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A New Species of Marine Tapeworm, *Diphyllobothrium orcini* n. sp. (Cestoda: Pseudophyllidea) Found from Killer Whale, *Orcinus orca* (Linnaeus, 1758) in Japan

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Abstract

A new species of marine tapeworm, *Diphyllobothrium orcini* n. sp. (Pseudophyllidea), is reported. The specimens were found in small intestine of killer whale, *Orcinus orca* (Linnaeus, 1758) which was captured off Kii Peninsula, Japan. The present new species is more comparable to *Diphyllobothrium polyrugosum* Deljamure *et* Skrjabin, 1966, although it is easily differentiable from *D. polyrugosum* by means of the relative position of cirrus-sac and seminal vesicle, and the connecting position of cirrus-sac and seminal vesicle as well as the winding pattern of uterine loops. The characteristic features of the new species are photographically demonstrated in detail.

Key words: *Diphyllobothrium orcini* n.sp., marine tapeworm, Pseudophyllidea, killer whale, *Orcinus orca*

In the previous paper, the authors have reported discovery of two mature tapeworms belonging to the genus *Diphyllobothrium* from a killer whale, *Orcinus orca* (Linnaeus, 1758) captured off Kii Peninsula, Japan (Hatsushika *et al.*, 1987). However, the taxonomical features of this species were not studied at that time. Since then, more detailed examination of the specimen has been done, and resulted in that the present tapeworm characterized in size and shape of scolex, in length of segments, in formation of winding uterine loops, in connecting position of cirrus-sac and seminal vesicle. These morphological characteristics have not so far been reported among the known species of diphyllobothriid tapeworms.

Therefore, the authors confirm those specimens are to be a new species as reported below.

Materials and Methods

Two mature tapeworms (one was devoid scolex) from small intestine of *Orcinus orca* had kept in 10% formalin solution for 7 years. Prior to new examination, the specimen with scolex was washed in running water overnight, stained with Semicon's carmine, and mounted in Canada balsam as whole mount preparation, after it was cut transversely 10cm long each. Some mature and gravid segments were serially sectioned at 10 μm in transverse and sagittal directions and stained with trichrome. Surface of genital area of gravid segment and egg-shell surface were studied with a Hitachi HHS-2R scanning electron microscope, after refixing in 2.5% glutaraldehyde and 1.0% osmotic acid, and dehydrating through graded series of ethanol and dried in a carbon dioxide critical point apparatus, followed by platinum palladium coating. Name of the species, *orcini,* represents the host animal.

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Diphyllobothrium orcini n. sp. (Figs. 1–10, Photos. 1–9)

Description: The strobila is muscular. The worm with scolex measures 120cm in length and 17mm in maximum width, and contains about 3,800 segments. All the segments are much wider than long, and lateral margins are markedly serrate (Photos. 2, 6). The scolex is relatively

small, with bothrial grooves extend full length of the scolex and typically onion-bug or heart-shaped in lateral view. It measures about 0.47mm long and 0.5mm width. The posterior edges of scolex are slightly overlapped with initial segments (Fig. 1, Photo. 1). The neck is indistinct. The length of segments is not so increased along strobila, measuring 0.04 to 1.2mm. The length/width ratio of immature segments (20 cm behind the scolex) is about 1:25, of mature segments (35cm behind the scolex), about 1:46, and of the terminal segment, about 1:16 (av. 1:22), respectively. The uterine loops are visible at 30cm behind the scolex (about 2,300th segment) and 35cm behind the scolex (about 2,400th segment) the eggs appeared first in the uterus (Fig. 2). A single set of reproductive organs is situated in the midline of each segment. The uterus extends quite parallel to each side with 4 to 6 loops (Figs. 2, 3, Photos. 2, 3). The genital opening is situated ventrally on midline at anterior margin of segment, and surrounded by fairly distinct papillae (Fig. 9, Photo. 8).

