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Light and Scanning Electron Microscopy of *Diphyllobothrium pacificum* Expelled from a Man

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Abstract

Morphological characteristics of *Diphyllobothrium pacificum* expelled from a man living in Matsuyama City, Ehime Prefecture, Japan were examined. By using light microscopy, the layer of longitudinal muscle is thick and muscle fibers are irregularly arranged. In scanning electron microscopy, the longitudinal elevation of the tegument with a few transverse grooves are seen along the median line. The vagina opens on the rear foot of hemispherical protuberance of the tegument, while the cirrus pore opens independently on the top of the protuberance, which itself caves in the body surface, forming a kind of hollow surrounded by the tegumental folds. No genital papillae are observed on the surface of the hemispherical protuberance. We observed a spike at the posterior end and deep pits densely distributed on the surface of eggshell.

Key words: Cestoda, *Diphyllobothrium pacificum*, scanning electron microscopy, human infection

Introduction

Although *Diphyllobothrium pacificum* is a natural parasite of seals, this parasite occasionally infects humans in the coastal areas of Peru (Baer *et al.*, 1967) and Chile (Atias and Cattan, 1976; Sagua *et al.*, 1976). In Japan, *D. pacificum* has been known to occur in marine mammals since Yamaguti (1951) recorded it from the fur seal, *Callorhinus ursinus*, and human infection with this parasite was first found in Okinawa (Kamo *et al.*, 1982). Since then, five additional human cases have been reported in Japan (Makiya *et al.*, 1987; Higo *et al.*, 1988; Tsuboi *et al.*, 1988; Yazaki *et al.*, 1990; Yamane *et al.*, 1991). The character of genital openings is one of the most distinguishable features of *D. pacificum*. Kamo *et al.* (1982) and Makiya *et al.* (1987) showed the surface structure of the genital openings of this worm obtained from human cases by using scanning electron mciroscopy (SEM), however, they did not observed the arrangement of cirrus, vaginal and uterine pores. So far, Maejima *et al.* (1981) only reported these characteristics about genital openings of this worm obtained from fur seals by using SEM. Some morphological differences were reported regarding the size of eggs and the structure of longitudinal muscles between *D. pacificum* obtained from human cases and those from fur seals (Makiya *et al.*, 1987; Yazaki *et al.*, 1990). As minute morphological study of *D. pacificum* from a human case has not been made, we investigated the detailed surface topography and the inner structure of *D. pacificum* from the human case reported by Tsuboi *et al.* (1988).

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Materials and Methods

The specimen of *D. pacificum* was the same one obtained from the patient reported by Tsuboi *et al.* (1988). The patient was a 59-year-old male living in Matsuyama City, Ehime Prefecture, Japan, and who sometimes ate raw squid or tuna ("sashimi"). He eliminated some segments of the tapeworm with feces without any complaint on September 29th in 1987.

A part of this specimen was fixed with 70% ethanol while compressed between two slide glasses, stained with borax carmine, and mounted with Canada balsam. Serial sections of mature proglottid fixed with 10% formalin solution were prepared in transverse and sagittal planes and stained with trichrome solution after sectioning.

For SEM, small pieces of proglottids or eggs naturally laid in saline solution were treated in phosphate buffered saline (pH=7.2) containing 2.5 mg/ml trypsin (DIFCO 1:250) for about 15 min at 37°C, fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH=7.4). The specimens were post-fixed in 2% osmium tetroxide, dehydrated through a graded series of ethanol and immersed in isoamyl-acetate. After drying by the critical-point method and sputter-coating with platinum, the specimens were examined with a Hitachi S-500A scanning electron microscope at 20 kV.

Results

Measurements of each part of the specimen were given in Table 1 comparing to the data obtained from specimens from fur seals (Maejima *et al.*, 1981). The strobila without scolex was shown in Fig. 1.

By light microscopy (LM) on the whole mount preparations, the cirrus sac was situated on the midline in the distance equal to about 29–48% of the segmental length from anterior margin. Behind the cirrus sac a series of the uterine loops extended one side to the other. The loops of the uterus were usually parallel form. The number of the loops was 4–6 on each side (Fig. 2).

