SURFACE ULTRASTRUCTURE OF EXCYSTED METACERCARIAE OF *HAPLORCHIS TAICHUI* (TREMATODA: HETEROPHYIDAE)

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Abstract. The morphology of newly excysted juvenile *Haplorchis taichui* was studied using a light and a scanning electron microscope (SEM). The reproductive organs were well developed. The whole body surface was covered with numerous transverse rows of scale-like spines, which had 3-11 points at the tip. The spines on the dorsal were similar with those on the ventral surfaces in shape, size and number of points. The spines in anterior were digitated into 10-11 points, then 8-9 points and 7 points. The presence of 3 points in each spine was observed in the area adjacent to the excretory pore. Two types of sensory papillae existed throughout the body: type I, ciliated knob-like swellings and type II, round swellings of the tegument. The rapid maturation of *H. taichui* results from the development of both internal and external organs during the newly excysted stage.

INTRODUCTION

Haplorchis taichui, a heterophyid fluke, is a minute intestinal fluke that parasitizes the small intestines of birds and mammals, including humans (Faust and Nishigori, 1926). This parasite is now recorded as the most common fluke found in the gastrointestinal tract of human populations of northern Thailand (Radomyos *et al*, 1998). Humans acquire this parasite via the consumption of raw and/or undercooked fresh water fish that contains an infective stage or metacercariae.

H. taichui rapidly matures in definitive hosts. Adult worms with immature eggs are found at 4 days after ingestion of the metacercariae (Faust and Nishigori, 1926). Scholz *et al* (1991a) revealed the external morphology of adult *H. taichui* under scanning electron microscopy. However, there is no study concerning the external and internal morphology of newly excysted worm. Therefore present study was performed to observe the internal and surface morphology of excysted *H. taichui* metacercariae, and to know the relationship between maturation of genital organs and development of surface ultrastructure.

MATERIALS AND METHODS

Collection of *H. taichui* metacercariae

Metacercarial cysts of *H. taichui* were collected from cyprinoid fish, *Thynnichthys thynnoides*, caught in the Mae Ngud reservoir of Mae Tang District, Chiang Mai Province, northern Thailand. The collected fish were rinsed in tap water until clean, eriscerated and ground with a mortar. The ground fish was digested by acid pepsin solution (concentrated hydrochloric acid 1 ml: porchine pepsin (Sigma®, Germany), 1 g; physiological saline 99 ml) in a water bath shaker for 1 hour and 30 minutes at 37ºC. The digested material was passed through 2 layers of wet gauze, rinsed with physiological saline and examined with a stereomicroscope. The collected metacercariae were identified by the general morphology of cysts (Scholz *et al* 1991b) and sclerites on the ventrogenital sac (Pearson and Ow-Yang, 1982).

Excystation of metacercariae

Approximately 300 metacercariae containing active juveniles were selected under the stereomicroscope and excysted with 1% trypsin solution (Sigma®, Germany) in a water bath shaker of 37ºC for 15 minutes.

Light microscopic observation

The excysted worms were fixed with hot alcohol-formalin-acetic acid, stained with Mayer's carmine, mounted with Permount® and observed with a light microscope.

Scanning electron microscopy

Some excysted juveniles were washed 3 times with physiological saline. Among them, fifty ac-

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tive flukes were fixed with 2.5% glutaraldehyde at 4ºC for 24 hours and subsequently postfixed with 1% osmium tetroxide. They were dehydrated in a graded alcohol series, dried in a critical-point dryer, coated with gold and observed with a JEOL-JSM840A scanning electron microscope at an accelerating voltage of 20 kV, and photographed with Kodak® Verichrome pan film VP 120.

