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A New Species of the Genus *Diphyllobothrium* Originated from Plerocercoids in Japanese Surf Smelt (*Hypomesus pretiosus japonicus*) and Olive Rainbow Smelt (*Osmerus eperlanus mordax*)

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Abstract

Diphyllobothrium hottai n.sp. was described on the basis of strobilae reared in golden hamsters (*Mesocricetus auratus*) from plerocercoids in Japanese surf smelts (*Hypomesus pretiosus japonicus*) and olive rainbow smelts (*Osmerus eperlanus mordax*).

D. hottai was readily distinguished from *D. ditremum,* one of the so-called freshwater species, in the histological details, in some biological aspects and in the deep and large pits on the eggshell surface. Among various marine species of the genus *Diphyllobothrium D. alascense, D. colymbi, D. dalliae* and *D. lashleyi* were somewhat resembled morphologically. However, they were differentiated from *D. hottai* in some morphological details.

Key words: *Diphyllobothrium, Hypomesus pretiosus japonicus, Osmerus eperlanus mordax,* tapeworm

Introduction

Different kinds of *Diphyllobothrium* plerocercoids have been found from a few species of freshwater, marine or anadromous fishes in northern Japan (Hotta *et al*., 1978). Although some have not been identified yet, others were identified as follows. Those from the musculature of masu salmons (*Oncorhynchus masu*) and pink salmons (*O. gorbuscha*) were recognized as *D. nihonkaiense,* which has recently been separated from *D. latum* Linne, 1758 by Yamane *et al.* (1986). The plerocercoids from three-spined stickle backs (*Gasterosteus aculeatus*) in Hokkaido was regarded as *D. dendriticum* by Hotta *et al.* (1980). In regard to plerocercoids from the body cavities of Japanese surf smelts (*Hypomesus pretiosus japonicus*) and olive rainbow smelts (*Osmerus eperlanus mordax*), Hotta *et al.* (1978) identified them tentatively as *D. ditremum* Creplin, 1825 on the basis of strobilae experimentally reared in golden hamsters. However, Yazaki *et al.* (1986) pointed out some morphological and biological differences between *D. ditremum* sensu Hotta *et al.* (1978) and *D. ditremum* sensu stricto, treating the former as *D.* sp. ind. Moreover, *D.* sp. ind. was not identical with any other known species of the genus *Diphyllobothrium.* So we proposed a new name, though its natural host has not been known yet. It is also aimed to avoid the confusion with two indetermined *Diphyllobothrium* species recently recorded from humans (Kamo *et al*., 1986).

Materials and Methods

Plerocercoids were collected from the body cavity of the Japanese surf smelts (*Hypomesus pretiosus japonicus*) and the olive rainbow smelts (*Osmerus eperlanus mordax*) sent from Hokkaido, Japan. All these plerocercoids were removed alive from these fishes, and placed in 1% saline. Three or five plerocercoids from each species of fishes were directly intubated into stomach of 25 golden hamsters (*Mesocricetus*

