, adult morphology is not reliable for indistinguishable sibling species. In Thai *Paragonimus*, though encysted metacercariae are easily differentiated from each other, excysted metacercariae are not. Tegumentary papillae as taxonomic characters have been used to distinguish subtle morphological differences in cercarial speices of *Paragonimus* and *Schistosoma,* and *Echinostoma* complexes [6-9]. The study of surface topography of newly excysted metacercariae of five species of Thai lung flukes has revealed that three morphological types of papillae are distributed and definitely arranged on the lips of the oral and ventral suckers in each species [10]. Therefore, the aim of this study was to compare the chaetotaxy of newly excysted metacercariae among five species of *Paragonimus* found in Thailand by scanning electron microscopy.

## Materials and methods

### Collection of *Paragonimus* metacercariae

Metacercariae of the *Paragonimus* fluke utilize crustaceans as the second intermediate host. Among the six species found in Thailand, metacercariae can be found in freshwater crabs, mountain or waterfall crabs, and rice-field crabs.

The waterfall crabs, *Larnaudia beusekomae* Bott 1970, used in this study were caught under rocks in shallow, fast-flowing mountain streams in Amphoe Pak Phli, Nakhon Nayok Province, where rice-field crabs, *Somanniathelphusa germaini* Rathbun 1902, were also caught. Metacercariae of *P. bangkokensis, P. harinasutai, P. heterotremus,* and *P. westermani* were isolated from waterfall crabs, while *P. siamensis* were isolated from rice-field crabs (Fig 1). After the carapaces were removed, the remainders of the crabs were ground by blender in physiological saline. The preparation was put into a sedimentation flask and allowed to stand for one hour at room temperature. The sediment was examined under a stereomicroscope for *Paragonimus* metacercariae. All metacercariae recovered were immediately processed for scanning electron microscopy.

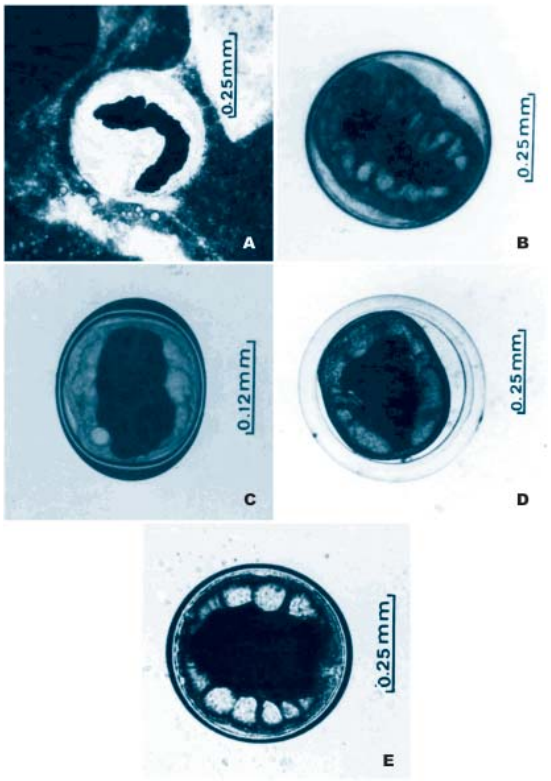


**Fig 1 Metacercariae recovered in this study. (A = *P. bangkokensis*, B = *P. harinasutai*, C = *P. heterotremus*, D = *P. siamensis*, E = *P. westermani*).**

### Silver nitrate impregnation

According to the method of Sakamoto and Ishii, the excysted metacercariae of each species were fixed in hot 0.5% silver nitrate solution for 10 minutes in the dark, rinsed in distilled water, then exposed to artificial light for 10 minutes and mounted in 10% glycerine solution [11].