RESULTS

Light microscopic findings

The newly excysted juvenile *H. taichui* was elongated oval in shape, approximately 286 μm long (range; 220-346) and 134 μm wide (range; 116-147), when measured in 11 fixed specimens. A scattering of dark cells was apparent in the whole region of the worm, with the most prominent being the group that will develop into several organs such as the testis, ovary and/or seminal vesicles (Figs 1A, 1B). The oral sucker was located near the anterior end of the body and was well developed. The pharynx was presented posterior to the oral sucker, while the prepharynx was not clear. The esophagus was relatively long and it bifurcated into the intestinal ceca, and extending excess testis. The testis appeared as a group of densely and darkly massed cells, slightly oblique in position, with a smooth outline and situated anterior to the excretory bladder. The ovary could be detected by the presence of a nearly circular to ovoid cell collection situated posterior to the ventrogenital sac. The seminal vesicle rudiments, which were visible in some specimens, could be made out as two cell groups densely packed on the side opposite the ovary. Vitelline cells were not clearly delineated. The excretory bladder was a swollen Y-shape with a short stem.

Scanning electron microscopic findings

The general appearance of newly excysted juvenile *H. taichui* including the oral sucker, ventrogenital sac and excretory pore are shown

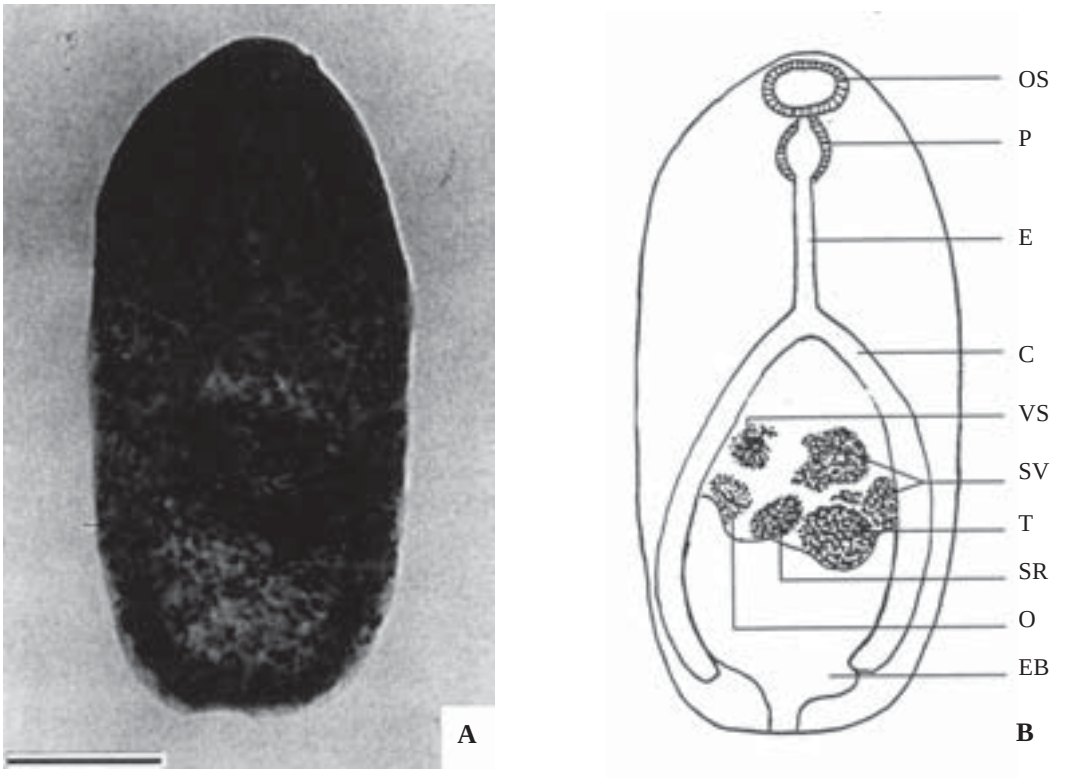


Fig 1–Newly excysted juvenile *Haplorchis taichui.* A: Light micrograph of a worm stained with Mayer's carmine. B: Diagrammatic of internal organs of a worm showing the oral sucker (OS), ventrogenital sac (VS), pharynx (P), esophagus (E), cecum (C), testis (T), ovary (O), seminal receptacle (SR), seminal vesicles (SV) and excretory bladder (EB). Bar = 0.1 mm.

ventrally in Fig 2. The oral sucker was large in size and located subterminally on the ventral surface (Fig 3). The ventrogenital sac lied ventrally somewhere in the middle region of the body and armed with 16 sclerites (Fig 4). The excretory pore was located in the most posterior end of the body (Fig 5).