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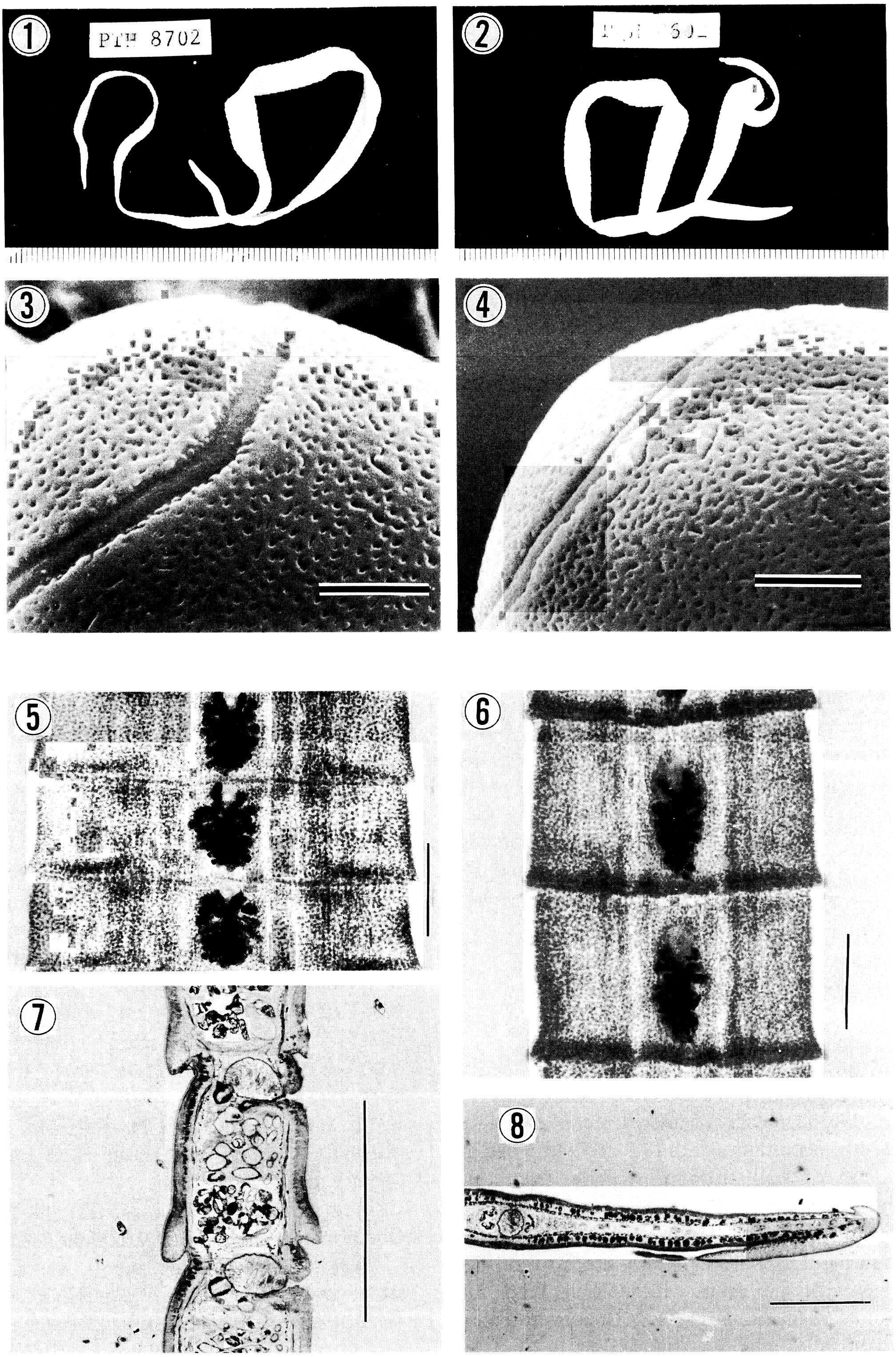
auratus). The hamsters autopsied on 7–15 days PI, and the strobilae removed from the intestine were transfered into tap water with a few drops of chloroform and placed in a refrigerator for 30–60 min at 4°C so that they were completely relaxed and dead. The stolobilae were fixed in 5% formalin in preparation for the morphometric and the histological study. Eggs for measurement were collected from the feces of each golden hamsters infected with single worm. Some plerocercoids were fixed for 24hr in 1% saline containing 4% formalin (formol-saline) for morphological observation and morphometric measurements. Some segments were stained in Semicon's acetic carmine, dehydrated through graded series of ethanol, and mounted in Eukit (a mounting reagent) after they were rinsed in xylene. Plerocercoids and portions of strobilae were embedded in paraffin, cut at 7 μm, and stained with modified trichrome stain solution. Some eggs from the uteri of gravid segments were put into 2.5% glutaraldehyde with phosphate buffer, and fixed for 2hr at 4°C in 1% osmium tetroxide. Then those eggs and some plerocercoids fixed in formol-saline were dehydrated through graded series of ethanol, transfered into amylacetate, and dried by the critical point dry method. Finally they were coated with gold-parasium alloy, and were observed under a Hitachi S-450 scanning electron microscopy. Measurements are recorded in millimeter, unless otherwise indicated.

