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Surface Morphology of *Echinostoma revolutum* (Digenea: Echinostomatidae): Excysted Metacercariae

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ABSTRACT

The surface ultrastructure of *Echinostoma revolutum* (Digenea: Echinostomatidae), obtained from freshwater snails, *Filopaludina* spp., naturally infected with the metacercariae, was studied by scanning electron microscopy. The excysted metacercaria was elongated, ventrally concave and pointed posteriorly. The anterior half body surface was covered with numerous peg-like tegumental spines. The oral sucker was circular and was situated subterminally at the antero ventral side of the body. The lip of the oral sucker was devoid of spines. The lip of oral sucker exhibited wrinkled cytoplasmic processes and had ciliated knob-like structure papillae. The head crown was surrounded by 37 retractable, horseshoe-shaped collar spines, which were embedded in cytoplasmic pockets. The ventral sucker was medial at one third of the body. The excretory pore was terminal, and showed a smooth tegument. A number of the ultrastructural features revealed may prove to represent taxonomically informative characters, some of which may be useful at the genus and species level.

INTRODUCTION

Echinostomes are intestinal parasitic trematodes whose adult stage infects a wide range of vertebrate host species, including mammals and humans, and whose larval stages are also parasitic on numerous invertebrate and vertebrate hosts [1]. Trematodes of the family Echinostomatidae (echinostomes) are food-borne parasitic zoonoses, which can cause a serious public health concern in many parts of the world [2]. Within the genus *Echinostoma*, the 37-collar spines group contains sibling species which resemble one another closely, so it's difficult to distinguish such parasites from one another by using only traditional morphological characteristics or conventional methods. For this reason, the systematics of *Echinostoma* have long been problematic due to the inter-specific homogeneity of characters and the poor differential diagnoses of newly established taxa [3]. Therefore, specific and accurate detections are needed for better classification, identification and epidemiological control programs. Due to the fact that the systematics of the genus *Echinostoma* still remain in a confused state, more studies are needed to improve the definition and reliability of *Echinostoma* identification.

Echinostoma revolutum is an intestinal trematode of birds and mammals including humans, and is the type species of this genus [4-5]. The actual morphological features of this species are required for setting an updated standard description. Therefore, in this study we described and illustrated the topographical features of newly excysted metacercariae of *E. revolutum* by using scanning electron microscopy (SEM) in order to improve knowledge of the topography of the tegumental surface as taxonomic characteristics of *E. revolutum*.

METHODOLOGY

Specimen preparation

Freshwater snails, *Filopaludina* spp. were collected from small streams in paddy fields of Hang Dong district (18º43′38.6″N 98º55′48.6″E), Chiang Mai province, Thailand. Encysted metacercariae of *E. revolutum* were removed from the pericardial region of naturally infected, *F. martensi martensi* and *F. sumatrensis polygramma* snails. Metacercariae were excysted under a cover slip, by quickly applying high pressure with a pin on the cover slip, and were observed morphologically.

LM study

The newly excysted metacercariae were compressed, fixed in 5‰ formalin, dehydrated in an ethanol series, stained with aceto-carmine, cleared with xylene and mounted in permount for permanent slides. Freshly isolated and permanent slides of adult worms were examined. Morphological traits of worms were studied and measured using the Olympus equipped microscope. Measurements and scales are in micrometers and illustrations were obtained with the aid of the camera lucida.

SEM study

The newly excysted metacercariae were rinsed several times in saline and fixed in 2.5% (w/v) glutaraldehyde at 4°C for 24 hours, washed several times in phosphate buffer and post fixed with 1% osmium tetroxide for 3 hours. Subsequently, they were dehydrated in a graded alcohol series, dried in a critical-point dryer, and then were

coated with gold. The specimens were observed and photographed under a JEOL JSM-5400LV scanning electron microscope.

RESULTS AND DISCUSSION

LM study

The general morphology of the encysted and excysted metacercariae were as follows: The encysted metacercaria, folded within a transparent cyst (Fig. 1), was spherical, 127.0-189.0 µm in diameter, with a bilayered wall (n=50). The cyst wall consisted of an outer and inner layer. Circumoral collar spines, 37 in total, excretory granules and suckers were visible through the cyst wall and the excretory organs filled with concretions through the cyst wall. They were chiefly found from the pericardial sac of the snail host.