The whole body of worms was covered with numerous transverse rows of backwardly directed scale-like spines, each of which comprised 3-11 densely packed points (Figs 6-11). The maximum width of tegumental spines was up to 3 μm at the anterior end and decreased downward to about 0.5 μm at the posterior end. The number of points in each spine gradually decreased posteriorly. Tegumental spines in anterior portion were digitated into 10-11 densely packed points and each spine in the same transverse row was close together (Fig 6). Those in anterior 2/5 were digitated into 8-9 points (Fig 7). Seven points were present on a majority of spines on the middle surface (Fig 8). Posteriorly, the spines had 6 points (Fig 9) followed by 5 points and downwards (Fig 10). In the posteriormost of the body, just in front of an excretory pore, the number of points decreased to 3 and interspinous space became wider (Fig 11).

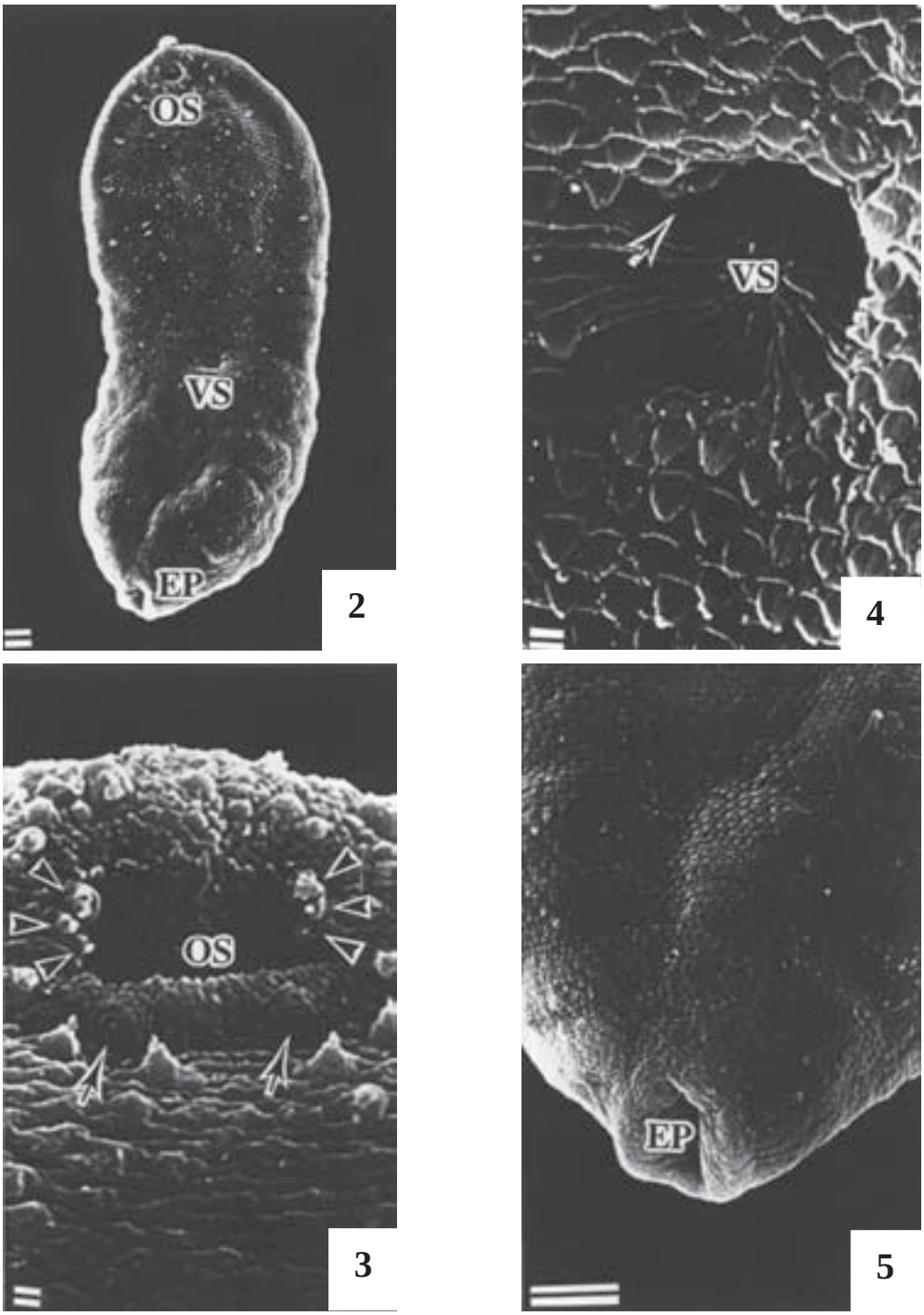
Several sensory papillae were presented on the body, both ventrally and dorsally, particularly on the anterior half of the region. Mainly, they comprised two different types as follows: type I, ciliated knob-like swellings (Figs 12-16), and type II, round swellings of the tegument (arrows in Fig 3), with only a pair observed on the ventral lip of the oral sucker.