Diphyllobothrium hottai sp. nov.
(Figs. 1–8)

Diagnosis: Strobila delicate, with slightly serrate margin, 174–250 long, composed of 159–235 segments. Segments increase rapidly in width, maximum width (3.9–6.0) attained at the end of first third of strobila, thereafter decrease gradually toward the end of strobila. Segments wider than long, with length slightly increasing posteriad. Length/width ratio of segments with maximum width about 1:4.6, of terminal segments about 1:2. Spaturate scolex with bothria extending full length, 1.20–1.55 long x 0.45–0.95 wide in lateral view. Neck very short, 0.20–0.30 long. Number of segments anterior to primordia 8–16, number of segments anterior to mature proglottids 56–63. Genital pore situated ventrally on midline in anterior fifth of segment. Genital atrium lined by rounded papillae. Cirrus sac oval, 0.290–0.293 in length and 0.203–0.220 in diameter, opening anteriorly into genital atrium. Cirrus sac situated somewhat obliquely in segment seen in sagittal sections. Seminal vesicle small, subspherical to spherical 0.101–0.108 x 0.081–0.089, situated dorsally to the cirrus sac. Vitellaria abundant, forming two lateral fields confluent across anterior and posterior margins of segments, and overlapping ends of uterine loops both dorsally and ventrally. Oval vitelline follicles 0.063–0.073 in greatest diameter. Subspherical testes, 0.150–0.170 in greatest diameter, arranged in single layer, forming two lateral fields confluent anterior to genital atrium and at posterior margin of segment. No distinct boundary between neighboring proglottids, and vitellaria and testes confluent from one proglottid to another. Vagina running anteriad, then turning ventrad into genital atrium and opening posterior to opening of cirrus sac. Bilobed ovary situated transversely near posterior margin of segment, dorsal to uterus. Gravid uterus with 6–7 loops extending laterad and anterolaterad, reaching level of anterior margin of genital atrium. Uterus opening through uterine pore posterior to genital pore, usually to right or left of midline. Eggs without apical knob, 0.061–0.070 x 0.039–0.045 (avg. 0.066 x 0.043) (Jap. surf smelt origin) and 0.063–0.070 x 0.038–0.045 (avg. 0.065 x 0.043) (olive rainbow smelt origin). Length/width ratio 1.5 (1.4–1.7). Eggshell surface distributed densely with deep, large pits, frequently joining each other.

Type locality: The coast of the Pacific Ocean in Hokkaido and Tohoku district, Japan.

Type: Slides bearing whole worm with scolex and serial sections of portions of strobila are deposited as the holotype (PTH8603) and paratypes No. 1 (PQH8701), No. 2 (PTH8111),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No. 3 (PTH8112), on No. 2 and No. 3, only whole worm slides without serial sections of portions of strobila, and two entire strobilae fixed and preserved in 5% formalin solution are deposited as paratypes No. 4 (PTH8702) and No. 5 (PQH8602) in Department of Medical Zoology, Tottori University School of Medicine, Yonago City 683, Japan.

Plerocercoid larva (Figs. 9–16)

Diagnosis: Plerocercoids killed in 4% formol-saline measured 5.2–8.6 (avg. 7.1) and 2.9–7.6 (avg. 6.1) in specimens from Japanese surf smelts and olive rainbow smelts, respectively. The maximum width attained at anterior one third of the body. The slightly flattened body tapers gradually to the posterior end. The tegumental surface, without transverse wrinkles, was covered with long microtriches. The parenchymal longitudinal muscles and transverse muscles were well developed forming thick layer circularly. Dorso-ventral muscle fibers were also prominent. The epidermal longitudinal musculature consisted of several layers of muscle fibers in cross section of the mid-body.

Host: Japanese surf smelt (*Hypomesus pretiosus japonicus*), olive rainbow smelt (*Osmerus eperlanus mordax*).