The newly excysted metacercaria was elongated and oval in shape, 610-1,150 µm long and 180-260 µm wide (Fig. 2). In the excysted metacercaria, the oral sucker, with a prominent head crown and collar spines, the ventral sucker, located near the equatorial line of the body, and 2 genital primordia were recognisable. The primordium of the cirrus pouch was seen near the anterior margin of the ventral sucker, and 1 primordium was visible in the posterior part of the body.

SEM study

Scanning electron microscopy (SEM) observations showed the topography surface of a newly excysted metacecaria (Fig. 3). The body of the excysted metacercaria was elongated, ventrally concave and pointed posteriorly. The anterior half body surface was covered with numerous peg-like tegumental spines and became slightly finer posteriorly. Peg-like tegumental spines were densely distributed on the anterior surface to the posterior margin of the ventral sucker level

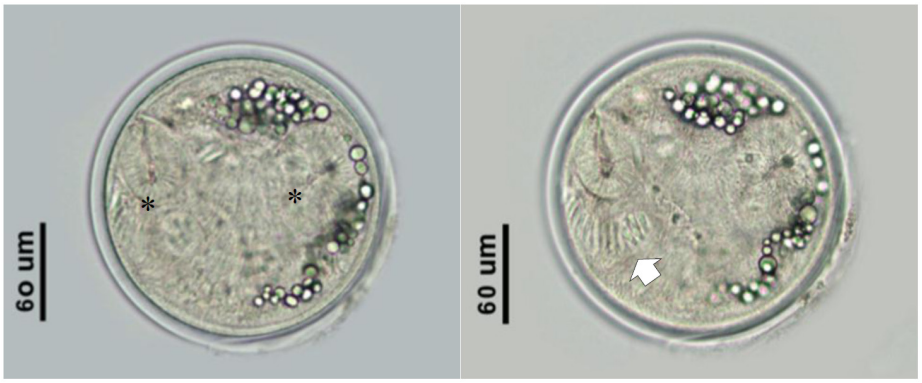

Figure 1 An isolated metacercariae of *Echinostoma revolutum* showing a well-developed oral and ventral sucker (asterisk) and head collar with collar spines (arrowhead).