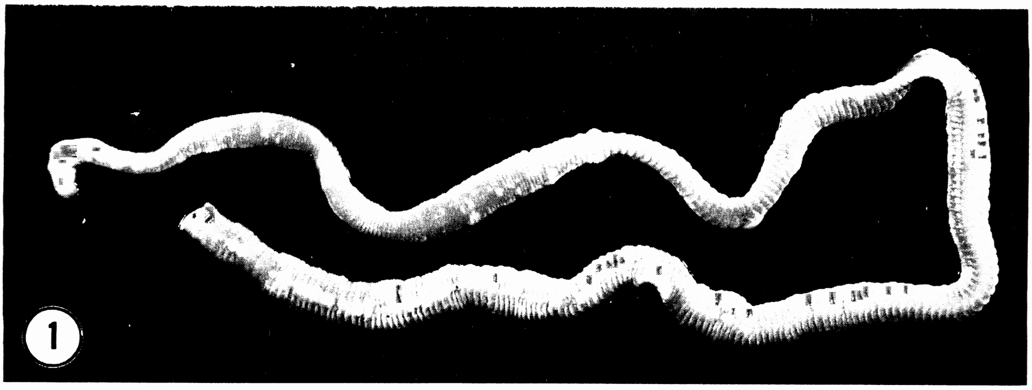
On the sectioned specimen, the cirrus sac, pyriform in sagittal sections, extended dorsally and a little anteriorly. The seminal vesicle, oval antero-posteriorly in sagittal sections and circular in transverse sections, was directly connected with the cirrus sac dorso-posteriorly. The vagina opened separately behind the cirrus pore (Fig. 3). The two main longitudinal nerve cords lay in the medullary zone, one on each side, about midway between the genitalia and the lateral margin of the segment. The elliptic testes were arranged in a single layer in the medullary parenchyma (Fig. 4). The layer of longitudinal muscle was thick (Fig. 4). Muscle fibers were irregularly arranged and the longitudinal muscle fibers were without spindle-shaped appearance in sagittal sections (Figs. 5, 6).

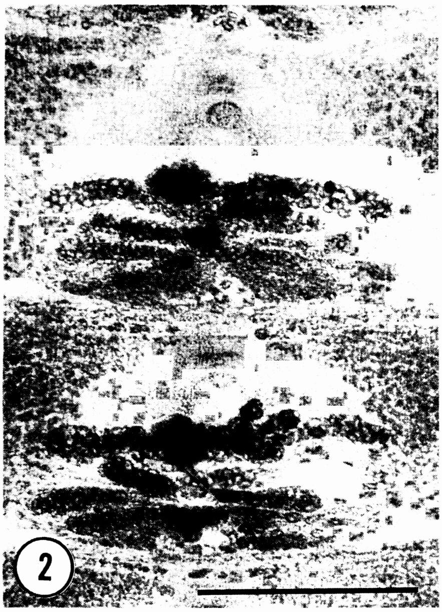
The surface of the segment observed by SEM

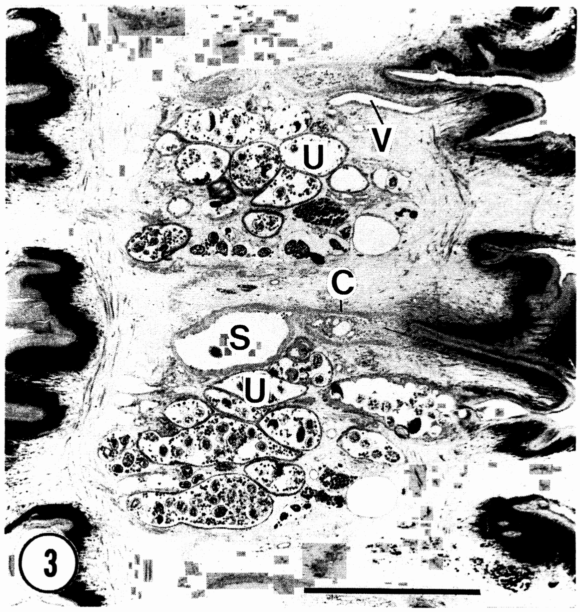
Table 1 Comparative observations of *Diphyllobothrium pacificum* between the present report and the specimens from fur seals by light microscopy