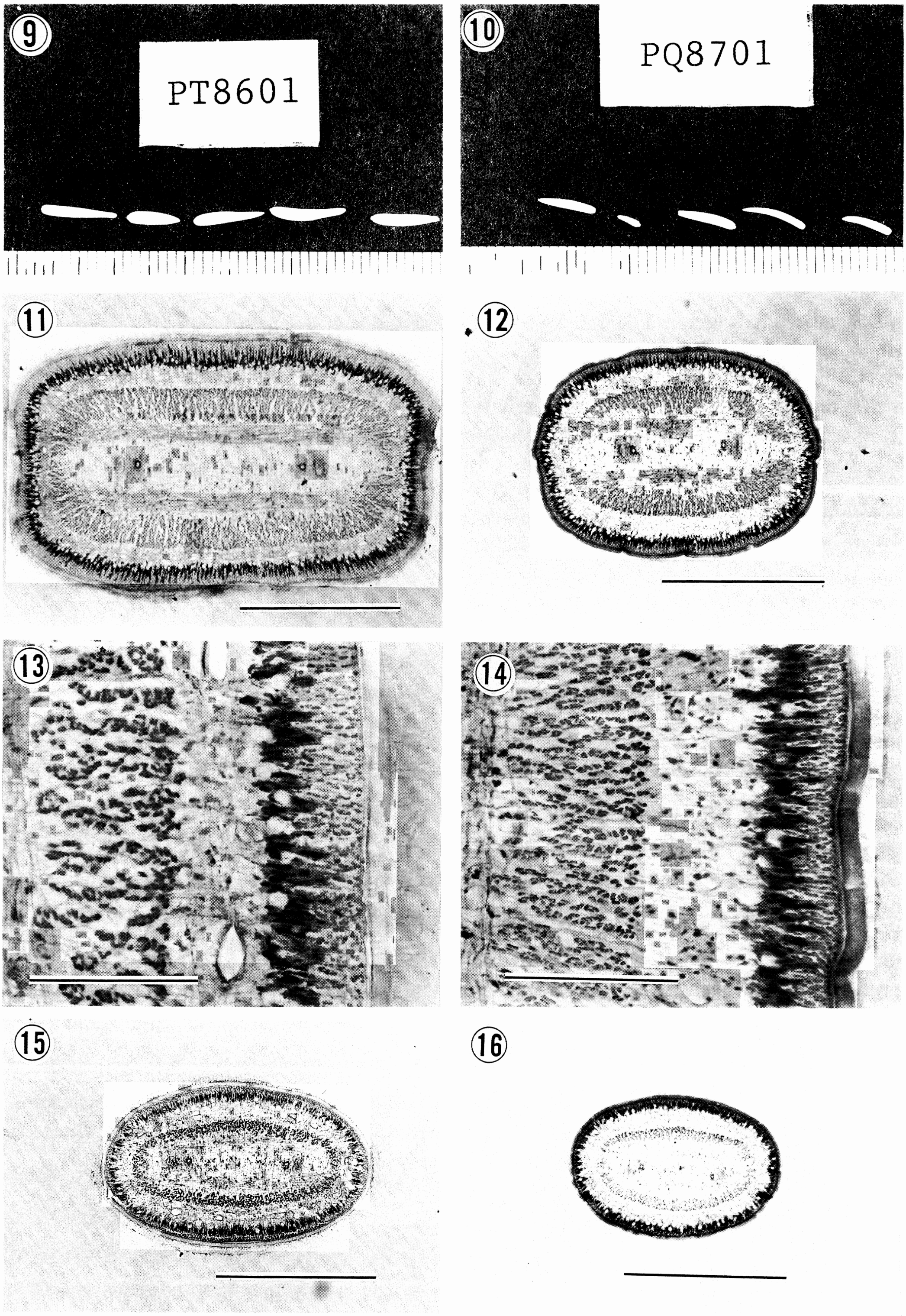
Habitat: In the body cavity of host fishes.

Type: Plerocercoids preserved in 70% alcohol prefixed in 4% formol-saline (PT8601, PQ8701) and serial sections of larva (PT8603) are deposited in Department of Medical Zoology, Tottori University School of Medicine, Yonago City 683, Japan.

Discussion

The strobila and plerocercoid of this species closely resemble those of *D. ditremum* (Creplin, 1825) in the general external morphology. As already shown by Yazaki *et al.* (1986), however, they were different from *D. ditremum* in every stages of the worm as follows: 1) Plerocercoids killed in formol-saline had larger body length tapering moderately to the posterior end, more layers of epidermal longitudinal muscle fibers in the mid-body, and more prominent parenchymal (especially transverse) muscle layer. 2) Strobilae raised in golden hamsters with higher infectivity weie matured more rapidly. The seminal vesicle attached closer to the dorsal end of the cirrus sac. 3) Eggs had larger length, with deep and large pits on the eggshell surface. 4) Coracidia adapted to sea water rather than to fresh water. In the same way, especially in the pits-pattern of eggshell surface this species is readily distinguishable from the so-called freshwater species related to *D. ditremum* such as *D. latum* (Linne, 1758), *D. dendriticum* (Nitzsch, 1824), *D. vogeli* Kuhlow, 1953. This species is also distinguishable from *D. ursi* Rausch, 1954 and *D. nihonkaiense* Yamane *et al.* 1986, as it are the anadromous species, in the similar way with special reference to the pits-pattern of eggshell surface. According to the recent revision of diphyllobothriid cestodes by Delyamure *et al.* (1985) 30 species of the genus *Diphyllobothrium* were recognized as valid. Subsequently Andersen (1987) proposed two new species, i.e. *D. rauschi* (=*D. hians* sensu Rausch, 1969) and *D. minutus* (=*D. elegans* sensu Rausch, 1969). Besides *D. colymbi* has been recorded from a red-throated diver, *Colymbus stellatus* by

