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Studies on the Diphyllobothriid Cestodes in Northern Japan

(4) Plerocercoids Recovered from the Alaskan Haddock, *Theragra chalcogramma*, and Adults Developed in the Golden Hamster

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The authors have studied the diphyllobothriid cestodes and diphyllobothriasis in northern Japan for several years (Hotta *et al.*, 1978, 1979, 1980; Hasegawa *et al.*, 1979). They recently detected two types of plerocercoids in the Alaskan haddock, *Theragra chalcogramma*, which is a common food fish for man. This paper deals with the morphology of these plerocercoids and of adults recovered in experimental infections.

Materials and Methods

The Alaskan haddock, *Theragra chalcogramma*, caught in the Sea of Japan (off Sado Island), were supplied by dealers. These fish were divided into organs, which were examined with the naked eye at first and then with artificial digestion technique.

Collected plerocercoids were preserved in physiological saline before use. For histological examination, they were fixed with formol saline (4% formaldehyde in 1% NaCl aqueous solution; Halvorsen, 1970), embedded in paraffine, sectioned at 7 μ and stained with azan or trichrome.

In order to obtain adult forms, golden hamsters and dogs were used as experimental final hosts. The hamsters were anaesthetized with ether before the plerocercoids were introduced into the stomach with a canule, while the dogs were forced to swallow a piece of bread containing the plerocercoids. Since one type of plerocercoids is too large for a hamster, only the scolex was given in one case (Table 2). Fecal examination was carried out daily from the seventh day after infection. If diphyllobothriid eggs were found, the infection experiment was taken as positive. The hosts were killed subsequently, cut open and the alimentary canals were incised in physiological saline.

The recovered adults were killed and relaxed in tap water containing a few drops of chloroform at 4 C. They were fixed with 5% formalin aqueous solution. Measurements of the strobilae were performed after Andersen (1971). Portions of the strobilae were stained in alum carmin or Meyer's hematoxylin. Sagittal and transverse sections were also prepared from a series of segments of the strobilae and stained as in the case of plerocercoids.

Table 1 Examination on plerocercoids in Alaskan haddock, *Theragra chalcogramma*, caught in the sea of Japan

Date of examination (in 1979)	No. of fish examined	Body length of fish (cm)	Body weight of fish (kg)	No. of plerocercoids recovered	
				Type A	Type B
Jan. 8	2	—	—	—	3
Jan. 16	15	41–55	—	—	4
Apr. 9	10	—	—	10	13
May 8	18	37–46	0.29–0.88	8	1
Oct. 9	10	35–48	—	0	0
Oct. 22	5	43–49	0.70–0.94	13	2
Nov. 5	3	51–55	1.24–1.51	25	0
Nov. 12	3	49–54	1.16–1.26	3	0
Nov. 20	2	57, 58	1.8, 2.0	2	0

Results

Plerocercoids.

Results of examination of the fish are summarized in Table 1. Two types of plerocercoids were detected.

Type A. This type were recovered from the stomach wall. Body is tadpole-like. When alive, plerocercoids of type A moved actively, expanding and contracting, and adhered to the bottom of a glass dish with the bothria. When killed in formol saline, they contracted (Fig. 1). Anterior part has bothria on dorsal and ventral areas. Bothrial part is the widest, and the scolex occupies from one-third to one-half of the total length of the plerocercoids in fixed condition. Body surface is smooth and not wrinkled. Body length: 0.55–1.00 mm, maximum width: 0.30–0.40 mm. Bothrial length: 0.23–0.41 mm.

Cross section of the scolex is round and bothria are V-shaped (Fig. 2). Microtriches are absent. Epidermal longitudinal musculature consists of one layer of muscle fibers. Frontal gland cells were found in medullar parenchyma of bothrial part, and their distribution forms an H-shape in cross section. In cross section of midbody, epidermal longitudinal musculature is one-layered, and parenchymal longitudinal musculature forms a thin elliptic band (Fig. 3). Parenchymal transverse musculature is dispersed and inconspicuous. Frontal gland is extended from the bothrial portion to near the posterior extremity (Fig. 4). Central invagination is not found at the posterior extremity.