	Present report	From fur seals*
Strobila (mm)		
length	280	75–160
maximum width	8.0	1.95–3.50
Segment (mm)		
maximum length	0.32–1.2	1.25–2.00
thickness	1.5	0.37–0.67
Diameter of nerve trunk (μm)	46 ± 5	30–50
Arrangement of testes	single layer	single layer
Diameter of testis (μm)	190 ± 35 × 64 ± 13	60–100 × 30–70
No. of testis in		
transverse section	43–53	20–40
sagittal section	17–22	12–21
No. of uterine loops	4–6	3–8
Cirrus sac (μm)		
length	290 ± 38	220–290
width	113 ± 24	140–160
wall thickness	18 ± 3	30–50
Seminal vesicle (μm)		
length	366 ± 51	150–190
width	182 ± 25	100–110
wall thickness	20 ± 8	40–50
Thickness of muscle layer		
longitudinal (μm)	278 ± 28	40–50
transverse (μm)	32.3 ± 4.5	10–20
% of segment width	15.5 ± 1.3	8–10
Diameter of muscle fiber (μm)		
longitudinal	3.3 ± 0.9	2–10
transverse	2.0 ± 0.7	1–10
Size of eggs (μm)		
length	54.2 ± 2.2	56.7–67.5
width	40.2 ± 1.8	40.5–45.9
thickness of eggshell	1.2 ± 0.3	–

*: the specimens reported by Maejima *et al.* (1981)







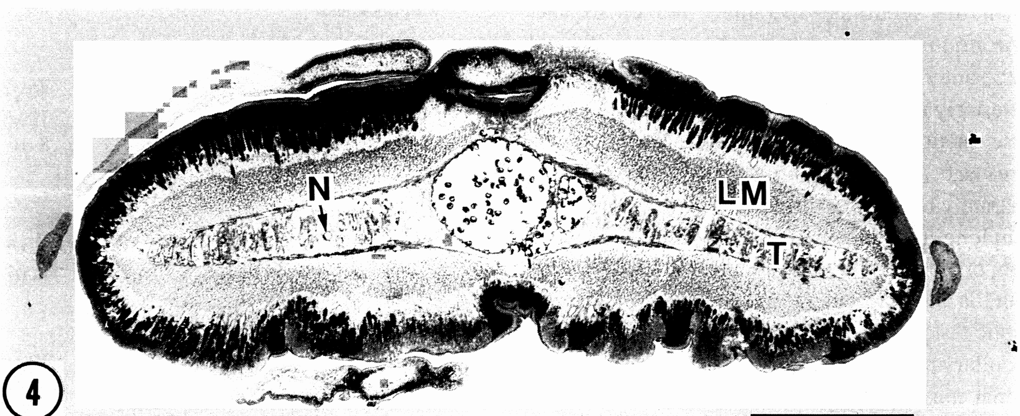


Fig. 1 Fragment of body.

Fig. 2 Whole mount of gravid segments. Scale bar = 1mm.

Fig. 3 Sagittal section of gravid segment, showing arrangement of genital organs. Scale bar = 0.5mm, C: cirrus sac, S: seminal vesicle, U: uterus, V: vagina.

Fig. 4 Transverse section of gravid segment. Scale bar = 1mm, LM: longitudinal muscle layer, N: nerve cord, T: testes.

