



Surface Topography of Newly Excysted Metacercariae of Thai *Paragonimus* Species

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Abstract

The surface topography of newly excysted metacercariae of five species of Thai lung flukes: *Paragonimus westermani*, *P. siamensis*, *P. heterotremus*, *P. harinasutai* and *P. bangkokensis* are described. The findings showed that the metacercarial bodies were ellipsoidal or oval in shape. Their surfaces were armed almost entirely with tegumentary spines. These spines were single-pointed and well developed in the antero-ventral region. Three morphological types of papillae were observed, as follows: a) large-domed papillae on the lips of the oral and ventral suckers; b) small-domed papillae over the entire body, persistently distributed around both suckers; and c) pit-type papillae which were limited to the oral sucker area.

Keywords: *Paragonimus*, metacercariae, topography, scanning electron microscopy, Thailand

Introduction

The lung fluke, *Paragonimus*, is one of the harmful parasites causing paragonimiasis in humans, mainly in Asia, some parts of West Africa, and South and Central America [1]. Six species of *Paragonimus* worms (*P. bangkokensis*, *P. harinasutai*, *P. heterotremus*, *P. macrorchis*, *P. siamensis* and *P. westermani*) have been found in Thailand, but only *P. westermani* and *P. heterotremus* are known to be causative agents of paragonimiasis in humans [2-5]. *Paragonimus* species have been extensively studied in several aspects in various parasitic stages. However, reports concerning *Paragonimus* species are mainly confined to the adult stage. In Thailand, a little information about the morphological characteristics of newly excysted metacercariae of *P. heterotremus* and *P. westermani*-like species was gained by light microscopy and scanning electron microscopy [6-7]. Although there have been some reports on newly excysted metacercariae, basic

information about the morphological characteristics of newly excysted metacercariae in the other *Paragonimus* species is still lacking and needs to be elucidated. Thus, the objective of this work was to study the tegumental appearance of newly excysted metacercariae 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, mountainous or waterfall crabs, and rice-field crabs. Waterfall crabs, *Larnaudia beusekomae* Bott 1970, used in this study were caught under rocks in shallow, fast-flowing mountain streams in Amphoe Pakphli, Nakhon Nayok Province. Rice-field crabs, *Somanniathelphusa germaini* Rathbun

1902, were also caught in Amphoe Pakphli, Nakhon Nayok Province. Metacercariae of *P. bangkokensis*, *P. harinasutai*, *P. heterotremus*, and *P. westermanni* were isolated from waterfall crabs (*L. beusekomae*), while *P. siamensis* was isolated from rice-field crabs (*S. germaini*) (Fig 1). After the carapaces were removed, the remainder of the crabs was 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* spp metacercariae. All metacercariae recovered were immediately processed for scanning electron microscopy.

Scanning electron microscopy

The scanning electron microscopy of excysted metacercariae of each species was studied 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), three changes, 15 minutes each, they were post 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 SEM. Here was the list of magnifications used in capturing the images of various parts of the worms' bodies. 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 types of papillae and spines distributed over the surface of each excysted metacercaria species were examined.

Results

General morphology

The shape of the excysted metacercarial bodies varied slightly depending on the state of their mobility at the time of fixation. The ones fixed during full extension were leaflike, dorsoventrally flattened and armed with cuticular spines. The oral sucker was situated at the anterior end of the ventral side of the worm's body. The ventral sucker was somewhat anteromedian in relation to the center of the body. The ranges of measurements for body size and sucker width are summarized in Table 1.

Paragonimus bangkokensis

The excysted metacercariae were 567 µm long and 303 µm wide. The bodies were covered with numerous single-pointed spines (Fig 2.6), which gradually reduced in number posteriorly. The spines on the anterior region of the ventral sucker were 1.1-1.4 µm long and 0.5-0.7 µm wide at the base, whereas those on the lateral and posterior region were approximately 1.1-2.2 µm long and 0.5-0.9 µm wide at the base. The oral sucker was subterminal, 12-30 µm in diameter and 36-52 µm wide. The ventral sucker, situated mid-ventrally on the body, was oval, 23-51 µm in diameter and 65-81 µm wide. The numbers of papillae around the oral sucker and their distribution on the dorsal lip, when compared to other parts of the body, were similar to *P. siamensis*. Three types of papillae were observed in this species; a large-domed type, having diameters ranging from 3.9-7.6 µm, was also confined to the lips of both suckers. It consisted of 2 pairs on the dorsal lip, 2 pairs with one individual papilla between these two pairs on the ventral lip of the oral sucker (Fig 2.1), and 6 regularly spaced papillae on the lip of the ventral sucker (Fig 2.4). Those on the dorsal lip of the oral sucker appeared to be fused, concentrated around the central region of the sucker and ill-defined (Fig 2.1). The small-domed type had diameters of 1.7-3.5 µm, with about 37 individual papillae

Table 1 Range of measurements (μm) of the metacercariae of five *Paragonimus* species.

Species	Body length	Body width	Oral sucker width	Ventral sucker width
<i>P. bangkokensis</i>	567 (1)	303 (1)	36-52 (2)	65-81 (2)
<i>P. harinasutai</i>	391-526 (3)	353-418 (3)	36-66 (4)	81-138 (3)
<i>P. heterotremus</i>	230-351 (2)	163-209 (2)	24-26 (2)	31-49 (3)
<i>P. siamensis</i>	260-414 (6)	254-320 (6)	52-70 (6)	72-100 (7)
<i>P. westermani</i>	348-806 (4)	306-461 (4)	45-67 (6)	66-87 (6)

() = numbers of metacercariae studied.

surrounding the 6 large-domed papillae of the ventral sucker (Fig 2.4). Pit-type papillae were the most prominent among the five species studied and their distributions were on the dorsal and dorso-lateral side of the oral sucker (Figs 2.1, 2.2, 2.3). Their diameters were 1.3-2.2 μm .