Type B. This type was found among the pyloric appendages, on the liver and in the abdominal cavity. The plerocercoids moved actively when transferred into physiological saline, contracting and expanding. The scolex protruded and was triangular in shape. When killed in formol saline, the plerocercoids contracted and became strongly wrinkled (Fig. 5). Body is widest near the posterior margin of bothrium. Body length: 14.0–42.7 mm, maximum body width: 1.5–2.0 mm and bothrial length: 1.6–3.0 mm. Body has shallow grooves on dorsal and ventral surface, and they extend to near caudal end. Body also has lateral grooves. Therefore, a cross section in midbody always shows four depressions in outline; two dorsoventral and two lateral (Fig. 7).

In cross section, outer epidermis lacks microtriches. Epidermal longitudinal musculature is composed of one-layered muscle fibers. Cross section of bothrial part shows deeply incised bothria which are also branched at the bottom (Fig. 6). Frontal gland cells are present in the medullary parenchyma surrounding the bothria. Their

Table 2 Experimental infection with plerocercoids recovered from Alaskan haddock, *Theragra chalcogramma*, to hamsters and dogs

Species of experimental host	Type of plerocercoid given	No. of plerocercoids given (per animal)	No. of animals used	Duration of infection (days)	No. of worms recovered	No. of mature worms
Hamster	A	5	1	14	2	1
〃	〃	〃	4	17	3	1
〃	〃	〃	3	21	1	1
〃	B	2	1	—	0	0
〃	〃*	2	1	—	0	0
Dog	A	10	1	—	0	0
〃	B	4	2	—	0	0
〃	〃	2	1	—	0	0

* Only bothrial portion was given.

distribution is restricted to the bothrial portion. In cross section of midbody, parenchymal longitudinal musculature is arranged in an elliptic band which is broadest mid-dorsally and mid-ventrally. This band looks as if it were pushed inward by the four grooves on the surface (Fig. 7). Central invagination is present at the posterior end (Fig. 8). Measurements of cross section in midbody are as follows: breadth of section: 1.37–1.75 mm, height of section: 1.31–1.56 mm, thickness of outer epidermis: 22.4–22.8 μ, thickness of epidermal longitudinal musculature: 5.7–6.8 μ, thickness of parenchymal longitudinal musculature: 195.8–322.9 μ, thickness of parenchymal transverse musculature: 125.5–227.2 μ.

Infection experiment.

The results of the experimental infections are summarized in Table 2. Only type A plerocercoids developed to adults in the golden hamsters. The prepatent period varied from 12 to 20 days.

Description of adults of type A.

External morphology (Figs. 9–11) (Based on 3 mature strobilae).

Strobila is robust and muscular, with slightly serrated margins, and widened gradually behind scolex. Length of strobila: 15.4–24.3 cm. Maximum width, 5.5–7.3 mm, attained in posterior half of strobila in two worms, but in anterior half in the other worm. Scolex is cordate or wide-oval in lateral view and club-shaped in dorsoventral view. Deep bothria open widely near apex but bothrial margins overlap in posterior part of scolex. Scolex length: 2.11–2.41 mm, scolex dorsoventral height: 1.70–2.11 mm. Neck is short and extended more anteriorly on lateral sides than on dorsoventral sides. Distance from posterior end of bothria to first interproglottid boundary is 1.55–2.85 mm. Number of segments anterior to genital primordia is 17–46, anterior to mature segments, 109–167. Total number of segments is 150–209. Length of segments at the widest part of strobila is 1.48–3.4 mm. Every segment has one set of reproductive organs, but one segment is exceptionally diplogonadic.

Internal morphology (Figs. 12–15).

Genital atrium is situated in the first fifth to first third of ventral surface of proglottids. Genital papillae, usually inconspicuous, are present on surface around genital atrium. In sagittal section, cirrus sac is oval in shape, 0.33–0.44 mm in diameter and 0.51–0.59 mm in length, and situated obliquely against ventral surface. In many segments, anteriormost portion of cirrus sac extends into preceding segment. Uterine