### Scanning electron microscopy

Scanning electron microscopy (SEM) of the excysted metacercariae of each species was performed as follows: the metacercariae were excysted by manipulation; the excysted metacercariae were washed several times in physiological saline, and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer solution at 4˚C for 2 hours. After washing with phosphate rinse buffer (pH 7.4), 3 changes, 15 minutes each, they were fixed in 1% osmium tetroxide for 1 hour, dehydrated through a graded series of ethanol (50, 70, 80, and 90%, and two changes of absolute alcohol, all for 15 minutes each), dried in a Hitachi critical point dryer (HCP-2), and mounted on aluminum stubs with double-sided adhesive tape. They were coated with gold using a sputter coater (Emitech, K550) and observed with Hitachi (S2150 and S-2360N) scanning electron microscopes at 15 KV accelerating voltage. Images of suitable specimens were captured under various magnifications using the imaging software SEMICAPS 1000 (Scanning Electron Microscope Image Capture and Processing System, version 1.13.13.1) c/o Image Transforms Pte Ltd, Singapore. This imaging software was loaded into a computer interfaced with a SEM. After the images were captured, they were processed (place micron bar and mark the area of interest) in SEMICAPS 1.13.13.1 and saved in Tag Image File Format (TIFF) as individual files, and further processed (labeled) using Paint Shop software. The papillae distributed over the surfaces of the oral and ventral suckers of each excysted metacercaria species were examined.

## Results

In normal practice, the papillae arrangements of worms are studied on specimens stained with

silver nitrate aqueous solution. However, in this study, the papillae of newly excysted *Paragonimus* metacercariae could not take up the silver nitrate stain. Therefore, the arrangement of the papillae of all species studied was investigated by SEM images of excysted metacercariae (Fig 2). The distribution of papillae was observed only on the ventral surface because most specimens toppled onto their dorsal surfaces while being attached to the specimen stubs. The papillae were found on the oral and ventral suckers and the ventral surface of the body.

**Distribution of papillae around oral sucker**

Numerous papillae were recovered around the oral sucker. They appeared in similar patterns for all species and could be grouped according to location: 1) papillae on the anterior lip of the oral sucker (PAOS); 2) papillae in the mouth of the oral sucker (PMOS); 3) papillae on the posterior lip of the oral sucker (PPOS); and 4) cervical papillae (CP) (Fig 3A). PAOS comprised four groups of papillae arranged in symmetry, two on the right side, PAOS1(R) and PAOS2(R), and two on the left side, PAOS1(L) and PAOS2(L).