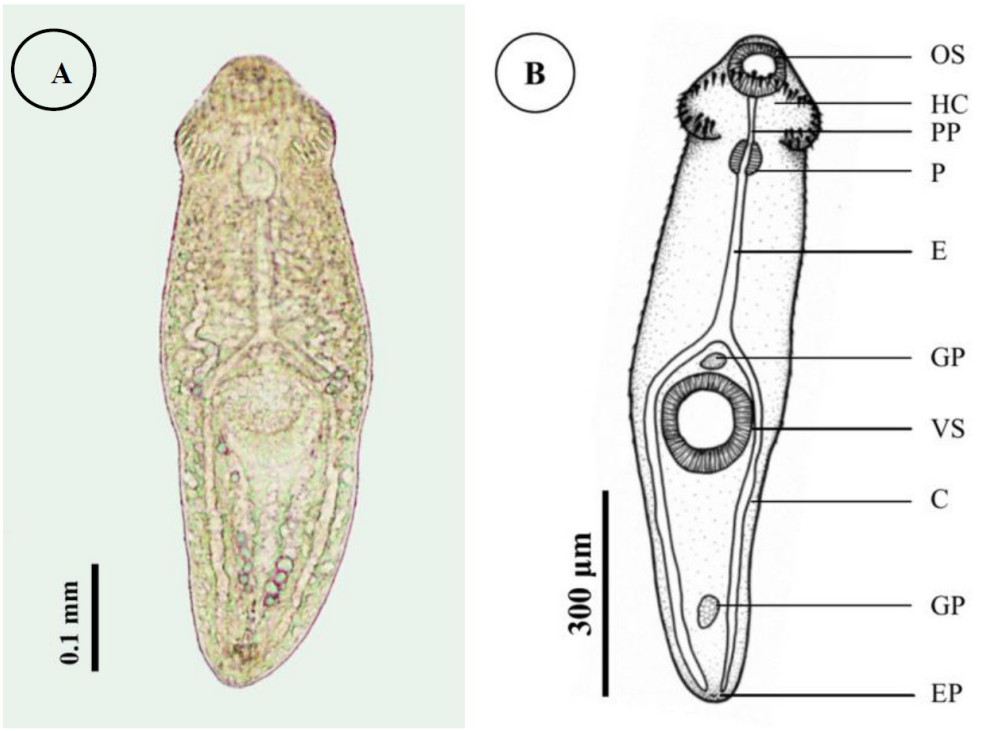

Figure 2 Illustration demonstrating excysted metacercaria of *Echinostoma revolutum*; (A) Photograph of excysted metacercaria compressed under a cover slip. (B) Drawing. Abbreviations: C, Caeca; E, Esophagus; EP, Excretory pore; GP, Genital primordia; HC, Head crown; OS, Oral sucker; P, Pharynx; PP, Prepharynx; VS, Ventral sucker.