Regarding the distribution of these type I papillae, they appeared as a bilaterally symmetrical arrangement. All of their numbers and distribution are represented diagramatically in Fig 17. When viewed ventrally, type I was the most abundant papillae to appear on the anteriormost region, with approximately 12-15 pairs of single papillae encircling the oral sucker. A group of three single papillae occurred laterally on the dorsal lip of the oral sucker (arrowheads in Fig 3). Three pairs in a group of 5 rounded papillae (Fig 15) were lined in the most lateral tracts of the ventral side. In only some cases, a group of 6 rounded papillae were presented instead of 5 (Fig 16). Papillae did not occur on the lip of the ventrogenital sac. A pair in a group of 3 (Fig 14) and 2 rounded papillae (Fig 13) were observed obliquely beyond and lower the ventrogenital sac, respectively (Fig 17). Regarding these papillae, some are ciliated while others are not (Figs 14, 16). Posteriorly, three pairs of single papillae are linearly and irregularly spaced, but symmetrically oblique to the excretory pore. When viewed dorsally, three pairs in a group of 5 rounded papillae were arranged alternately in a zigzag pattern with single papillae (Fig 17). A smaller number of papillae appeared in the dorsal view than those shown in the ventral one.

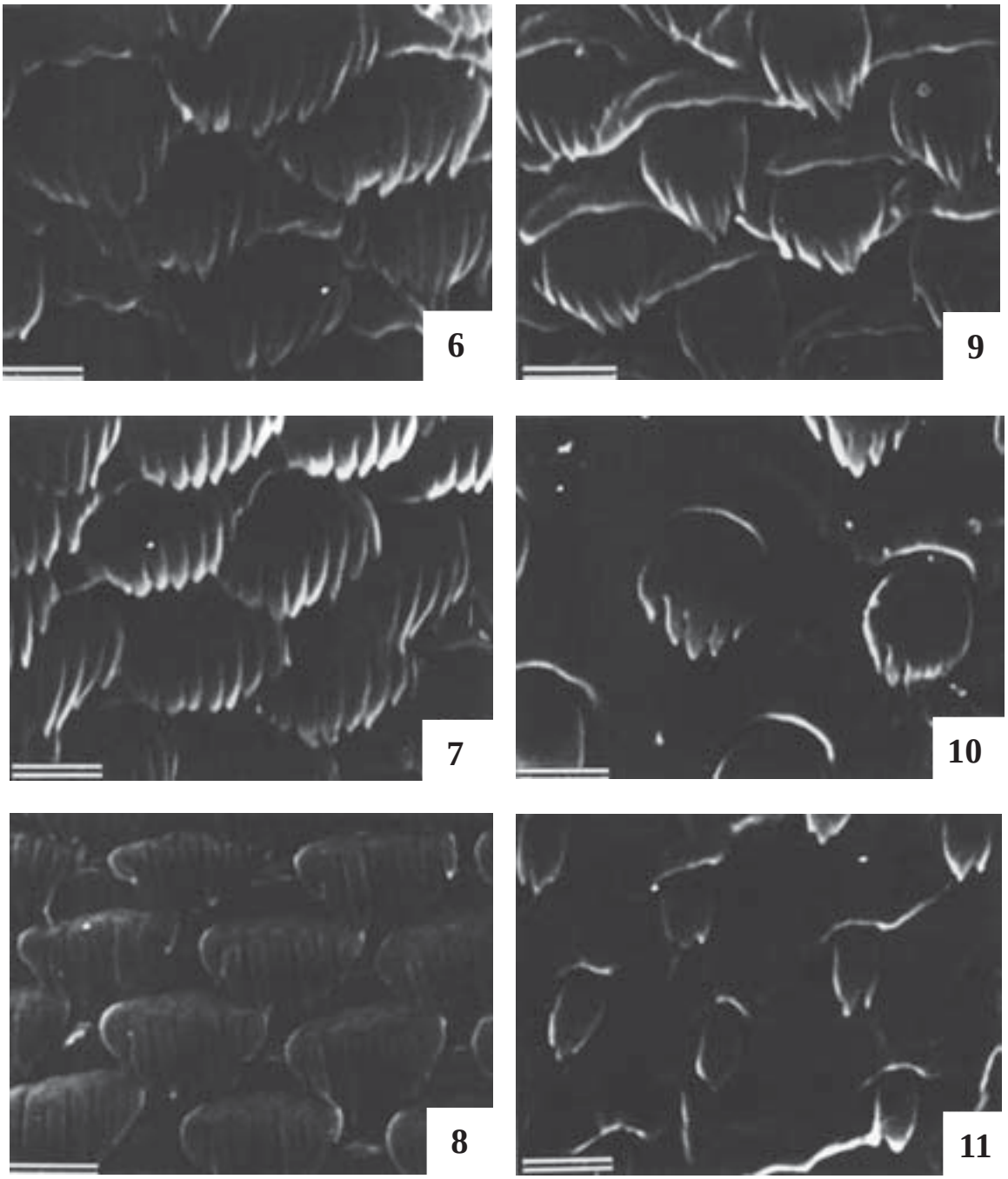
DISCUSSION

The results of this study revealed that both the internal organs and external morphology (tegumental spines and sensory papillae) of newly excysted juvenile *H. taichui* were well developed. This implied that the function of these structures was to help the worm establish and/or survive efficiently in the intervillous crypts of the small intestine of the host. Regarding the function of tegumental spines in trematodes, Bennett (1975) suggested that they are implicated in the locomotory and feeding process. The more pointed and enlarged spines that occurred between the oral and ventral suckers of adult flukes are likely related to the abrasion of host tissues for feeding and, to a lesser degree, anchorage. The presence of spines on the entire worm surface help the newly excysted worms to maintain good contact with the intestinal wall, which in turn, prevent them from being passed in feces (Srisawangwong *et al*, 1989). In accordance with Hong *et al* (1991), the structure of the tegumental spines of flukes is closely related to migratory behavior, route and the final niche of each species in the host. The absence of spines in the tegument overlaying the oral sucker is probably an adaptation, which allow the sucker to make a smooth seal with the substrate prior to suction (Bennett, 1975).