Paragonimus harinasutai

The metacercarial body did not differ from other species in shape, at 391-526 μm long, and 353-418 μm wide. The body was covered with single-pointed spines (Figs 3.3, 3.4, 3.6), densely distributed anteriorly, but further apart toward the posterior end. On the area anterior to the ventral sucker, each spine measured 1.8-3.4 μm long, and 0.6-1.0 μm wide at the base while that on the lateral and posterior regions of the ventral sucker were 2.1-3.1 μm by 1.1-1.6 μm . The oral sucker was subterminal, oval, 16-33 μm in diameter and 36-66 μm wide. The ventral sucker was 41-69 μm in diameter, 81-138 μm wide and located in the same region as the other species studied.

Comparing other parts of the body, the numbers and patterns of papillae distribution around the oral sucker were almost the same as *P. siamensis*. In contrast, papillae around the ventral sucker consisted of three rings (Fig 3.5), *ie*, inner, median and outer. Lines of papillae, radiating from the oral sucker, were also observed in the anterior half of the body in this species. All three types of papillae were observed in this species. Large-domed papillae, bearing diameters 3.7-8.3 μm , were about 2 pairs on the dorsal lip, 2 pairs with one individual papilla between them on the ventral lip of the oral sucker (Fig 3.1) and 6

regularly spaced papillae on the median ring of the ventral sucker lip (Fig 3.5). Those on the dorsal lip were obscure in shape and fused (Fig 3.1). Small-domed papillae were 2.3-4.2 μm in diameter. There were 42 individual papillae around the oral sucker (Fig 3.1), 6 regularly spaced papillae on the inner ring of the ventral sucker lip, and 10-14 individual papillae on the ventral sucker (Fig 3.5). Pit-type papillae, scarcely seen in this study, were 1.0-1.7 μm in diameter. They were observed on the lateral region of the oral sucker (Fig 3.4).

Paragonimus heterotremus

The metacercarial body was ellipsoidal or oval in shape, 230-351 μm long, 163-209 μm wide, and covered with single-pointed spines (Figs 4.2, 4.5), which were quite prominent and abundant at the anterior half of the body, and gradually reduced in number towards the posterior end. The spines anterior to the ventral sucker were 0.6-1.1 μm long and 0.3-0.5 μm wide at the base, while those on the lateral and posterior regions of the ventral sucker were 0.9-1.2 by 0.4-0.9 μm . The oral sucker was subterminal 6-11 μm in diameter and 24-26 μm wide. The ventral sucker was circular or oval, 11-28 μm in diameter, and 31-49 μm wide. Only two types of papillae were found in this study, large-domed and small-domed types. Their diameters were 1.9-3.5 μm (large-domed type) and 1.5-3.1 μm (small-domed type).

Three rings of papillae were observed on the ventral sucker in this study (Fig 4.4), *ie*, the inner, median and outer rings bore the small-domed type, whereas the median ring bore the large-domed type.

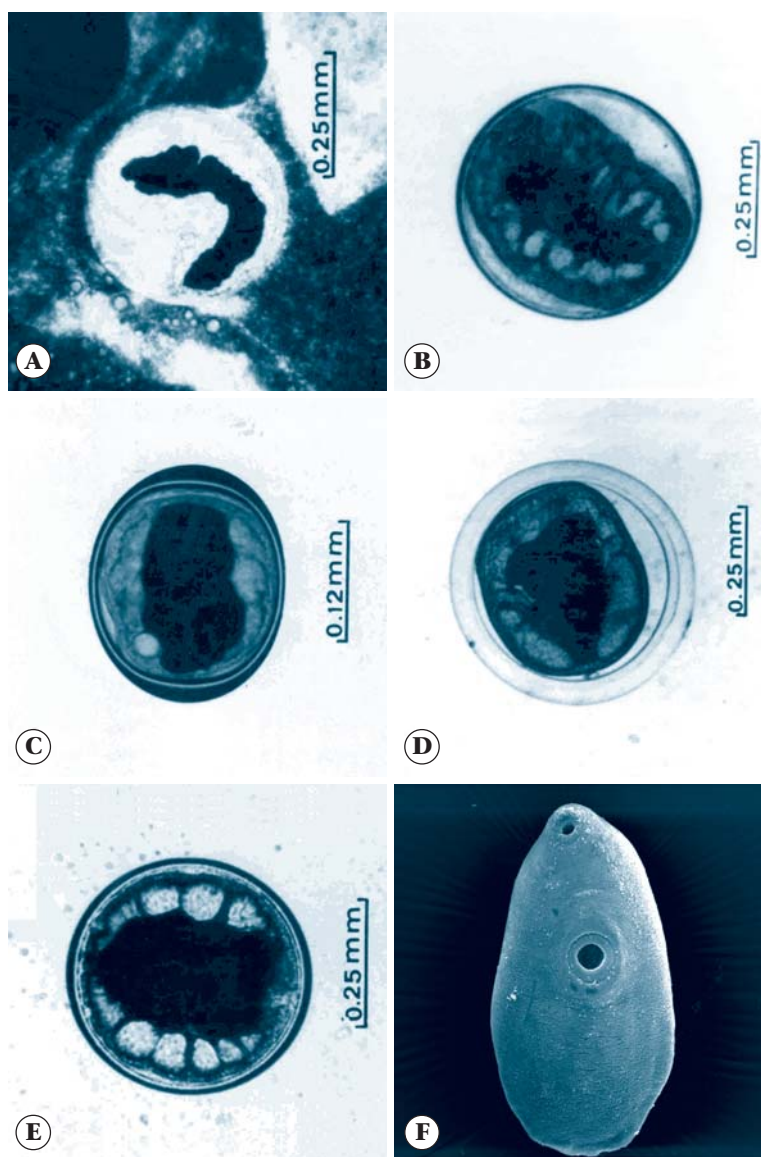


Fig 1 Photographs of metacercariae recovered in this study. A = *P. bangkokensis*, B = *P. harinasutai*, C = *P. heterotremus*, D = *P. siamensis*, E = *P. westermani*, F = Excysted metacercaria.