pore is situated 0.20–0.25 mm behind genital atrium. Ovary consists of two lateral lobes and a median isthmus. Each lateral lobe is wing-shaped with a protruding posterior horn. Vagina arises from seminal receptacle behind ovarian isthmus and runs anteriad in ventral parenchyma, undulating moderately. It extends to near the level of uterine pore, abruptly turns dorsad and extends to level of the end of cirrus sac, again turns ventrad and then runs directly to the opening in floor of genital atrium. Uterus consists of 8–13 loops on each side of midline. Anteriormost uterine loop extends to level of anterior third of cirrus sac. Vas deferens arises in middle third of segment and runs in dorsal parenchyma forming complex loops laterally. It also forms several ventral loops. Seminal vesicle is round, 0.12–0.16× 0.10–0.13 mm, and situated caudally to cirrus sac. Its long axis is transverse in some segments. Vitellaria, 50–73 μ in diameter, are numerous and distributed in lateral fields. They are often confluent at anterior and posterior margins of segments. Testes are oval-shaped, 0.12–0.15 mm in diameter, and arranged in two to three layers in medullar parenchyma. Their extension is the same as that of the vitellaria but they do not overlap the uterine coils and only touch the ovarian lobes laterally. Number of testes in a segment is about 500. Although there is a constriction between the neighboring segments, vitellaria and testes seem to be confluent from one segment to another in sagittal section (Fig. 15). Egg is elliptical and operculated (Fig. 16) and surface is finely sclobiculate. Operculum tip is very thick in some eggs. Size of eggs is 63–78×37–46 μ.

Discussion

The final hosts of the diphyllobothriid plerocercoids in the Alaskan haddock might be some marine mammals. Up to date more than 50 diphyllobothriid species have been recorded from marine mammals. However, there are many discrepancies among the classification systems of various investigators (e.g. Markowsky, 1952a, b; Delyamure, 1955; Yamaguti, 1959; Wardle *et al.*, 1974) and the number of valid species may be far fewer.

Type A larva closely resembles *Diphyllobothrium* sp. larvae recorded by Markowsky (1952a, Fig. 75) from Weddel seal and leopard seal. He thought they might be *D. wilsoni* (Shipley, 1907) or *D. mobile* (Rennie and Reid, 1912), but the present adults differ greatly from the adult forms of these species.

To the authors' knowledge, four types of *Diphyllobothrium* plerocercoids have been found in fishes which spend their entire lives in marine environment. The authors could not find the original description of the plerocercoids of *D. lanceolatum* (Krabbe, 1865) and *D. schistochilus* (Germanos, 1895) by Linstow (1878, 1901; Cited by Ginetsinskaya, 1958 and Delyamure, 1955), but the adult forms of these two species clearly differ from the present ones. Diphyllobothriid plerocercoids obtained from the gadid fish, *Micromestius potasson*, by Andersen (1977) and Grabda (1978) differ from the present species in having a flat body and a developed scolex. The present plerocercoids are also distinguishable from *Diphyllobothrium* sp. larvae recovered from *Sciaena deliciosa* and *Polyclemus peruanus* by Tantalean (1975) in having a smaller body and a different shape of scolex.

Morphological characteristics of type A adults resemble those of *Diphyllobothrium cordatum* (Leuckart, 1863), *D. pacificum* (Nybelin, 1931) and *D. alascense* Rausch and Williamson, 1958.

D. cordatum is parasitic to pinnipeds and was also reported from dogs and man. The description of *D. cordatum* are very confused. For example, the cirrus sac is somewhat pyriform or oval, and the testes are arranged in two or three layers according to Markowsky (1952a), while Delyamure

(1955) wrote that the former are elongated and the latter are all in one layer. In both descriptions, the cirrus sac is horizontal in contrast to the oblique direction in the present specimens.

Diphyllobothrium pacificum is a parasite of the fur seal and is also known in man on the Pacific coast of South America (Baer *et al.*, 1967; Sagua *et al.*, 1976). This species is recorded from seas adjacent to Japan (Yamaguti, 1951). *D. pacificum* is distinguishable from the present adults in having one-layered testes, smaller eggs and a wide space with pits anterior to the genital atrium (Markowsky, 1952a; Yamaguti, 1951; Baer *et al.*, 1967).

The present species is very similar to *D. alascense* which occurs in dogs in Alaska but is supposed to be a marine species (Hilliard, 1960, 1972). However, the adults of type A differ from *D. alascence* in having papillae around the genital atrium and a larger egg width, and in the shape of the junction between scolex and neck.

At the present time, the authors conclude that no species previously described has enough affinity to the type A adults. Nevertheless, there remains some possibility that the present adults belong to an already-known species, because the morphology of diphyllobothriid is greatly variable.