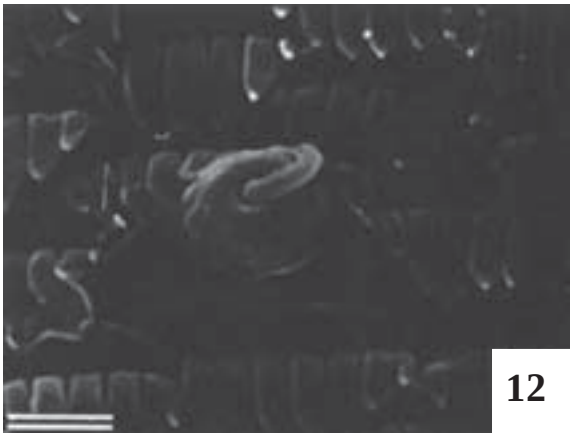
With regard to the morphology of tegumental spines, the number of points on an individual spine of newly excysted *H. taichui* is similar to those that occurred in its adult stage, in which the points gradually decrease posteriorly (Scholz *et al*, 1991a). This similarity in the pattern of tegumental spines of newly excysted and adult worms could be because the small intestine, the habitat of the adult, was the same as where the worm excysted. Worms do not necessarily migrate to other parts of body, such as the bile duct in the case of liver flukes, *Fasciola hepatica*, *Clonorchis sinensis* and *Opisthorchis*

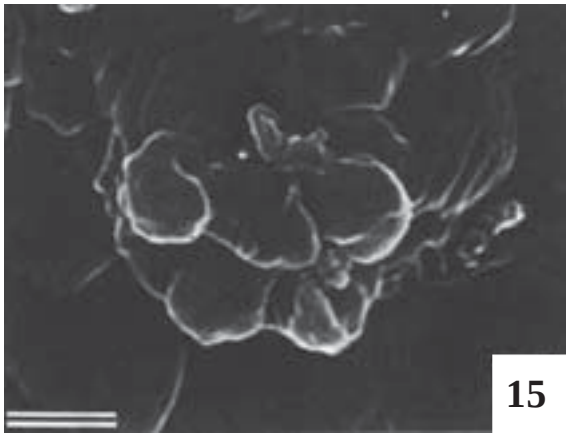


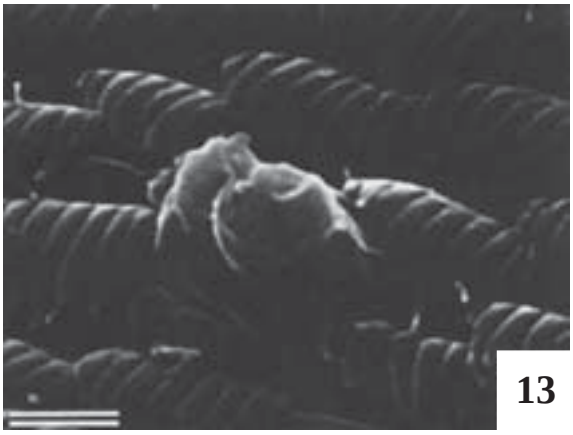
Figs 2-5–Scanning electron micrographs of newly excysted juvenile *Haplorchis taichui*. (2) Whole ventral view showing the tegumental spines covering the whole body surface. Bar = 10 μm. (3) Enlarged view of the oral sucker. Arrowhead and arrow indicate type I and type II sensory papillae, respectively. Bar = 1 μm. (4) Enlarged view of the ventrogenital sac that bears 16 sclerites. Arrow indicates the genital opening. Bar = 1 μm. (5) Enlarged view of an excretory pore. Bar = 10 μm. Abbreviation: OS = Oral sucker, VS = Ventrogenital sac, EP = Excretory pore.