The thickness of segments is 1.23 to 1.75mm (av. 1.50). The tegument (cuticle) is about 17 μm in thickness (Figs. 7, 8). The longitudinal grooves on the tegumental surface are distinguished and about 0.01 to 0.03mm deep in transverse sections of gravid segment (Fig. 5, Photo. 4). The number of longitudinal grooves is about 43 dorsally and 33 ventrally. The cortical zone in the dorsal and ventral parts is 0.09 to 0.52mm (av. 0.49) in thickness, in which there exists well-developed longitudinal muscle fibers and vitelline glands excepting uterine field. Longitudinal muscle layer measures 0.03 to 0.32mm (av. 0.28) in thickness (Figs. 6–8, Photos. 5–7). The vitelline follicles are elongated and relatively large, measuring 0.03 to 0.10mm in maximum diameter. The medullary zone is about 0.06 to 1.17mm (av. 0.17) in thickness. The layer of transverse muscles is relatively thin, measuring 0.01 to 0.17mm (av. 0.08) in thickness. The testes are arranged in a single layer and widely distributed in the medullary zone excepting uterine field. There are 48 to 60 in a transverse section on each side of the segment and 12 to 17 in sagittal section, about 1,500 in each segment. They are elliptical to oval, measuring 0.11 to 0.22×0.04 to 0.13mm (av. 0.14×0.09) in diameter in transverse section.