Type B may be the same species as the large plerocercoid recorded as *Pyramicocephalus phocarum* (Fabricius, 1780) from *Eleginus gracilis, Myoxocephalus quadricornis* and *Megalocottus platycephalus laticeps* by Rausch *et al.* (1967, 1970) in Alaska and from *Theragra chalcogramma* by Zhukov (1960), Mamaev and Baeva (1963) and Grabda (1977) in Far East waters. The so-called *P. phocarum* larvae were generally thought to be recovered first by Wardle (1932) from fingerling codfish, *Gadus ograc*, in arctic Canadian waters. Yamaguti (1959) doubted this identification by Wardle since the scolex of the plerocercoid was not cauliflower-like.

The adult *P. phocarum* was recorded not only from phocids but also from dogs and even man (e.g. Yamaguti, 1959). However, as in the present experiments, repeated attempts to infect dogs and man with so-called *P. phocarum* plerocercoids have failed (Rausch *et al.*, 1967, 1970). The host-specificity of *P. phocarum* should be critically re-examined. Machida *et al.* (1979) recently recovered *Pyramicocephalus* sp. from Steller sea lion in Japan.

The epidemiological significance of these plerocercoids in the Alaskan haddock as human pathogens is the subject for a future study.

Summary

Two types of plerocercoids were recovered from the Alaskan haddock, *Theragra chalcogramma*, caught in the Sea of Japan.

One type was obtained from the stomach wall and developed to adult stage when administered to the golden hamster. Adult of this type closely resembles *Diphyllobothrium alascence* Rausch and Williamson, 1958, but differs in details.

The other type was found among the pyrolic appendages, on the liver or in the body cavity, and identified as so-called *Pyramicocephalus phocarum* larva.

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References

1) Andersen, K. (1971): Studies of the helminth fauna of Norway XVII: Morphological comparison of *Diphyllobothrium dendriticum* Nitzsch 1824, *D. norvegicum* Vik 1957 and *D. latum* (Linné 1758) (Cestoda: Pseudophyllidea). Norw. J. Zool., 19, 21–36.
2) Andersen, K. (1977): A marine *Diphylloboth-*

rium plerocercoid (Cestoda: Pseudophyllidea) from blue whiting (*Micromesistius poutasson*). Z. Parasitenk., 52, 289–296.