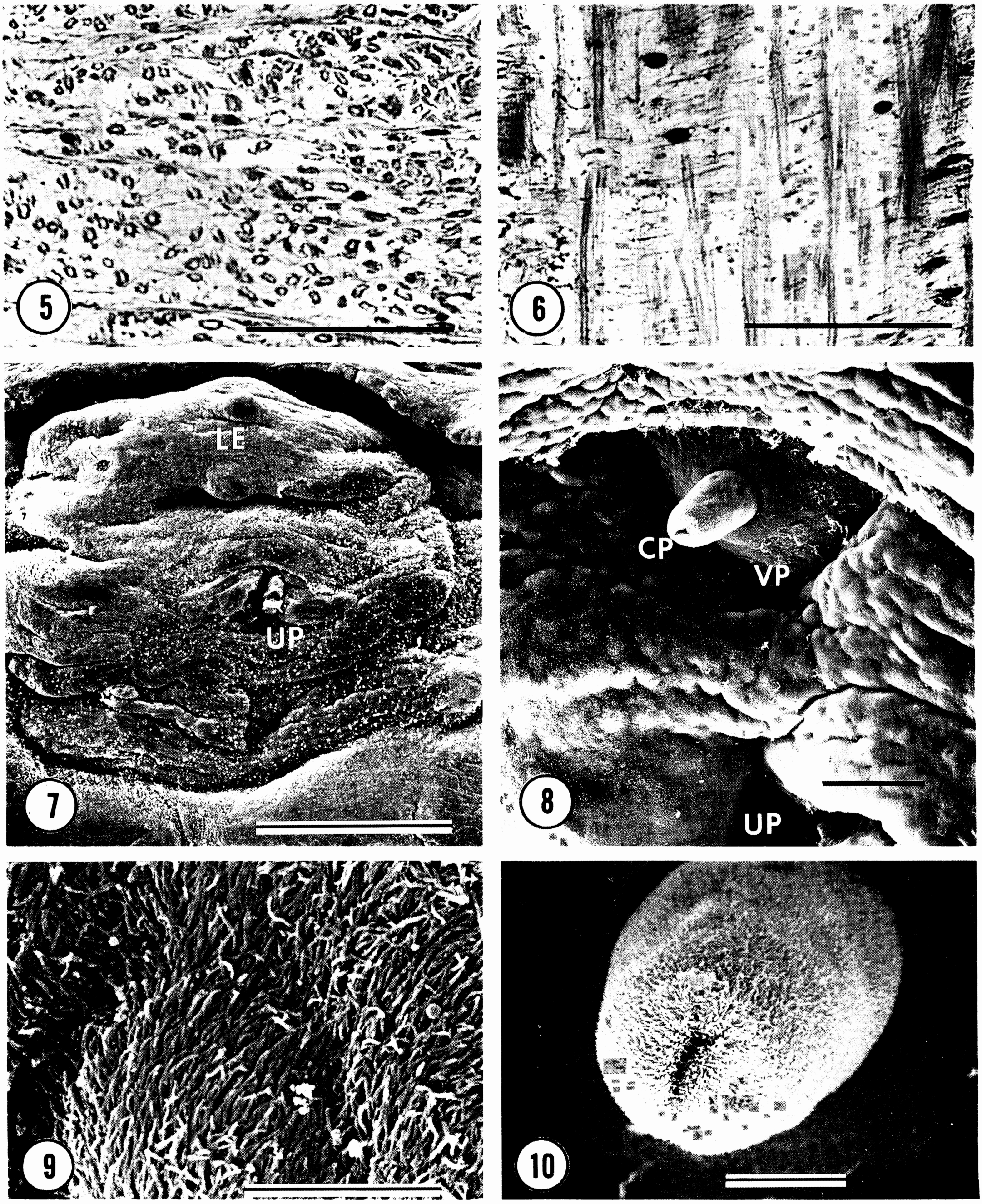


Fig. 5 Transverse section of gravid segment, showing details of the longitudinal muscle fibers. Scale bar = 50 μm.

Fig. 6 Sagittal section of gravid segments, showing details of the longitudinal muscle fibers. Scale bar = 50 μm.

Fig. 7 Body surface of gravid segment by scanning electron microscopy. Scale bar = 500 μm, LE: longitudinal elevation of the tegument, UP: uterine pore.

Fig. 8 Genital openings, showing cirrus (CP), vaginal (VP) and uterine (UP) pores. Scale bar = 50 μm.

Fig. 9 The surface of the longitudinal elevation of the tegument, showing the dense growth of microtriches. Scale bar = 5 μm.

Fig. 10 Cirrus pore covered with microtriches. Scale bar = 10 μm.

showed four to five transverse grooves of the wrinkled tegument elevated longitudinally along the median line (Fig. 7). The cirrus pore opened on the top of hemispherical protuberance of the tegument. The vaginal pore lay at about 50 μm distance behind from the cirrus pore, opening separately on a rear foot of the hemispherical protuberance. The uterine pore was at about 200 μm distance behind the cirrus pore (Fig. 8). The hemispherical protuberance was usually caved in the body surface, forming a kind of hollow. The hollows were seen as slit-like appearance (Fig. 7). The longitudinal elevation of the tegument (Fig. 9) or the other part of the segment was covered with the same microtriches, and the microtriches were also seen around the cirrus pore (Fig. 10).

The shape of eggs was oval, and the eggshell was thin (Fig. 11). Deep pits were densely distributed on the surface of eggshell (Fig. 12). The eggshell was usually furnished with a small spike at posterior end (Fig. 14). The opercular suture was relatively deep groove in which a defined linear elevation was seen (Fig. 13).

Discussion

Table 1 showed some size differences of *D. pacificum* between from human case and from fur seals. Especially, the size of seminal vesicle of this specimen was larger and the thickness of longitudinal muscle layer was thicker than that from fur seals. However, the size of these two of the specimen from fur seals is almost the same as that from human case (Makiya *et al.*, 1987). Accordingly, these size differences of *D. pacificum* were not due to the difference of its host.