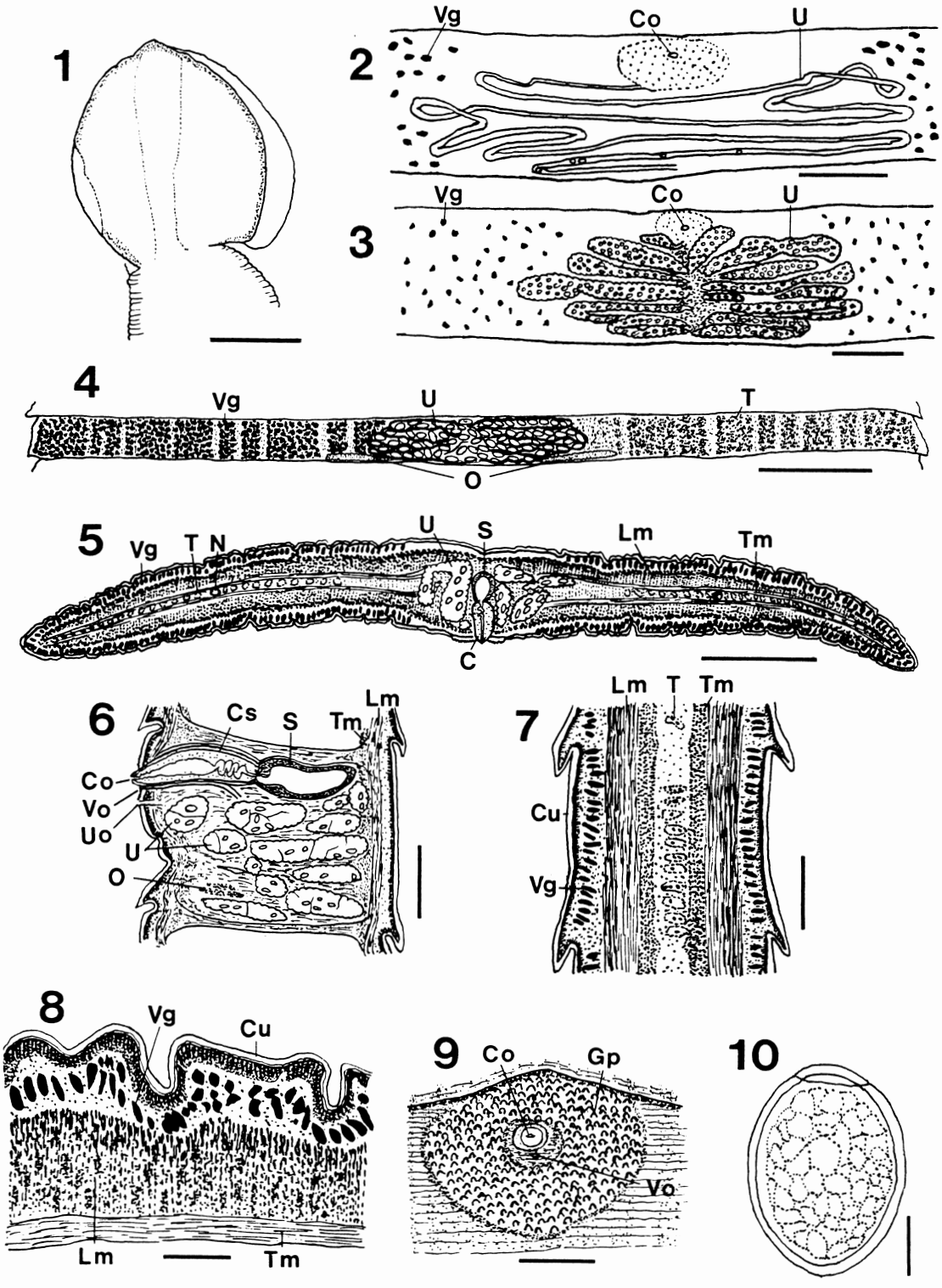
The gravid bilobed ovary is situated transversely at posterior margin of segment, measuring about 4.6mm wide which occupies approximately 1/3 of total width of the segment. The lateral end of ovarian lobes extends beyond the lateral end of uterine loops (Fig. 4, Photo. 4). A single pair of longitudinal nerve trunks is seen about halfway between the lateral end of uterine loops and lateral margin of segment in transverse sections (Fig. 5, Photo. 7). It measures 0.06 to 0.10mm in diameter. The excretory canals are not visible. Locations of cirrus and vaginal opening are separated each other. The cirrus is relatively large, and usually protruded ventrally (Fig. 6, Photos. 5, 8). The cirrus-sac is long and dorsally extends from genital atrium, measuring 0.71 to 0.81mm dorsoventrally and 0.20 to 0.25mm in diameter in sagittal sections. Cirrus-sac wall is 10.4 to 34.7 mm (av. 20.8) in thickness, and cirrus opening is located about 0.3mm posterior to anterior margin of the segment in sagittal section. The seminal vesicle is also elongated, and situated behind the cirrus sac, which is horizontally connected each other (Fig. 6, Photo. 5). The seminal vesicle measures 0.52 to 0.58mm dorsoventrally and 0.15 to 0.36mm in diameter in sagittal section. Seminal vesicle wall is quite thick compared with that of cirrus-sac, measuring 20.8 to 86.8mm (av. 41.6). Vaginal opening is located posterior to the cirrus opening and the vaginal duct runs dorsally along under wall of cirrus-sac. Uterine opening is located about 0.6mm from anterior end of segment (Fig. 6, Photo. 5). Eggs are ovoid without apical knob, measuring 66.7 to 70.1 μm (av. 66.9) in length and 46.8 to 57.5 μm (av. 50.2) in width (Fig. 10). Egg-shell is 2.4 to 4.5 μm (av. 3.2) in thickness, having scattered pits on the surface (Photo. 9).

Host : *Orcinus orca* (Linnaeus, 1758).

Habitat : Small intestine.

Location and Date : Kii Peninsula (the central part of Japan, facing the Pacific Ocean), Japan; June 3rd, 1979.

Type specimens : In the Department of Parasitology, Kawasaki Medical School,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Kurashiki 701-01, Japan. Slides Do-90 WM(1)–(13), Do-90 TS(1)–(19), Do-90 SS(1)–(60), including the sectioned preparations.