The large-domed-type papillae, 4 pairs and individual papillae were situated on the lips of the oral suckers (Figs 4.1, 4.2) and six papillae were distributed in regular spaces on the median rings of the ventral sucker lips (Fig 4.4). Many small-domed-type papillae were distributed over the whole body, about 12 papillae around the oral sucker (Figs 4.1, 4.2), 6 regularly spaced papillae on the inner ring of the ventral sucker lips, and 6-

7 individual papillae surrounding the 6 large-domed types around the ventral sucker (Fig 4.4).

Paragonimus siamensis

The excysted metacercariae had various shapes. However, the bodies of most specimens resembled *P. westermani*, being 260-414 μm long and 254-320 μm wide. The body was covered with numerous single-pointed spines with a semilunar

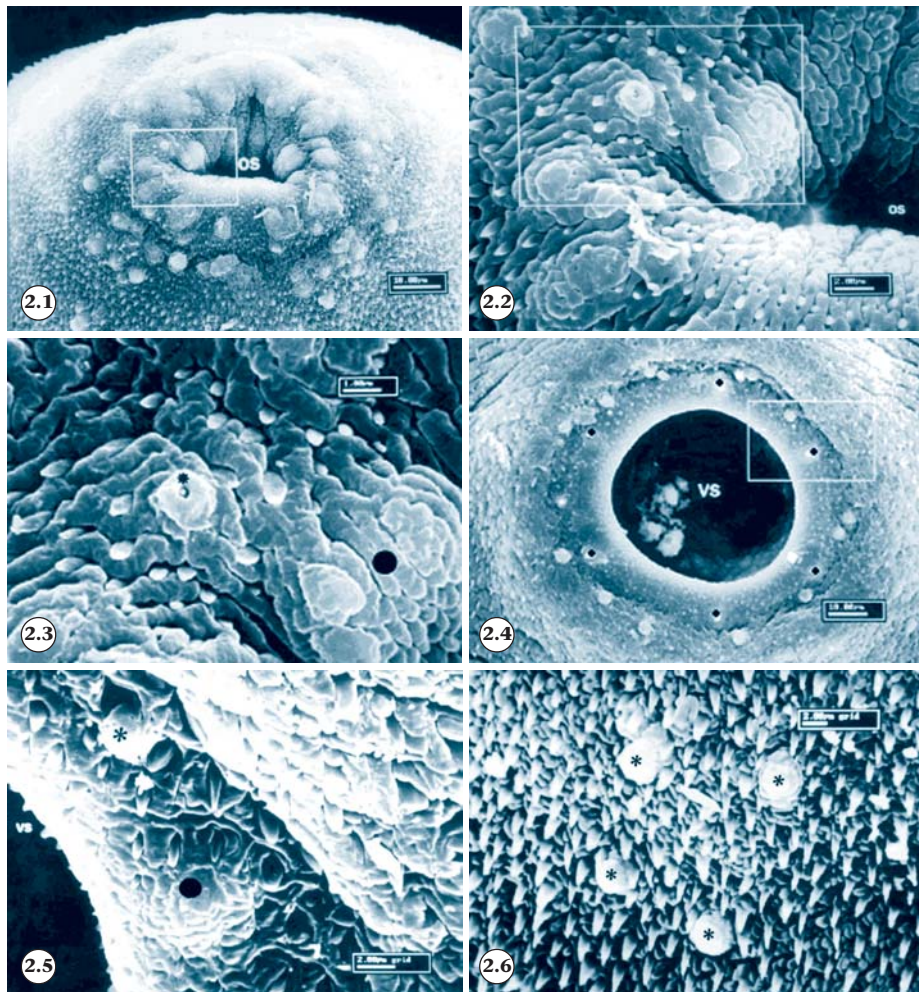


Fig 2 Scanning electron micrographs of excysted metacercariae of *P. bangkokensis*.

2.1 Whole view of oral sucker.

2.2 Enlarged view of rectangular area in Fig 2.1.

2.3 Magnification of rectangular area in Fig 2.2, displaying large-domed papillae (●) and pit- type papillae (*).

2.4 Ventral sucker exhibiting six inner large-domed papillae (●) and outer small-domed papillae.

2.5 Enlargement of rectangular area in Fig 2.4, showing large-domed papillae (●) and small- domed papillae (*).

2.6 Tegumentary spines on the posterior portion of the oral sucker. Some small-domed papillae (*) are seen.

tegumental process at the front base. They gradually reduced in size and number towards the posterior end of the body (Figs 5.8, 5.9). Spines appearing on the area anterior to the ventral sucker were 0.8-1.7 μm long and 0.4-0.9 μm wide at the base while those on the area lateral and posterior

to the ventral suckers were devoid of tegumental spines.