Fig. 1. Strobila originated from plerocercoid in Japanese surf smelt (PTH8702). Scale: 1mm
Fig. 2. Strobila originated from plerocercoid in olive rainbow smelt (PQH8602). Scale: 1mm
Fig. 3. Eggshell surface through SEM (Japanese surf smelt origin). Bar: 5 μm
Fig. 4. Eggshell surface through SEM (olive rainbow smelt origin). Bar: 5 μm
Fig. 5. Mature segments (PTH8603). Bar: 1mm
Fig. 6. Gravid segments (PTH8603). Bar: 1mm
Fig. 7. Longitudinal section of mature segment (PTH8603). Bar: 1mm
Fig. 8. Transverse section of mature segment (PTH8603). Bar: 2mm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Yamaguti (1951). In all 34 species are recognized as valid at present. Twenty eight species of them can be comprised in a group of marine origin, most of which have special characteristics in their external and/or internal morphology, and are distinguishable enough from the present species. Of these only four species such as *D. alascense* Rausch et Williamson, 1958, *D. colymbi* Yamaguti, 1951, *D. dalliae* Rausch, 1956 and *D. lashleyi* (Leiper et Atkinson, 1914) are somewhat related to the present species. The present species was distinguished from *D. alscense* in the size of scolex, the arrangement of testes, the distribution of testes and vitelline follicles, and egg-size. *D. alascense* have the large scolex (2x2), the testes arranged in 2 to 3 deep, the testes and vitelline follicles distributed in separate lateral field, and large eggs (0.073x0.048). The species was distinguished from *D. dalliae* in the arrangement of testes, the connecting point of the seminal vesicle with the cirrus sac, and thickness of layers of the epidermal muscle fiber in the plerocercoid. In *D. dalliae* testes are arranged in 2 to 3 deep, the seminal vesicle connects with the cirrus sac at the point closer to the middle of caudal margin of the cirrus sac. Epidermal muscles form layer 8–16 μ thick. The species was differentiated from *D. lashleyi* in the area occupied by the gravid uterus in a segment, and egg-sizes. In *D. lashleyi* the gravid uterus occupied only small part of the segment and the eggs are 0.053–0.056 x 0.040–0.044, while in the species the gravid uterus occupies larger part of segment, reaching at the posterior end of the segment, the eggs are larger than those of *D. lashleyi*. The species was differentiated from *D. colymbi* in the size of scolex, the number of uterine loops and the marginal serration of strobila. *D. colymbi* is distinctive in the broader scolex (1.5–1.6 x 1.1–1.2) with foliate margins, the remarkable serration of body margin, and the greater number of uterine loops (dozen on each side). Plerocercoids and adult originated from the Japanese surf smelt were compared with those originated from the olive rainbow smelt. On the morphological and biological data, no differences were recognized between the two strains. Fukumoto *et al.* (1987) did not reveal any differences between the two strains in the soluble protein profiles and the isoenzyme patterns by isoelectric focusing. On the other hand, the soluble protein profile of *D. ditremum* demonstrated by Bylund and Djupsund (1977) were different from those of *D.* sp. ind. (now *D. hottai* n. sp.) reported by Fukumoto *et al.* (1987).

The natural final host of the present species is not known yet. Judging from the habitat of the second intermediate host i.e. the estuary or coastal waters of northern Japan, it is estimated that some kinds of marine birds play a role of the final host.

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Fig. 9. Plerocercoids killed in folmol-saline from Japanese surf smelt. Scale: 1mm
Fig. 10. Plerocercoids killed in formol-saline from olive rainbow smelt. Scale: 1mm
Fig. 11. Transverse section of plerocercoid at level of midbody from Japanese surf smelt. Bar: 0.5mm
Fig. 12. Transverse section of plerocercoid at level of midbody from olive rainbow smelt. Bar: 0.5mm
Fig. 13. Higher magnification of part of Fig. 11. Bar: 0.1mm
Fig. 14. Higher magnification of part of Fig. 12. Bar: 0.1mm
Fig. 15. Transverse section of plerocercoid at level of posterior body from Japanese surf smelt. Bar: 0.5mm
Fig. 16. Transverse section of plerocercoid at level of posterior from olive rainbow smelt. Bar: 0.5mm

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