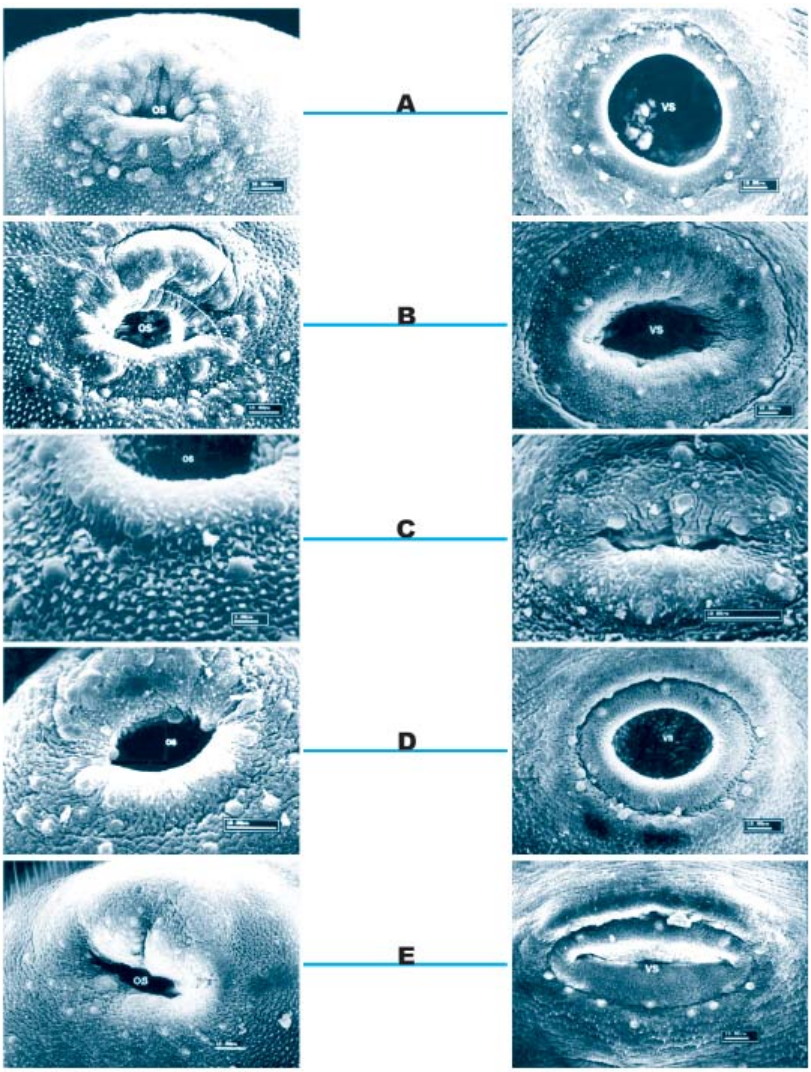


**Fig 2 Papillae on the oral (OS) and ventral (VS) suckers of *Paragonimus* excysted metacercariae recovered in this study. (A = *P. bangkokensis*, B = *P. harinasutai*, C = *P. heterotremus*, D = *P. siamensis*, E = *P. westermani*).**

PMOS were less complicated, usually comprising two papillae, one on each inner side of the mouth. PPOS were arranged in a half circle on the surface of the posterior lip. CP had papillae closer around the oral sucker on both ventral and lateral sides. The number of papillae in each group of five species of *Paragonimus* metacercariae are shown in Fig 4 and summarized in Table 1.

**Distribution of papillae around ventral sucker**

The papillae around the ventral sucker were fewer in number than those around the oral sucker. The patterns of distribution appeared quite definite as a ring of papillae, or two or three rings: 1) papillae on the surface of the ventral sucker (PSVS), and 2) papillae around the ventral sucker (PAVS) (Fig 3B). PSVS consisted of one and two rings of six regularly arranged papillae, *ie,* one ring in *P. bangkokensis* and *P. siamensis*; two rings in *P. harinasutai, P. heterotremus*, and *P. westermani* (Fig 5). These six papillae, on both inner and middle rings, were arranged as three each on the anterior and three on the posterior lips. Outside the regularly spaced six papillae, there was a ring of papillae, the numbers of which seemed to vary with species. The number of papillae distributed in each group of five species of *Paragonimus* metacercariae are shown in Fig 5 and summarized in Table 2.

**Differentiation between species using chaetotaxy**

The number and distribution of papillae seemed to differ among species. Use of the papillae on the ventral and oral suckers to differentiate the five species of *Paragonimus* metacercariae found in Thailand was proposed as shown in Table 3.

**Table 1 Comparative numbers of papillae distributed around the oral sucker among five *Paragonimus* species classified by location.**

| Species | PAOS1 | | PAOS2 | | PMOS | | | PPOS | CP |
|---|---|---|---|---|---|---|---|---|---|
| | R | L | R | L | R | L | A | | |
| *P. bangkokensis* (2) | 5-6 | 4-6 | 4-5 | 4-7 | 3 | 3 | - | 5-6 | 42 |
| *P. harinasutai* (2) | 4-5 | 4-5 | 5-9 | 5-7 | 2 | 2 | - | 5-6 | 46 |
| *P. heterotremus* (2) | 1 | 1 | 1 | 1 | 1 | 1 | - | 5 | 12 |
| *P. siamensis* (3) | 4-6 | 5 | 4 | 3-4 | 1 | 1 | 1-2 | 5-6 | 10-15 |
| *P. westermani* (3) | 9 | 7 | 6 | 4 | 1 | 1 | - | 3 | 26 |

PAOS = papillae on the anterior lip of the oral sucker; PMOS = papillae in the mouth of the oral sucker;
PPOS = papillae on the posterior lip of the oral sucker; CP = cervical papillae; R= right-hand side;
L = left-hand side; A = anterior side; ( ) = numbers of excysted metacercariae studied.

**Table 2 Comparative numbers of papillae distributed around the ventral sucker among five *Paragonimus* species classified by location.**

| Species | PSVS (inner ring) | PSVS (middle ring) | PAVS (outer ring) |
|---|---|---|---|
| *P. bangkokensis* (2) | - | 6 | 13-14 |
| *P. harinasutai* (2) | 6 | 6 | 10-14 |
| *P. heterotremus* (2) | 6 | 6 | 6-7 |
| *P. siamensis* (4) | - | 6 | 13-14 |
| *P. westermani* (4) | 6 | 6 | 9-13 |

PSVS = papillae on the surface of the ventral sucker; PAVS = papillae around the ventral sucker;
( ) = numbers of excysted metacercariae studied.