The oral sucker widths and orifices were 52-70 μm and 22-31 μm , respectively. The ventral suckers, larger than the oral ones, were 72-100 μm wide and their orifices 40-72 μm . Except for

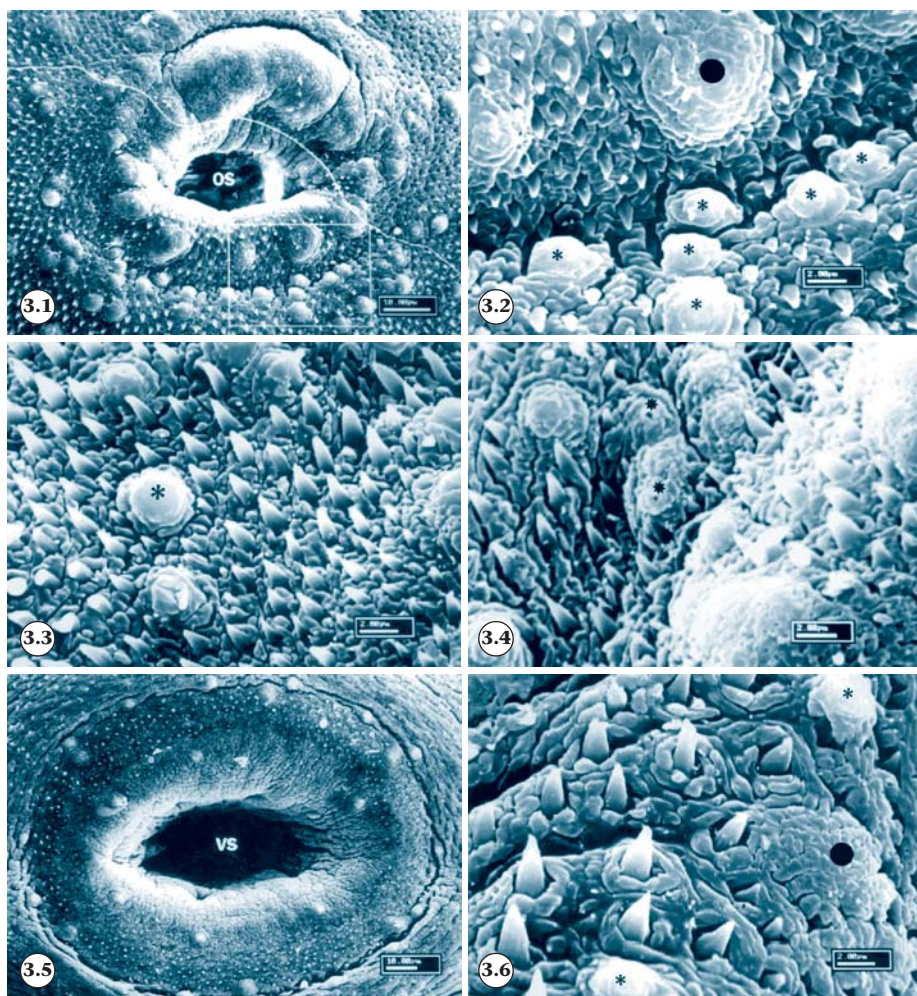


Fig 3 Scanning electron micrographs of excysted metacercariae of *P. harinasutai*.

3.1 Whole view of the oral sucker.

3.2 Higher magnification of rectangular area in Fig 3.1, exhibiting large-domed papilla (●) and small-domed papillae (*).

3.3 Higher magnification of small-domed papillae (*) and spines on the area surrounding the oral sucker.

3.4 Some pit-type papillae (*) on the right-hand side of the oral sucker.

3.5 The ventral sucker (VS) bears rings of papillae.

3.6 Enlarged view of the area around the ventral sucker, displaying large-domed papillae (●), small-domed papillae (*), and spines.

number and distribution, the types and shapes of the papillae found were almost the same as *P. westermani*. Their diameter sizes were 2.6-4.9 μm (large-domed type), 1.9-4.3 μm (small-domed type), and 0.8-1.5 μm (pit-type).

The numbers of papillae around the oral sucker, when compared to other parts of the body

were prominent and larger (the same as *P. westermani*).

Large-domed papillae were also recovered on the lips of both suckers. Three pairs were on the ventral lip of the oral sucker while those on the dorsal lip were concentrated around the central region of the sucker, often obscure in shape, ill-

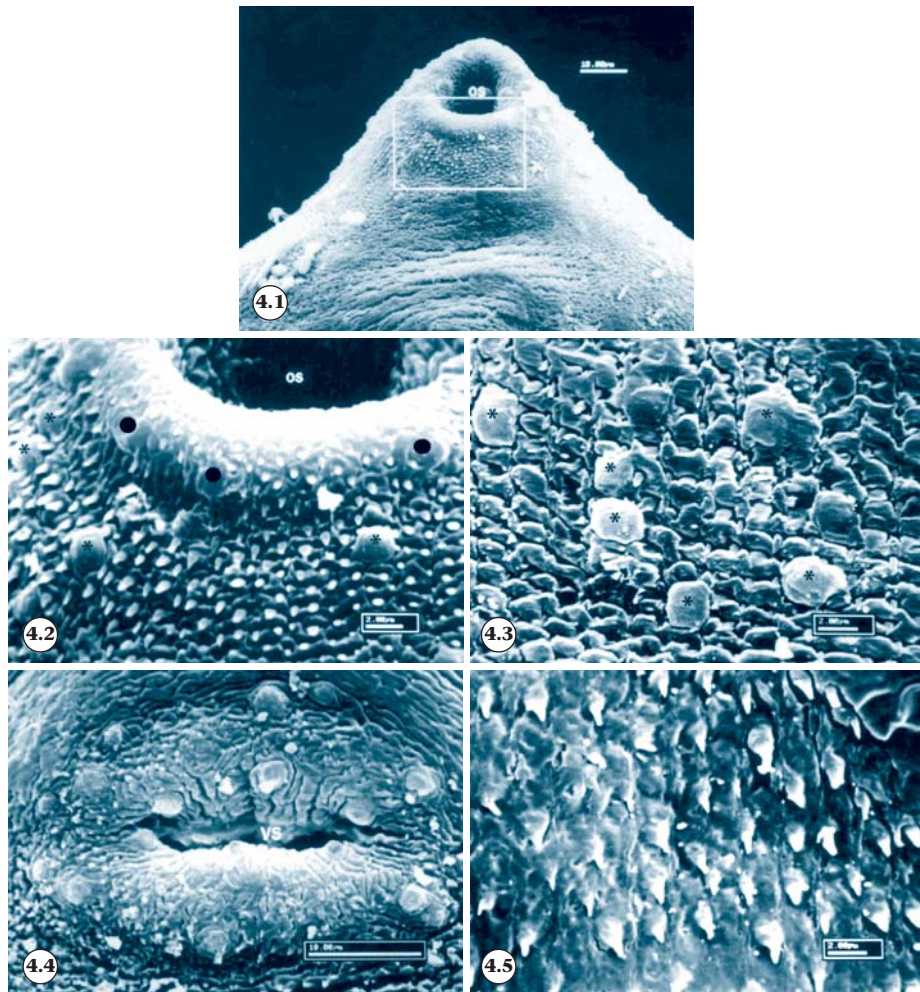


Fig 4 Scanning electron micrographs of excysted metacercariae of *P. heterotremus*.