3) Baer, J. G., Miranda, C. H., Fernandez, R. W., Medina, T. J. (1967): Human diphyllobothriasis in Peru. Z. Parasitenk., 28, 277–289.
4) Delyamure, S. L. (1955): The helminth fauna of mammals in the light of their ecology and phylogeny. Izdatel'stvo Nauk SSSR, Moscow, 517 pp. (In Russian)
5) Ginetsinskaya, T. A. (1958): The life cycles of fish helminths and the biology of their larval stages. In "Basic problems of the parasitology of fishes" (ed. by Dogiel, V. A., Petrushevsky, T. P. and Olyansky, Y. L.) p. 144–183, Izdatel'stvo Leningradskogo Universitata. (In Russian, English edition by T. F. H. Publ., USA, 1970, p. 140–179)
6) Grabda, J. (1977): Studies on parasitisation and consumability of Alaska pollack, *Theragra chalcogramma* (Pall.). Acta Ichthyol. Piscat., 7, 15–34.
7) Grabda, J. (1978): Studies on parasitic infestation of blue whiting (*Micromesistius* sp.) with respect to the fish utilization and consumption. Acta. Ichthyol. Piscat., 8, 29–41.
8) Halvorsen, O. (1970): Studies of the helminth fauna of Norway XV: On the taxonomy and biology of plerocercoids of *Diphyllobothrium* Cobbold, 1858 (Cestoda: Pseudophyllidea) from north-western Europe. Nytt. Mag. Zool., 18, 113–174.
9) Hasegawa, H., Hotta, T., Chiba, K., Sekikawa, H. and Otsuru, M. (1979): Studies on the diphyllobothriid cestodes in northern Japan. (2) The growth of *Diphyllobothrium* sp. from the Japanese surfsmelt, *Hypomesus pretiosus*, in the golden hamster. Jap. J. Parasit., 28, 427–434. (In Japanese)
10) Hilliard, D. K. (1960): Studies on the helminth fauna of Alaska. XXXVIII. The taxonomic significance of eggs and coracidia of some diphyllobothriid cestodes. J. Parasit., 46, 703–716.
11) Hilliard, D. K. (1972): Studies on the helminth fauna of Alaska. LI. Observations on eggshell formation in some diphyllobothriid cestodes. Can. J. Zool., 50, 585–592.
12) Hotta, T., Chiba, K., Hasegawa, H., Sekikawa, H. and Otsuru, M. (1978): Studies on the diphyllobothriid cestodes in northern Japan. (1) Plerocercoids recovered from several species of fishes and their adult forms. Jap. J. Parasit., 27, 357–368. (In Japanese)
13) Hotta, T., Chiba, K., Hasegawa, H., Sekikawa, H. and Otsuru, M. (1979): Epidemiological surveys on diphyllobothriasis in Niigata Prefecture. Niigata Med. J., 93, 374–383. (In Japanese)
14) Hotta, T., Hasegawa, H., Chiba, K., Sekikawa, H. and Otsuru, M. (1980): Studies on the diphyllobothriid cestodes in northern Japan. (3) Plerocercoids recovered from three-spined sticklebacks caught in the Lake Tofutsu of Hokkaido and an adult developed in the hamster. Jap. J. Parasit., 29, 157–161. (In Japanese)
15) Machida, M., Yamaguchi, K. and Seki, M. (1979): Parasites of Steller sea lion caught off southern Hokkaido. (presentation at 48st Ann. Meet, Jap. Soc. Parasit., April 5–6, Tokyo.)
16) Mamaev, J. L. and Baeva, O. M. (1963): Helminth fauna of *Theragra chalcogramma* (Gadiformes) from Kamtchatka waters and on the use of this fish as food. Helminthologie, 4, 318–331. (In Russian)
17) Markowsky, S. (1952a): The cestodes of pinnipeds in the arctic and other regions. J. Helminth., 26, 171–214.
18) Markowski, S. (1952b): The cestodes of seals from the antarctic. Bull. Brit. Mus. (Nat. Hist.), Zool., 1, 123–150.
19) Rausch, R. L. and Hilliard, D. K. (1970): Studies on the helminth fauna of Alaska. XLIX. The occurrence of *Diphyllobothrium latum* (Linnaeus, 1758) (Cestoda: Diphyllobothriidae) in Alaska, with notes on other species. Can. J. Zool., 48, 1201–1219.
20) Rausch, R. L., Scott, E. M. and Rausch, V. R. (1967): Helminths in Eskimos in western Alaska, with particular reference to *Diphyllobothrium* infection and anaemia. Trans. Roy. Soc. Trop. Med. Hyg., 61, 351–357.
21) Rausch, R. L. and Williamson, S. L. (1958): The description and occurrence of *Diphyllobothrium alascense* n. sp. (Cestoda). Z. Tropenmed. Parasit., 9, 64–72.
22) Sagua, H., Miranda, E., Fuentes, A., Vladillo, V. (1976): *Diphyllobothrium pacificum* (Nybelin, 1931), Margolis, 1956. Primeros dos casos de infección humana en el notre de Chile. Bol. Chil. Parasit., 31, 33.
23) Tantalean, M. (1975): Hallazgo de larvas plerocercoides de Diphyllobothriidae Lühe, 1910 (Cestoda) en peces del mar peroano. Bol. Chil. Parasit., 30, 18–20.
24) Wardle, R. A. (1932): The cestodes of Canadian fishes II. The Hudson Bay drainage system. Contr. Can. Biol. Fish., n.s. 7, No. 30, p. 377–403.
25) Wardle, R. A., McLeod, J. A. and Radinovsky, S. (1974): Advances in the zoology of tapeworms. University of Minnesota Press, Minneapolis, 247 pp.

26) Yamaguti, S. (1951): Studies on the helminth fauna of Japan. Part 47. Cestodes of marine mammals and birds. Arb. Med. Fak. Okayama, 7, 307–314.
27) Yamaguti, S. (1959): Systema Helminthum Vol. II. The cestodes of vertebrates. Interscience Publ., New York, 626 pp.
28) Zhukov, E. V. (1960): Endoparasitic worms of the fishes in the Sea of Japan and South-Kuril shallower waters. Trudi Zool. Inst. Akad. Nauk. SSSR, 28, 3–146. (In Russian)