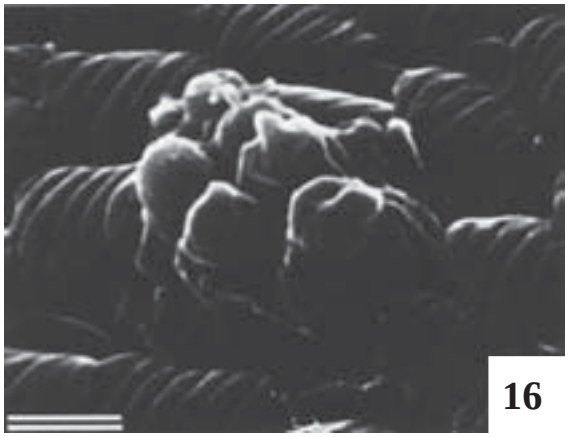


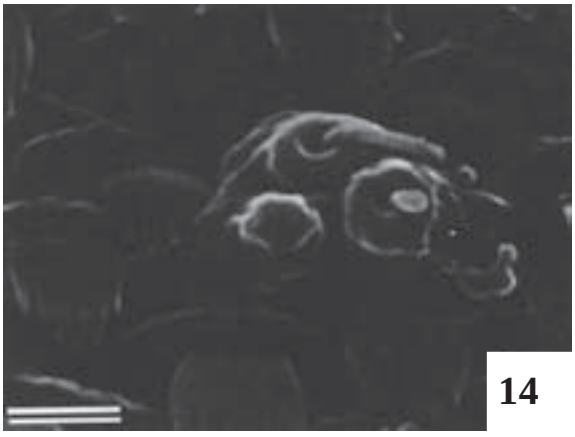
Figs 6-11–Scanning electron micrographs of newly excysted juvenile *Haplorchis taichui*. (6) Dorsal surface of the anteriormost of the body showing the tegumental spines with 10-11 points. (7) Dorsal surface of two-fifths of the body showing the tegumental spines with 8-9 points. (8) Ventral surface on the middle region of the body showing the tegumental spines with 7 points. (9) Ventral surface of the posterior part of the body showing the tegumental spines with 6 points. (10) Ventral surface of the posterior part of the body showing the tegumental spines with 5 points. (11) Ventral surface of the posterior region of the body, just beyond the excretory pore, showing the tegumental spines with 3 points. Scale bar = 1 μm for all figures.











Figs 12-16–Scanning electron micrographs of sensory papillae of newly excysted juvenile *Haplorchis taichui*. (12) Single cilium. (13) A group of 2 rounded papillae. (14) A group of 3 rounded papillae (with and without any cilium in the same swelling tegument). (15) A group of 5 rounded papillae. Non-ciliated papillae are shown in the same swelling tegument. (16) A group of 6 rounded papillae (with and without any cilium in the same swelling tegument). Scale bar = 1 μm for all figures.

viverrini (Bennett and Threadgold, 1975; Fujino *et al*, 1979; Apinhasmit *et al*, 1993). The changes in the tegumental spines between the newly excysted and adult worms of these liver flukes existed so as to serve their migration more efficiently.

Sensory papillae are a common feature on tegument of trematode juveniles and adult worms. They enabled the internal parasite to establish itself via a perception of considerable information of such factors as the proximity of food, the presence of a noxious substance or the nature of the substratum (Ip and Desser, 1984). Regarding the