4.1 Anterior region of the ventral surface.

4.2 Enlarged view of rectangular area in Fig 4.1, showing large-domed (●) and small-domed (*) papillae.

4.3 Posterior portion of the oral sucker; some small-domed papillae (*) are seen.

4.4 Ventral sucker exhibits rings of papillae.

4.5 Tegumentary spines on the area posterior to the ventral sucker.

defined, with a nodular appearance (Figs 5.1, 5.2, 5.3). Their distribution and numbers around the ventral sucker were the same as *P. westermani*.

The small-domed type had 9-10 individual papillae on the oral sucker (Fig 5.1) and 13-14 individual papillae surrounding the 6 large-domed type papillae of the ventral sucker (Fig 5.6). Lines of these papillae, observed in the anterior half of the body, radiated from the oral sucker.

Pit-type papillae were rarely observed in this

study. When they were found, the locations were the lateral region of the oral sucker (Figs 5.4, 5.5).

Paragonimus westermani

The metacercarial body was flattened dorso-ventrally and tapered anteriorly. Whole bodies measured 306-461 by 348-806 μm . The antero-ventrally directed mouth had an opening diameter of 15-36 μm , and a width of 45-64 μm . The ventral suckers, larger than the oral ones, were situated

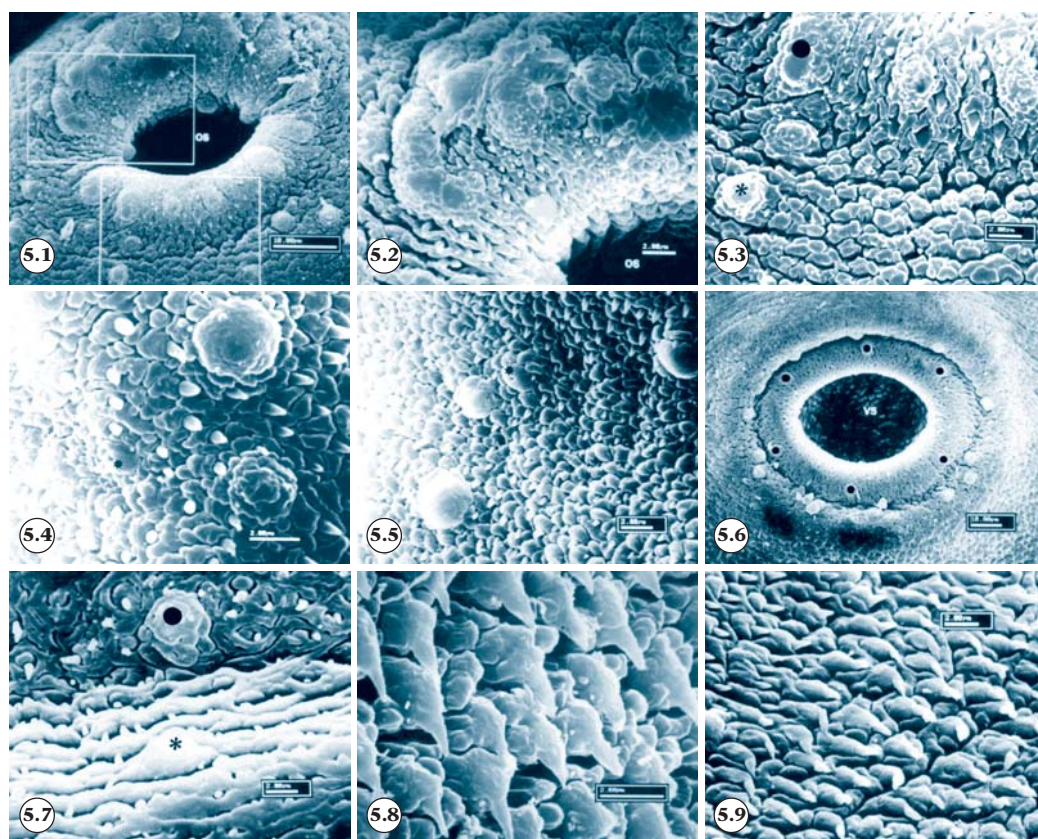


Fig 5 Scanning electron micrographs of excysted metacercariae of *P. siamensis*.

5.1 Oral sucker region.

5.2 Magnification of upper rectangular area in Fig 5.1, displaying fused and irregular papillae of the large-domed type.

5.3 Large-domed type (●) and small-domed type (*) in an enlarged view of the lower rectangular area in Fig 5.1.

5.4 Pit-type papillae (*) on left-hand side of the oral suckers lip.

5.5 Pit-type papillae (*) on right-hand side of the area posterior to the oral sucker.

5.6 Ventral sucker bears 6 large-domed-type papillae (●) while many small-domed-type encircle them.

5.7 Magnification of large- (●) and small-domed papillae (*) on the ventral sucker region.

5.8 Single-pointed spines on the area between the oral and ventral sucker.

5.9 Tegumental spines on the area lateral to the oral sucker.

in the mid-ventral part, slightly anteriorly of the body. Their diameters and widths were 38-65 μm and 66-87 μm , respectively.