DISCUSSION

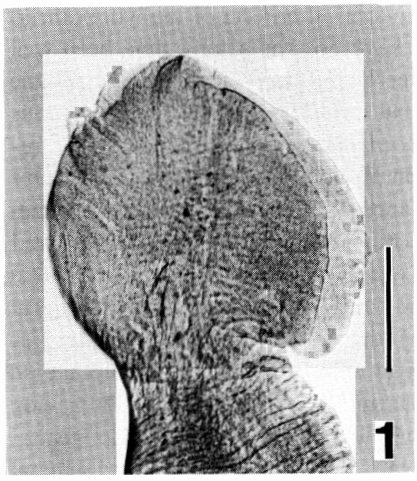
The tapeworm of the genus *Diphyllobothrium* has so far been reported almost 80 species which began with a description of *Diphyllobothrium stemmacephalum* sp. nov. as the type species of this genus by Cobbold (1858) (Andersen, 1987). Among these, about 35 species are considered to be parasitic species of marine mammals. Recently, Andersen (1987) has reported that marine species of *Diphyllobothrium* can be classified systematically into 4 groups, (I) *Diphyllobothrium stemmacephalum* Gobbold, 1858 (syn. *D. yonagoense* Yamane *et al.*, 1981) for model species, including *D. macroovatum* Yurakhno, 1973, and as re-described by Kamo *et al.* (1980), *D. polyrugosum* Deljamure *et* Skrjabin, 1966 and *D. fuhrmanni* sensu Yazaki *et al.* (1982) for related species; (II) *Diphyllobothrium elegans* (Krabbe, 1865) for model species, including *D. lashleyi* (Leiper *et* Atkinson, 1914), *D. scotti* (Shipley, 1907) and *D. pacificum* sensu Maejima *et al.* (1981) for related species; (III) *Diphyllobothrium cordatum* (Leuckart, 1863) for model species, including *D. cameroni* Rausch, 1969, *D. lanceolatum* (Krabbe, 1968), *D. hians* (Diesing, 1850) and *D. rauschi* sp. nov. (=*D. hians* sensu Rausch, 1969) for related species; and (IV) *Diphyllobothrium wilsoni* (Shipley, 1907) [syn. *D. mobile* (Rennie *et* Reid, 1912)] for model species, including *D. minutus* sp. nov. (=*D. elegans* sensu Rausch, 1969) and *D. quadratum* (Linstow, 1892) for related species.

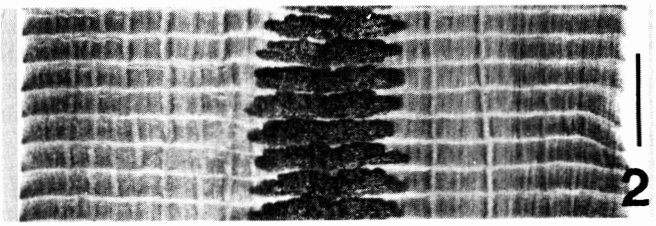
The taxonomical criteria of diphyllobothriid tapeworm have not for a long time been well defined, whereas Kamo (1978) suggested reliable and practical criteria for identifying the genus *Diphyllobothrium* in the process of re-examination of diphyllobothriasis in Japan. Our specimens appeared to be diphyllobothriid species of marine origin because there loosely scattered pits on the egg-shell surface (Hilliard, 1960, 1972) were found. Judging from the morphological features of our specimens, its characteristics resemble very closely to that of group I type *Diphyllobothrium* by Andersen (1987) having large strobila with small scolex, distinct genital papillae, relatively large and thick-walled seminal vesicle, well-developed longitudinal muscle layers, and genital atrium and uterine opening at some distance from each other. Consequently our specimen was clearly differentiable from species of groups II to IV type of *Diphyllobothrium*, with the exception of *D. cordatum* which was somewhat resembling to those of our specimen in its external appearance. Accordingly, morphological aspects of our specimen were compared with that of group I type *Diphyllobothrium,* respecting Kamo's (1978) criteria for specific identification.