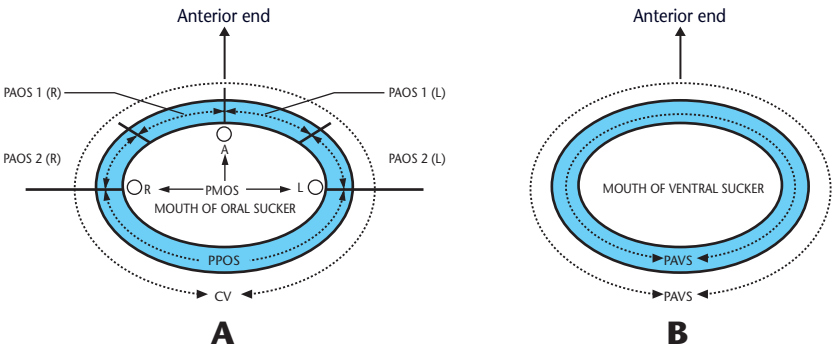


**Fig 3 Schematic diagram illustrating groups of papillae around the oral sucker (A) and ventral sucker (B) classified by location. (PAOS = papillae on the anterior lip of the oral sucker, PMOS = papillae in the mouth of the oral sucker, PPOS = papillae on the posterior lip of the oral sucker, CP = cervical papillae, PSVS = papillae on the surface of the ventral sucker, PAVS = papillae around the ventral sucker, A = anterior, L = left-hand side, R = right-hand side).**

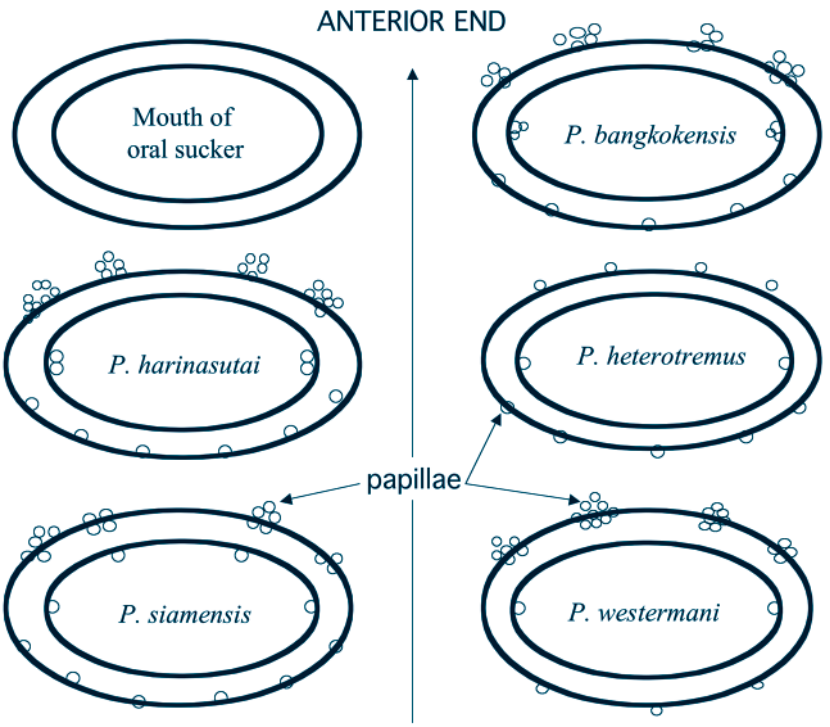


**Fig 4 Diagram of papillae distributed around the oral sucker of *Paragonimus* metacercariae.**

## Discussion

Papillae in or on the teguments of trematode cercariae have been reported in a number of distantly related groups. The use of cercarial papillae as taxonomic characters has been suggested by Vercammen-Granjean for *Schistosoma mansoni* [6], and Kuntz for *Schistosoma* complexes [7], but the papillae for *Paragonimus* cercaria was only reported by Ishii and Miyazaki [8]. They proposed that to