The tegument of the whole body was covered with single-pointed spines, which were fairly flattened in shape. Their tips were sharp and became broader near the base (Figs 6.3, 6.5). On the areas anterior to the ventral sucker, each spine

measured 1.9-3.3 μm long and 1.0-1.4 μm wide at the base. In contrast, spines appearing on the areas lateral and posterior to the ventral sucker gradually decreased in number and size, and each spine measured 1.2-1.7 μm long and 0.5-0.8 μm wide at the base. The inner surfaces of the suckers, the lips of the ventral suckers and the ventro-median surfaces of the metacercarial bodies, were devoid

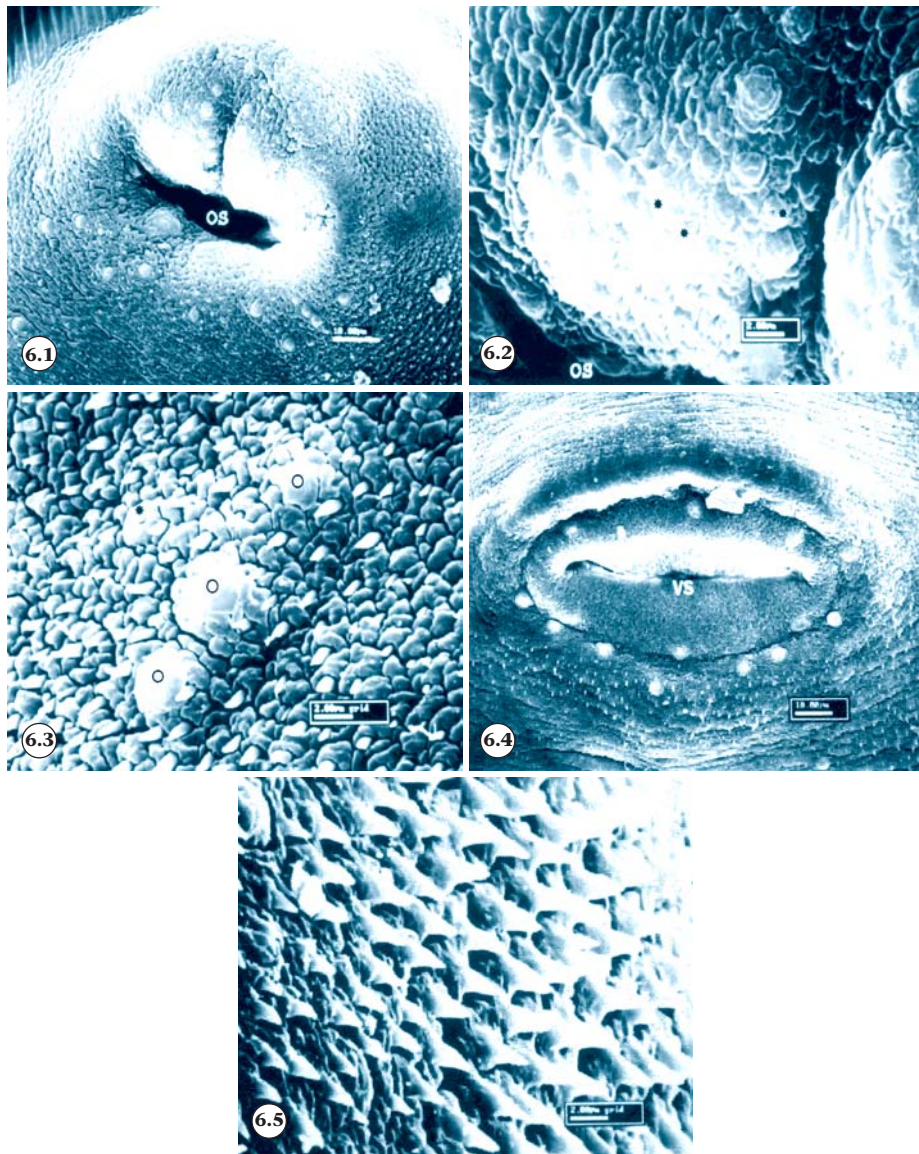


Fig 6 Scanning electron micrographs of excysted metacercariae of *P. westermani*.

6.1 Whole view of the oral sucker (OS).

6.2 Magnification of upper lip of oral sucker (right-hand side); some pit-type papillae (*) are seen.

6.3 Small-domed papillae (O), pit-type papillae (*) and tegumentary spines on area between oral and ventral sucker.

6.4 The ventral sucker exhibits rings of papillae.

6.5 Tegumentary spines on area lateral to oral sucker (left-hand side).

of tegumental spines.

Three types of papillae were observed, large-domed papillae, small-domed papillae, and pit-type papillae. Large-domed papillae were tegumental swellings with ragged or many fine

and short projections or folds on the surface and were 2.1-4.4 μm in diameter. Small-domed papillae were hemispherical, dome-shaped with a smooth surface and almost the same size as the large-domed ones (2.1-4.4 μm). Pit-type papillae, having

diameters of 1.1-1.8 μm , were tegumental protuberances with irregularly shaped pits.

The papillae located around the oral sucker were numerous, exceeding the papillae located in other parts of the body; their distribution showed some differences both within and among the species studied.

The papillae around the ventral sucker were arranged in three circles, *ie*, inner, median and outer rows. The inner and median-ring papillae were large-domed in type and six in number among all individuals examined. These six papillae were regularly arranged, three on the anterior lip and three on the posterior one (Fig 6.4). Outside the regularly spaced six inner papillae, the small-domed type appeared as a ring. Their numbers varied from 9-13 (Fig 6.4).

Other than areas around the suckers, the small-domed papillae were distributed bilaterally especially on the anterior halves of the body surfaces. Lines of papillae, radiating from the oral sucker, were observed on the anterior half of the body, but on the posterior half, they were irregularly distributed.