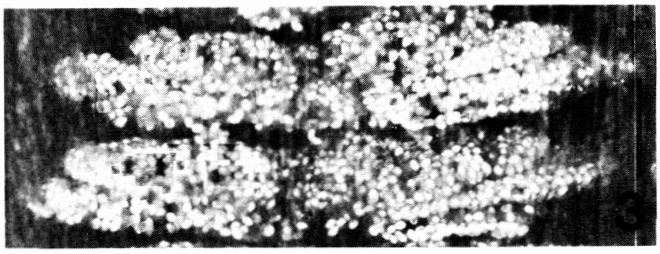
D. stemmacephalum Cobbold, 1858 [including Yamaguti (1935), Stunkard (1949), Deljamure (1968), Kamo *et al.* (1982a) and Andersen (1987)] obviously differs from our specimen in the size and shape of scolex, in the number of testis per segment and uterine loops, in the size of cirrus-sac, and in the relative position of cirrus-sac and seminal vesicle.

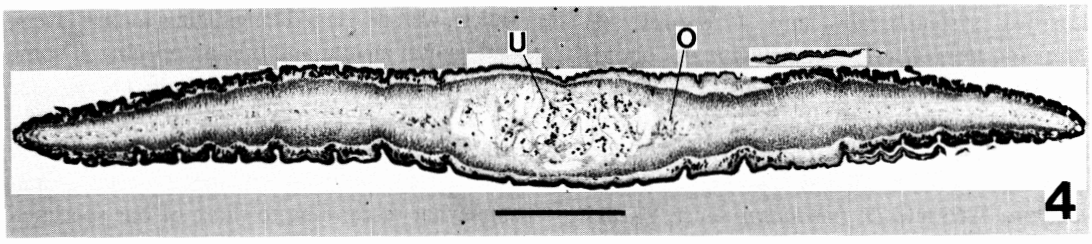
D. yonagoense Yamane *et al.* (1981) differs from our specimen in the shape and size of scolex, in the occurrence of genital organ per

Figs. 1–10. *Diphyllobothrium orcini* n. sp. from killer whale, *Orcinus orca* (Linnaeus, 1758).
Fig. 1. Scolex, lateral view (Scale bar = 0.2mm).
Fig. 2. Uterine loops of mature segment (about 35cm behind the scolex), ventral view (Scale bar = 0.2mm).
Fig. 3. Uterine loops of gravid segment, ventral view (Scale bar = 1.0mm).
Fig. 4. Gravid segment (about 90cm behind the scolex), ventral view (Scale bar = 2.0mm).
Fig. 5. Transverse section of gravid segment passing through the level of the genital opening (Scale bar = 2.0mm).
Fig. 6. Sagittal section of gravid segment passing through the genital field (Scale bar = 0.3mm).
Fig. 7. Sagittal section of gravid segment passing through the lateral field (Scale bar = 0.5mm).
Fig. 8. Transverse section of gravid segment, showing details of the dorsal musculature (Scale bar = 0.2mm).
Fig. 9. Cirrus opening and genital papillae of gravid segment, ventral view (Scale bar = 0.4mm).
Fig. 10. Egg (Scale bar = 0.02mm).