By LM, it has been described that the spindle-shape fibers and the network structure of well developed longitudinal muscles has been regarded as one of the characteristics of *D. pacificum* (Markowski, 1952; Baer *et al.*, 1967; Maejima *et al.*, 1981; Kamo *et al.*, 1982). But the spindle-shape fibers were not distinctly observed in our case. Similar result were reported on the specimens obtained from human infection (Yazaki *et al.*, 1990). According to these findings, it does not seem that the shape of longitudinal muscle fibers is the definitive characteristics of *D. pacificum.*

On the whole mount preparations of *D. pacificum*, Stunkard (1948) and Yamaguti (1951) described that the vaginal pore was opening to the exterior independently behind the male pore. By the examination of sagittal sections, Margolis (1956) showed that the male and female pores opened independ-

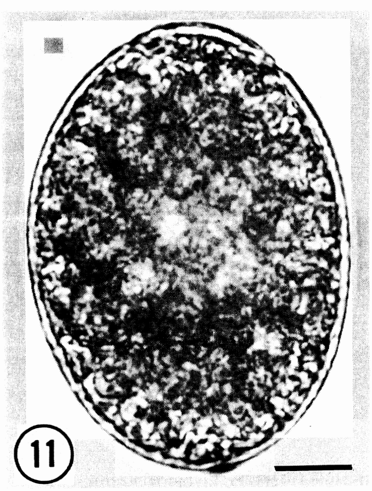
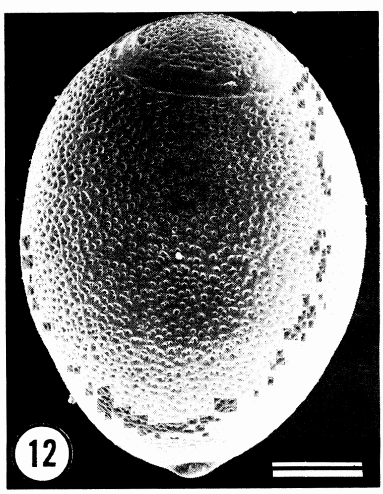
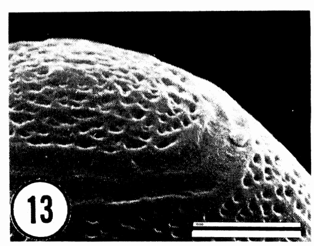
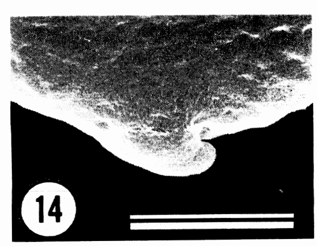





Fig. 11 Egg. Scale bar = 10 μm.
Fig. 12 The surface of the eggshell, showing deep pits. Scale bar = 10 μm.
Fig. 13 The surface at the anterior end of the eggshell, showing opercular suture. Scale bar = 5 μm.
Fig. 14 The surface at the posterior end of the eggshell, showing a small spike. Scale bar = 5 μm.

ently into a very shallow common genital atrium, and the vagina opened posterior to the cirrus at the posterior margin of the genital atrium. By SEM, the arrangement of cirrus, vaginal and uterine pores was observed exactly on the specimens from fur seals (Maejima *et al.* 1981). In the present SEM observations, the location of cirrus, vaginal and uterine pores was arranged almost in the same manner as observed on the specimen from fur seals (Maejima *et al.* 1981). These observations are the first report about the arrangement of genital openings of the specimen obtained from human case.

Papillae around the genital atrium were reported from various species of diphyllobothriids (Andersen, 1975; Yamane *et al.*, 1976). However, no genital papillae was observed on the surface of the hemispherical protuberance of *D. pacificum*. As Maejima *et al.* (1981) suggested, the loss of genital papillae might be due to a certain degree of maceration of the tegumental surface. Yet, the tegument surrounding the hemispherical protuberance looked like the genital papillae because of the strong muscle contraction.

Hilliard (1972) reported that the shell of fully developed eggs was deeply pitted in the species having marine intermediate and final hosts, while the eggshell was only superficially pitted in the species having the aquatic phase of the life cycle in a freshwater habitat. The formation of the opercular suture seemed to be concurrent with the enlargement and deepening of the pits on the eggshell. We observed the spike at the posterior end of the egg and densely distributed deep pits on the surface of eggshell. The opercular had a relatively deep groove in which a defined linear elevation was seen. These characteristics of our specimen were similar to that from fur seals. The size of the eggs of our specimen were smaller than that from fur seals and almost the same size as that of specimens from human case (Maejima *et al.*, 1981; Kamo *et al.*, 1982; Makiya *et al.*, 1987; Yazaki *et al.*, 1990).

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