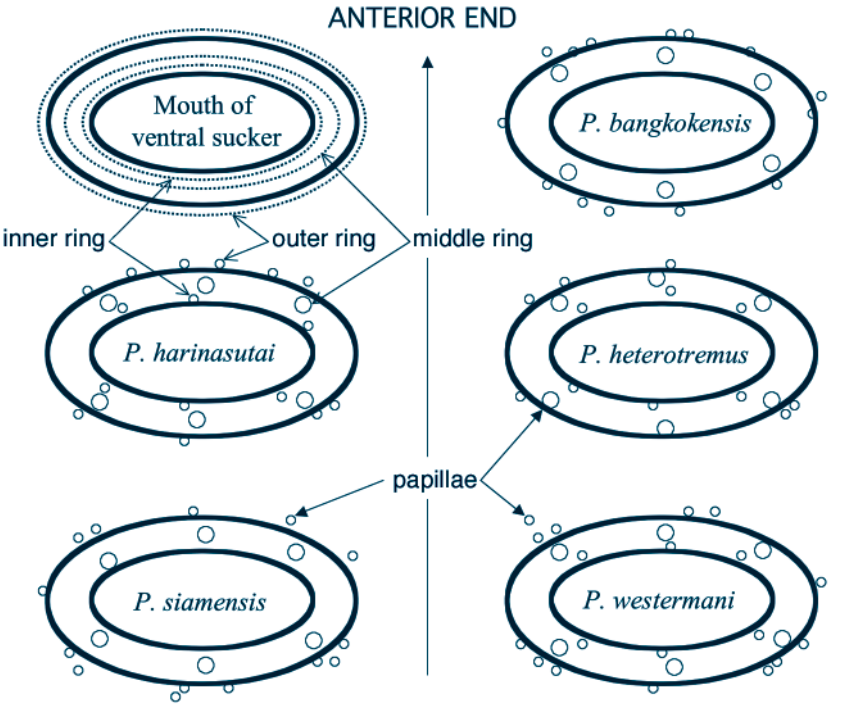


**Fig 5 Diagram of papillae distributed around the ventral sucker of *Paragonimus* metacercariae.**

**Table 3 A key to species of Thai *Paragonimus* excysted metacercariae using chaetotaxy.**

| | | |
|---|---|---|
| 1 | Ventral sucker with 2 rings of papillae | 2 |
| | Ventral sucker with 3 rings of papillae | 3 |
| 2 | Mouth of oral sucker with 2-4 single papillae | *P. siamensis* |
| | Mouth of oral sucker with a group of 3-4 papillae on each side | *P. bangkokensis* |
| 3 | Mouth of oral sucker with two papillae on each side | *P. harinasutai* |
| | Mouth of oral sucker with one papillae on each side | 4 |
| 4 | Anterior to oral sucker with 4-6 single papillae | *P. heterotremus* |
| | Anterior to oral sucker with 4 groups of 4-9 papillae | *P. westermani* |

differentiate species of the genus *Paragonimus* not only the flame cell pattern of the cercaria, but also the structure of its body surface should be investigated. They obtained images of the papillae as sensory endings on the oral suckers of *P. sadoensis* and intended to compare them with other species of *Paragonimus* by the same method. Higo and Ishii studied the papillae distribution on newly excysted metacercariae of *Paragonimus* worms found in Japan, and stated that the arrangement of papillae on the body surface, except the suckers, varied within individual worms, and regular patterns of body papillae could not be determined [12-13]. In the present study, the papillae on the ventral surface of the worm's body also varied within individual worms and definite patterns could not be found.

Silver nitrate impregnation method was used in previous studies on chaetotaxy. In the present study, this method was also used, but the papillae

could not be detected, perhaps because the parasitic stage used differed from the others, and they were not suitable for this method. Instead of using the above method, therefore, we used the SEM images to study papillae distribution.

The papillae nomenclature, classified by location, was proposed in the present study. The arrangements of papillae on the oral suckers of the five species were considered location by location, and are summarized in Table 1. In the present study, the ventral suckers of all five species showed a definite pattern of six papillae; three papillae located on the anterior half and another three on the posterior half of the surface of the ventral sucker. An additional six papillae were arranged in a similar manner, but closer to the mouth of the ventral sucker in *P. westermani, P. heterotremus,* and *P. harinasutai*. According to location, the papillae arranged on the ventral sucker in this study were classified into two groups: PSVS (papillae on the surface of the ventral sucker) and PAVS (papillae around the ventral sucker). For the five *Paragonimus* species studied, these arrangements are summarized in Table 2.

There were no reports about this aspect of *Paragonimus* worms in the reviewed literature. Thus, this seemed to be the first paper describing the distribution of papillae on Thai *Paragonimus* worms by their location, and in which a nomenclature of papillae classification according to area and a key for species identification of newly excysted metacercariae, were also proposed.

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