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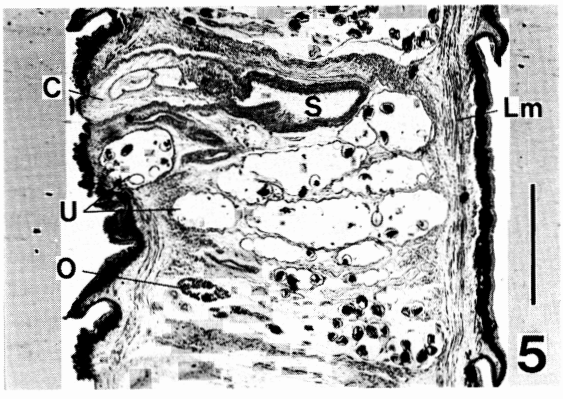
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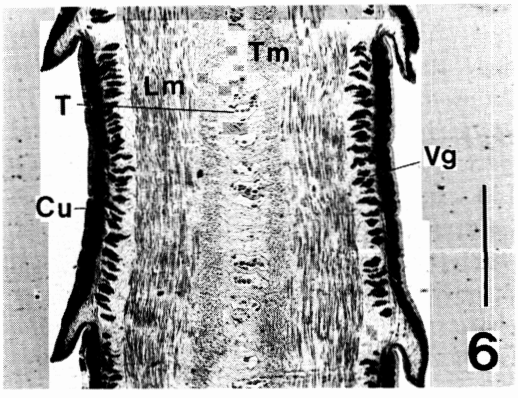
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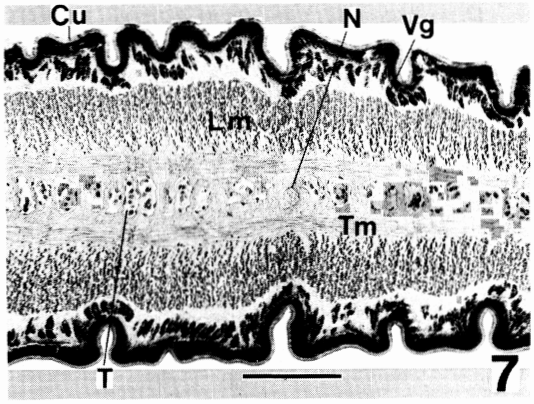
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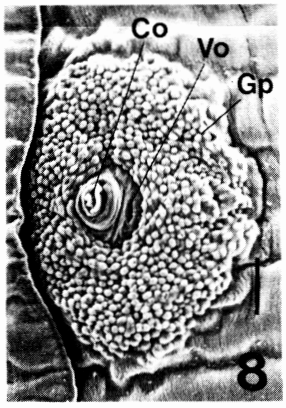
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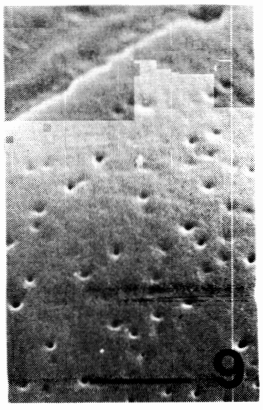

segment, in the number of uterine loops, in the relative position of cirrus-sac and seminal vesicle, and in the structural feature of pits on the egg-shell surface. Although there is no description in Andersen's paper (1987), the specimen of so-called "Koga-Okamura type" of diphyllobothriid tapeworm by Kamo *et al.* (1977, 1982b) is closely comparable to our specimen in the winding pattern of uterine loops, in the relative position of cirrus-sac and seminal vesicle, and other features. According to the current study, however, the "Koga-Okamura type" is well consisted with *D. yonagoense* [Yamane *et al.* (1981), Kamo *et al.* (1982b)], and furthermore, this type was identical to *D. yonagoense* [Hasegawa *et al.* (1989)]. Therefore, the "Koga-Okamura type" differs from our specimen.

D. fuhrmanni sensu Yazaki *et al.* (1982) [including *D. fuhrmanni* Hsü, 1935 and Kamo *et al.* (1982a)] differs from our specimen in the length/width ratio of posterior segments, in the number of uterine loops and in the relative position of cirrus-sac and seminal vesicle.