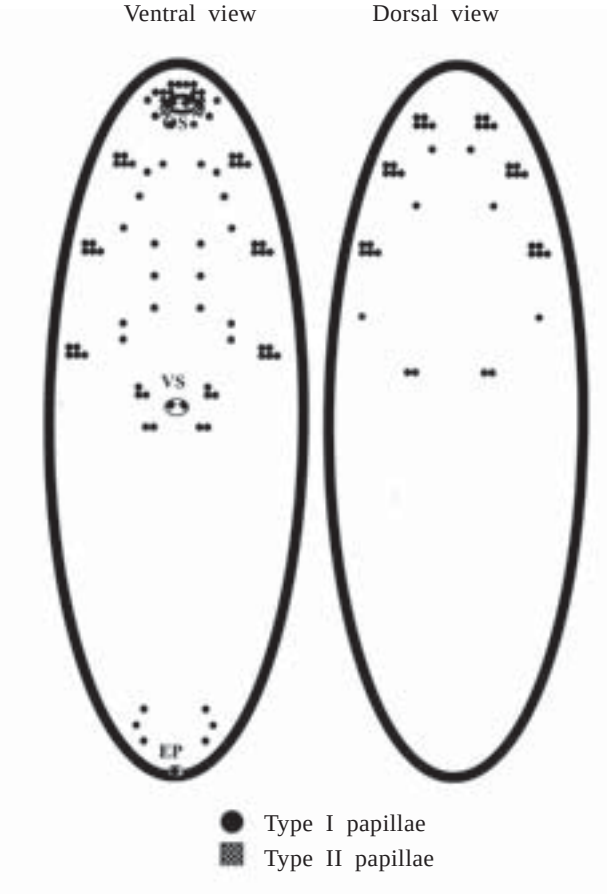


Fig 17–Papillae distribution on the tegument of newly excysted juvenile *Haplorchis taichui*. Abbreviation see Figs 2-5.

heterophyid flukes, both type I and type II sensory papillae have been reported from several species, *eg*, *Metagonimus yogokawai* (Lee *et al*, 1984), *Heterophyopsis continua* (Hong *et al*, 1991), *Heterophyes nocens* (Chai *et al*, 1992), *Metagonimus miyatai* (Chai *et al*, 1998).

Sensory papillae with a cilium at the apex are observed on the tegumental surface of trematode species. Sensory papillae with a cilium that arises from a hollow basal bulb (type I papillae) were the most predominant papillae occurring in newly excysted *H. taichui*, particularly around the oral sucker. Transmission electron microscopic (TEM) studies showed that the ciliated type papillae are compose of a neurone containing microtubules, which penetrated the peripheral body musculature and tegumental basal lamina and formed a "winged" bulb within the tegumental syncytium (Bennett, 1975). The numerous ciliated papillae around the oral sucker could be involved in actively probing the host environs for feeding (Hong *et al*, 1991) or they might be tangoreceptors responsible for producing locomotor movements (Bennett, 1975). The study of Sobhon *et al* (1986) on *O. viverrini* tegument suggested that the ciliated papillae are probably involved in tactile recognition, since they bear cilia. Fujino *et al* (1979) suggested that papillae with cilia may function as chemoreceptors. Since type I papillae appeared both singularly and as a group on newly excysted *H. taichui* (*eg* a group of 3 on the dorsal lip of the oral sucker), it is suggested that grouped sensory papillae are more sensitive to the changes of their environs than single ones (Hong *et al*, 1991). They may also be used to determine the nature of the environment into which the oral sucker is projecting during its migration in the definitive host (Bennett, 1975).

A pair of type II sensory papillae of excysted *H. taichui* were similar to those in other heterophyid flukes (Lee *et al*, 1984; Hong *et al*, 1991; Chai *et al*, 1992; 1998). TEM studies of the "domed" type by Bennett (1975) on *F. hepatica* showed that it consists of a neuronal bulb that contains two electron-dense collars, a typical ciliary basal body and a striated rootlet. Because of the location, arrangement and structure of the type II papillae, it is suggested that they may be tangoreceptors and/or pressure receptors in function (Bennett, 1975; Sobhon *et al*, 1986; Hong *et al*, 1991).

When viewed internally, alimentary and reproductive systems of newly excysted juvenile *H. taichui* were well developed, as seen by the obvious internal organs in both systems. This information provide the explanation as to why this fluke rapidly matures, according to the presence of eggs in the uterus as early as 3 days post-infection (Sukontason, unpublished data). The rapid maturation of *H. taichui* is similar to that of *Centrocestus formosanus* (Srisawangwong *et al*, 1997). Hence, the rapid maturation of *H. taichui* results from the development of both internal and external organs during the newly excysted stage.

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