The large-domed papillae were confined to the lips of the suckers, *ie*, 6 regularly spaced on the median ring of the lip of the ventral sucker (Fig 6.5), whereas those on the oral sucker were not observed in this study (Figs 6.1, 6.2).

The small-domed-type papillae were distributed over the entire body, about 2-3 pairs on the dorsal lip and 10-13 individual papillae on the ventral lips of the oral sucker and 9-13 individual papillae surrounding the 6 large-domed type papillae of the ventral suckers (Figs 6.1, 6.4).

Pit-type papillae were rarely observed on the middle region of the upper lips of the oral suckers (Fig 6.2).

Discussion

A number of attempts to distinguish metacercariae of *Paragonimus* spp through surface ultrastructure were carried out using the numbers of small-dome-shaped papillae around the ventral sucker [8-11]. Intraspecies variation in the numbers of papillae on the ventral sucker was evident. In 1984, Higo and Ishii reported that 7-11 papillae were found in Japanese *P. westermani*,

whereas in their study in 1987, 5-13 papillae were found in the same species [9, 12]. They also reported the numbers of large-domed-type papillae, which were confined to areas around the suckers, as follows: 7-10 pairs on the lip of the oral sucker and 6 regularly spaced on the lip of the ventral sucker. In 1987, they found only 5-6 pairs of this type of papillae on the lips of the oral sucker while those on the ventral sucker appeared to be the same [9]. For the small-domed-type papillae, 10 pairs were distributed around the oral sucker, while their study in 1987 revealed several pairs. In 1984, they found 7-11 papillae surrounding the large-domed type ventral sucker, but in 1987 only 5-13 papillae were found [9, 12].

In the Thai *P. westermani*-like species, which was examined by Kanla [6], 10-13 papillae of this type were recovered. In 1984, Higo and Ishii [12] reported pit-type papillae, which were numerous around the oral sucker, as follows: 6 pairs on the dorsal, 9 pairs on the dorsolateral, and 3-5 pairs on the ventral side. Their report for this type of papillae around the oral sucker in 1987 was 19-25 pairs, whereas the study of Thai *P. westermani*-like by Kanla [6] found 27-30. The results of each paper on *P. westermani* are summarized and tabulated in Table 4. Thai *P. heterotremus* were examined by Sugiyama [7], and the results are summarized in Table 5.

Jiang and Xia [13] also examined the papillae on the surface of *P. heterotremus*. They reported that there were 2 and 3 rings of sensory papillae on the oral and ventral suckers, respectively, but they did not mention about the number of papillae, except for six papillae on the innermost ring on the ventral sucker.

Six large-domed-type papillae, regularly distributed around the ventral sucker, were also observed in the metacercariae of *Fasciola hepatica* [14], *Clonorchis sinensis* [15], *P. peruvianus* [8], Japanese lung flukes: *P. westermani*, *P. pulmonalis*, *P. miyazakii*, *P. ohirai* and *P. iloktsuenensis* [9], Thai *P. heterotremus* [7] and *Opisthorchis viverrini* [16]. Higo and Ishii [12] also proposed that these 6 papillae might generally exist in all metacercarial species of digenetic trematodes.

In the present study, no remarkable morphological differences among the 5 species

Table 2 Numbers of papillae around the oral suckers of *Paragonimus metacercariae*.

Species	LP	SP	PP
<i>P. bangkokensis</i> (2)	Dorsal: 4 papillae Ventral: 5 papillae	16 papillae	Prominent
<i>P. harinasutai</i> (2)	Dorsal: 4 papillae Ventral: 5 papillae	42 papillae	Scarcely seen
<i>P. heterotremus</i> (2)	Dorsal: 4 papillae Ventral: 5 papillae	12 papillae	Not observed
<i>P. siamensis</i> (3)	Dorsal: obscure Ventral: 6 papillae	9-10 papillae	Scarcely seen
<i>P. westermani</i> (3)	Not observed	Dorsal: 2-3 pairs Ventral: 10-13 papillae	Scarcely seen

LP = large-domed-type papillae, SP = small-domed-type papillae, PP = pit-type papillae.

() = numbers of metacercariae studied.

Table 3 Numbers of papillae around the ventral sucker of *Paragonimus metacercariae*.

Species	Inner ring	Middle ring	Outer ring
<i>P. bangkokensis</i> (2)	-	6	13-14
<i>P. harinasutai</i> (2)	6	6	10-14
<i>P. heterotremus</i> (2)	6	6	6-7
<i>P. siamensis</i> (4)	-	6	13-14
<i>P. westermani</i> (4)	6	6	9-13

() = numbers of metacercariae studied.

Table 4 Variations in papillae found on newly excysted *P. westermani*.

Oral sucker		Ventral sucker	
LP	7-10 pairs (Higo & Ishii, 1984) 5-6 pairs (Higo & Ishii, 1987) none (present study)	LP	6 papillae (Higo & Ishii, 1984) 6 papillae (Higo & Ishii, 1987) 4 papillae (Kanla <i>et al</i> , 1997) 6 papillae (present study)
SP	10 pairs (Higo & Ishii, 1984) several pairs (Higo & Ishii, 1987) 2-3 pairs on dorsal lip and 10-13 papillae on ventral lip (present study)	SP	7-11 papillae (Higo & Ishii, 1984) 5-13 papillae (Higo & Ishii, 1987) 10-13 papillae (Kanla <i>et al</i> , 1997) 15-19 papillae (present study)
PP	18-20 pairs (Higo & Ishii, 1984) 19-25 pairs (Higo & Ishii, 1987) 27-30 papillae (Kanla <i>et al</i> , 1997)		

LP = large-domed-type papillae, SP = small-domed-type papillae, PP = pit-type papillae.

studied were evident, except for the size and arrangement of the sensory papillae around the oral and ventral suckers. Though many authors tried to use the numbers of papillae around the ventral sucker to discriminate *Paragonimus* worms, it was quite difficult due to variations in various steps, as follows:

Species variation: the papillae distributed around the oral sucker, in this report, consisted of 3 types, with the exception of *P. heterotremus* and *P. westermani*, in which only 2 types were found. *P. westermani* had small-domed and pit-type papillae, whereas *P. heterotremus* had large-domed and small-domed type. The types of papillae around the oral and ventral suckers among the five *Paragonimus* species studied were quite different, as summarized in Table 6.