D. macroovatum Yurakhno, 1973 [including Kamo *et al.* (1980)] is easily differentiable from our specimen by the size of scolex and eggs, the number of uterine loops and the length/width ratio of most segments. *D. cordatum* (Leuckart, 1863) [including Markowski (1952), Deljamure (1955) and Kamo *et al.* (1982a)] differs evidently from our specimen in the size of strobila, scolex and cirrus-sac, in the arrangement of testis which is commonly 2 to 3 layers, and in the numbers of testis per segment and uterine loops. In addition, 2 species of *D. gondo* Yamaguti, 1942 [including Kamo *et al.* (1982a)] and *D. nihonkaiense* (Yamane *et al.*, 1986, 1989) recorded from some marine mammals in Japanese environs are quite different with our specimen in the size of scolex, in the size and the relative position of cirrus-sac and seminal vesicle, and in the number of uterine loops, and other features.

Our specimens show marked resemblance to *D. polyrugosum* Deljamure *et* Skrjabin, 1966 which was found from *Orcinus orca* captured in the 3rd fishing zone of Atlantic Ocean. It is rather difficult to distinguish the difference between *D. polyrugosum* and our specimen by general appearances but there are striking difference in the relative position and the connecting position of cirrus-sac and seminal vesicle, and in the winding pattern of uterine loops. In our specimen the seminal vesicle is situated behind cirrus-sac, and both of which are horizontally connected each other in sagittal section (Fig. 6, Photo. 5). According to Fig. 3 of Deljamure *et* Skrjabin's paper (1966), the cirrus-sac of *D. polyrugosum* extends horizontally to dorsal longitudinal muscle layer, while the seminal vesicle exists underneath the cirrus-sac. Both of which are connected the under side of cirrus-sac with the upper side of seminal vesicle from each other. In the view of these features, we are convinced that the relative

Photos. 1–9. *Diphyllobothrium orcini* n. sp. from killer whale, *Orcinus orca* (Linnaeus, 1758).

Photo. 1. Scolex, lateral view (Scale bar = 0.2mm).

Photo. 2. Gravid segment (about 60cm behind the scolex), ventral view (Scale bar = 2.0mm).

Photo. 3. High-magnification of Photo. 1, showing details of uterine loops (Scale bar = 0.5mm).

Photo. 4. Transverse section of gravid segment (Scale bar = 2.0mm).

Photo. 5. Sagittal section of gravid segment passing through the genital field (Scale bar = 0.3mm).

Photo. 6. Sagittal section of gravid segment passing through the lateral field (Scale bar = 0.5mm).

Photo. 7. Transverse section of gravid segment, showing details of the lateral field (Scale bar = 0.4mm).

Photo. 8. Genital papillae and cirrus opening of gravid segment by scanning electron microscopy (Scale bar = 0.2mm).

Photo. 9. Egg-shell surface by scanning electron microscopy (Scale bar = 2.0 μm).

Abbreviation in Figures and Photographs

C: cirrus, Cs: cirrus-sac, Co: Cirrus opening, Cu: cuticle, Gp: genital papillae, Lm: longitudinal muscle layer, N: nerve trunk, O: ovary, S: seminal vesicle, T: testis, Tm: transverse muscle layer, U: uterus, Uo: uterine opening, Vg: vitelline gland, Vo: vaginal opening.

position and the connecting position between the cirrus-sac and seminal vesicle of our specimen can quite easily be differentiable from that of *D. polyrugosum*. Furthermore, the winding pattern of uterine loops in our specimen was characterized as mentioned above, but no detailed description on uterine loops of *D. polyrugosum* is given by Deljamure *et* Skrjabin (1966).

Hasegawa *et al.* (1989), on the other hand, have reported that the morphological characteristics of diphyllobothriid tapeworm; such as the general appearance, shape and number of the furrows on the tegumental surface, and the shape and the relative position of cirrus-sac and seminal vesicle are very due to different fixation method. Although the segments of our specimen appeared to be shrank in antero-posterior direction with 10% formalin solution, the connecting position of cirrus-sac and seminal vesicle did essentially not changed, regardless of fixation method as described by Kamo (1978) and Maeda (1986).

Acknowledgments

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