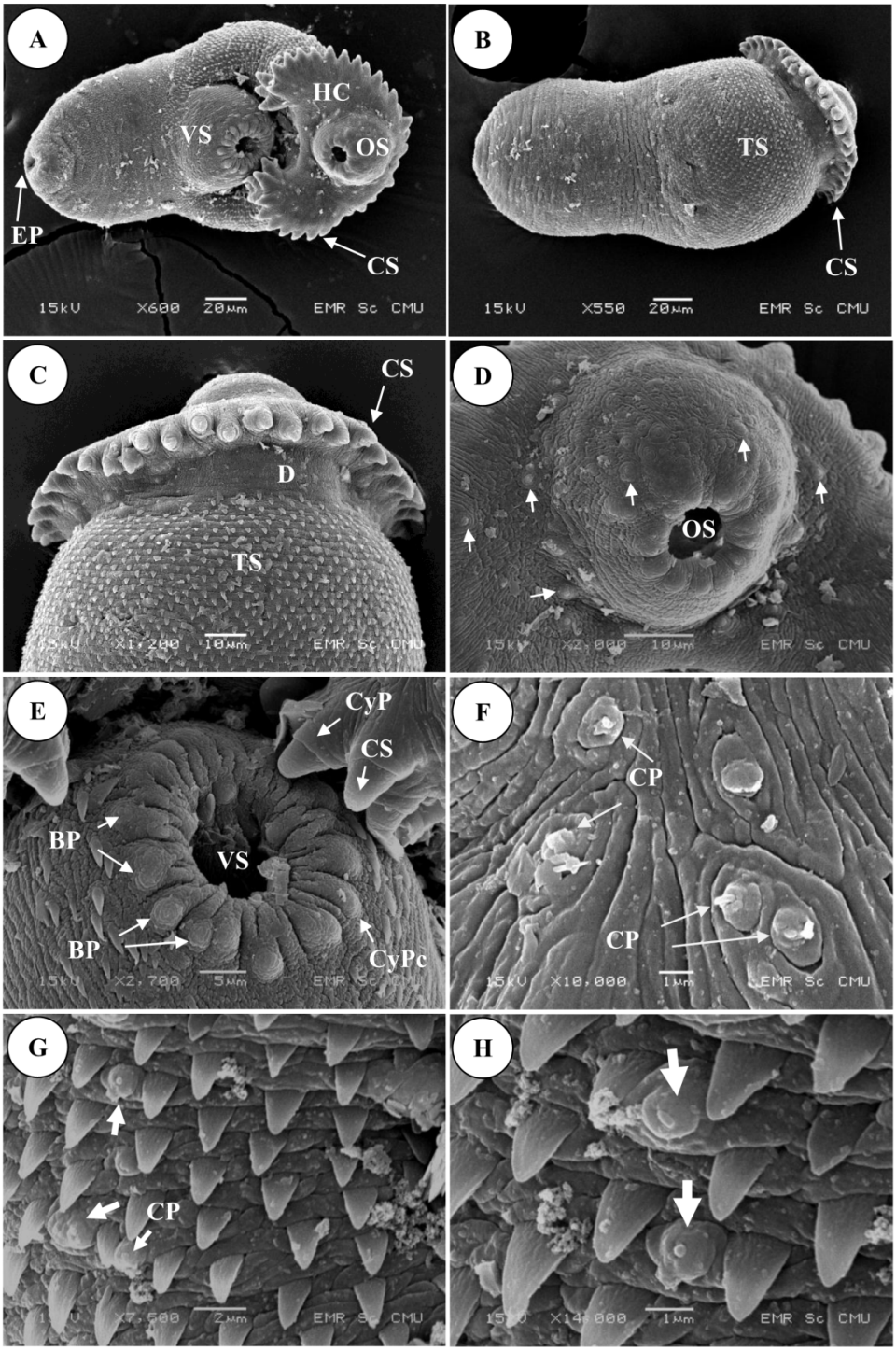

Figure 3 Scanning electron micrographs of an excysted metacercaria of *Echinostoma revolutum*: (A) The whole body (ventral view). (B) The whole body (dorsal view). (C) Dorsal view of anterior tip of the body. (D) Head collar region showing the oral sucker and distribution of sensory papillae (arrowhead); (E) Middle part of body showing the ventral sucker is devoid of spines. (F) Tegumental surface of head collar. (G) Anterior tegumental surface showing the peg-like tegumental spines and ciliated knob-like structure papillae between the tegumental spines. (H) Enlarged view of tegumental surface showing the ciliated knob-like structure papillae and tegumental spines.
Abbreviations: BP, button shaped papillae; C, Caeca; CP, ciliated knob-like papillae; CS, collar spines; CyP, cytoplasmic pockets; D, a space devoid of spines; E, Esophagus; EP, excretory pore; HC, head collar; OS, oral sucker; VS, ventral sucker.

except the adjacent areas of the head crown, which were devoid of spines, and became sparsely distributed and disappeared posteriorly. The anterior peg-like tegumental spines were larger than the posterior tegumental spines. The oral sucker was circular and was situated subterminally at the antero ventral side of the body. The lip of the oral sucker was devoid of spines. The lip of oral sucker exhibited wrinkled cytoplasmic processes and had ciliated knob-like structure papillae (Fig. 3F). Ciliated knob-like structure papillae appeared around the oral sucker, the areas of the head crown and sparsely over the body surface between the tegumental spines (Fig. 3F-H). The head crown

was surrounded by 37 retractable, horseshoe-shaped collar spines, which were embedded in cytoplasmic pockets. The collar spines were arranged in a double row (oral and aboral), and had end grouped spines (corner spines) (Fig. 3A).

The ventral sucker was medial at one third of the body (Fig. 3E). The surface of the ventral sucker was covered with cytoplasmic processes arranged radially. The lip of the ventral sucker was encircled with button shaped papillae (Fig. 3E). The circle of button shaped papillae was located along outer margin of the ventral sucker. The excretory pore was terminal, and showed a smooth tegument (Fig. 3A).

Studies on the surface ultrastructure of *E. revolutum* have been undertaken with various hosts, i.e. birds and mammals [6-8]. However, the surface ultrastructure of excysted metacercaria of *E. revolutum* was observed in this study. The surface ultrastructure of the collar was found to be similar to that of *E. trivolvis* and *E. caproni* described by Smales and Blankespoor [6], Fried and Fujino [7], Kruse *et al*. [9], Ursone and Fried [10] and Fujino *et al.* [11]. In the aforementioned papers, surface ultrastructure information and details for the collar region were incomplete. For this reason, detailed comparisons between this study and the aforementioned studies were not possible. However, it is well known that *E. revolutum* and the 37-collar-spined allies of the *'revolutum'* group and their avian and mammalian hosts, including humans, are considered relatively new in an evolutionary sense. The tegumental ultrastructure of *E. revolutum* was generally similar to that of other echinostomes in the shape of the tegumental spines and cytoplasmic processes, and the distribution pattern of tegumental spines and sensory papillae [12-14]. However, some features such as the morphological change of tegumental spines and the appearance of sensory papillae on the ventral sucker according to development, and number, shape and arrangement of collar spines were characteristic, which may be of taxomic and bioecological significance. The shape and distribution of tegumental spines of digeneans is closely related to worm maturation and parasitic niche. In some intestinal digeneans, tegumental spines enlarge and become more pointed as the worm matures [15-18].

CONCLUSION

The present study extends knowledge of echinostome digenean by taking into account details of the species, *E. revolutum*. The results provide valuable information useful for identification of this species and other species in the genus, combined with morphological criteria that establish the higher standard description.

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