Study variations: when the present study is compared with previous reports, which are valid only for *P. westermani* and *P. heterotremus*, all 3 types of papillae were recovered, with the differences summarized in Tables 4 and 5. For *P. westermani*, the number of small-domed papillae was higher than that of Higo and Ishii [12], but pit-type papillae, had the least. For *P. heterotremus*, this study found more large-domed type, less small-domed type, and no pit-type.

The numbers of pit-type papillae in this study (Tables 2, 4, 5) differed from other reports on *Paragonimus* spp (Tables 4, 5), because among the 5 species studied, only *P. bangkokensis* had prominent pit-type papillae, while they were

hardly seen in the other species. For the 3 species in which pit-type papillae were rarely seen (*P. harinasutai*, *P. siamensis* and *P. westermani*), it was necessary to investigate around the oral suckers as in the previous reports and use higher magnifications to find only one or two.

The types of papillae distributed around the ventral sucker corresponded to other reports, in which both large- and small-domed types were found, while in the present study, an additional ring on the surface of the ventral sucker was observed. Two rings of papillae, around the ventral sucker, were reported in most of the *Paragonimus* spp reviewed, except for *P. heterotremus* by Jiang and Xia [13], who observed 3 rings of papillae on this sucker, which were similar to the present study of the same species.

The other 2 species, in the present study, which were found to have 3 rings of papillae around the ventral sucker, were *P. westermani* and *P. harinasutai*. This was the first report for *P. harinasutai* of its morphology by SEM, but for *P. westermani*, it differed from others in the additional ring found in the present study. The inner ring was the one close to the mouth of the ventral sucker (MVS), while the outer ring was on the outermost one. The middle ring was between these 2 rings. The inner and middle rings were on the surface of the ventral sucker while the outer ring was around its surface. The papillae in the inner and middle ring were constantly 6 in number in each, and were regularly distributed, 3

Table 5 Variations in papillae found on newly excysted *P. heterotremus*.

Oral sucker		Ventral sucker	
LP	4 on dorsal lip & 2 on ventral lip (Sugiyama <i>et al</i> , 1990) 2 pairs on dorsal lip, 2 pairs & 1 pair on ventral lip (present study)	LP	6 (Sugiyama <i>et al</i> , 1990) 6 (present study)
SP	15 (Sugiyama <i>et al</i> , 1990) 12 (present study)	SP	9-13 (Sugiyama <i>et al</i> , 1990) 12-13 (present study)
PP	30 (Sugiyama <i>et al</i> , 1990) none (present study)		

LP = large-domed-type papillae, SP = small-domed-type papillae, PP = pit-type papillae.

Table 6 Types of papillae found around oral and ventral suckers among five *Paragonimus* species.

Species	LP		SP		PP OS only
	OS	VS	OS	VS	
<i>P. bangkokensis</i>	+	+	+	+	prominent
<i>P. harinasutai</i>	+	+	+	+	rare
<i>P. heterotremus</i>	+	+	+	+	rare
<i>P. siamensis</i>	+	+	+	+	rare
<i>P. westermani</i>	-	+	+	+	rare

LP = large-domed-type papillae, SP = small-domed-type papillae, PP = pit-type papillae, OS = oral sucker, VS = ventral sucker.

on the upper lip and 3 on the lower (Table 3). These corresponded to the numbers, proposed by Higo and Ishii [12], and the above-mentioned trematodes. However, in Thailand, some reports opposed this proposal, *ie*, in newly excysted metacercariae of *P. westermani*-like [6], where only 4 large-domed papillae around the ventral sucker were found. The variations in numbers of papillae in the outer ring were indistinct, as summarized in Table 6, compared to previous reports of the numbers of papillae in this type of *P. westermani* in Table 4, and *P. heterotremus* in Table 5. In this report, *P. westermani* was in the same range, while *P. heterotremus* was below it (Table 3). However, further observations using more specimens are required to confirm this finding.

The tegumental spines found in this study were similar to others described for newly excysted *Paragonimus* metacercariae, *ie*, single-pointed spines rather different in shape among the species studied. Hong [17] explained that the structure of the tegumental spines of flukes was closely related to the migratory behavior, route and final niche of each species in the host. When fluke age was not a consideration, the spines exhibited the maximal numbers of points, and spines with maximal size appeared at the dorsal and ventral anterior surfaces; their point and size numbers decreased on the lateral and posterior portions of the body. Among trematodes, the tegumental spines were more simply pointed on the surface of juveniles than adults, *eg*, *Metagonimus yokogawai* [18], *Cryptocotyle lingua* [19], or *Fibricola seoulensis*

[20] and on migratory stages than non-migratory ones, *Fasciola hepatica* [21], *P. westermani* [22], or *P. iloktsuenensis* [23]. The published data suggest that the simple spines on juvenile flukes probably function in locomotion and anchorage [17].

Compared to the tegumentary spines of other flukes, *O. viverrini* have two types of edge-shaped spine [16], *ie*, a serrated edge on the anterior part, and a single sharp edge on the middle part. This was similar to those reported in the same stage of *C. sinensis* [15]. However, the serrated spines of *O. viverrini* were much larger in size. In contrast, the spines of *F. hepatica* juvenile appeared to be smallest and were single-pointed type [14].

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