北日本における裂頭条虫類の研究

(4) スケトウダラ *Theragra chalcogramma* より得られたプレロセルコイドとその成虫

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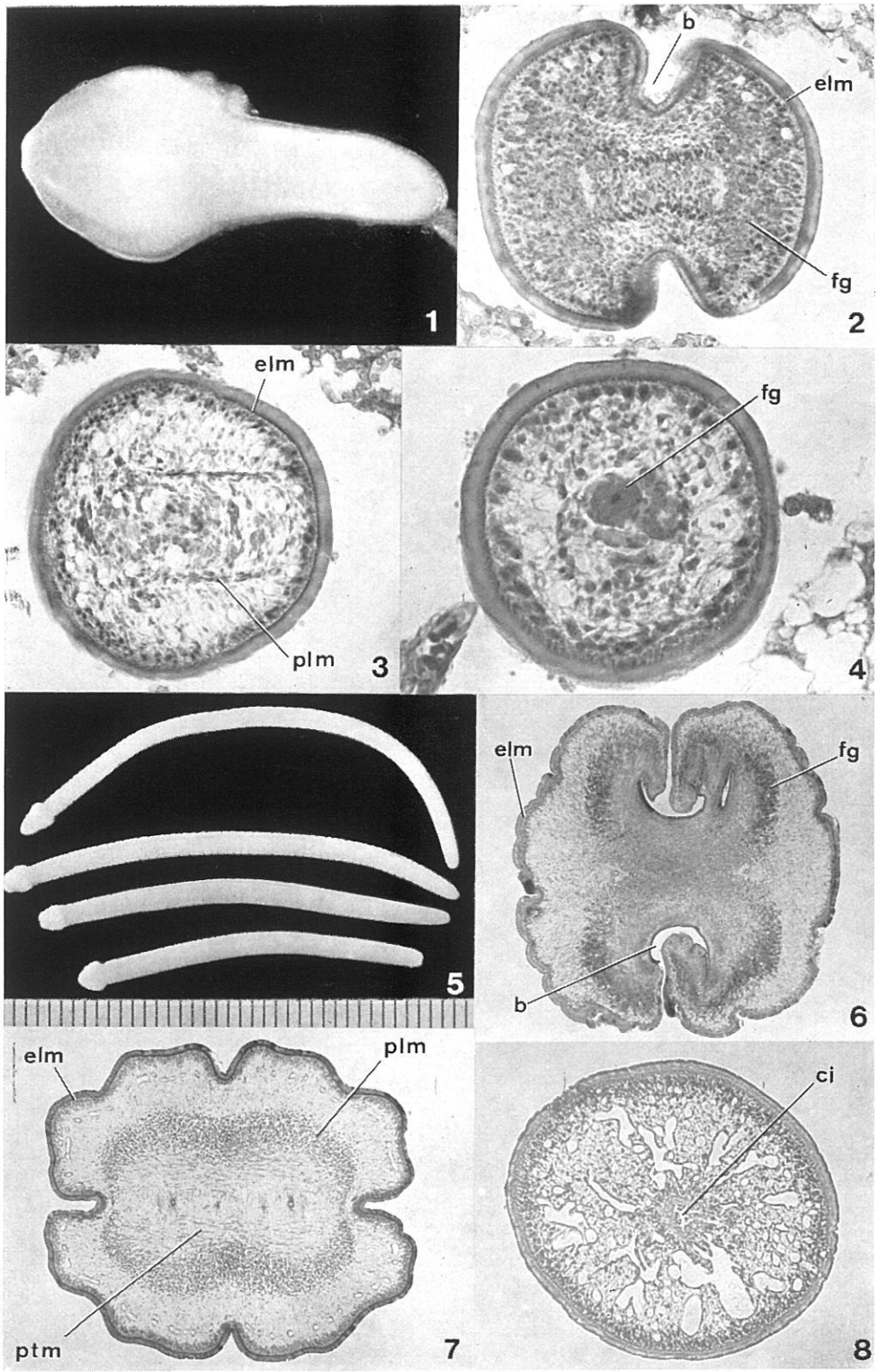
日本海で捕獲されたスケトウダラ *Theragra chalcogramma* より2種のプレロセルコイドが得られた.

1種は胃壁に寄生しており，ハムスターに投与すると成虫に発育した．成虫は *Diphyllobothrium alascense* Rausch and Williamson, 1958 に類似していたが，細部において異っていた.

他の1種は幽門垂間，肝表面，腹腔内に見られ，イヌ，ハムスターへの感染実験で成虫を得ることはできなかったが，プレロセルコイドの形態から，いわゆる *Pyramicocephalus phocarum* と考えられる.

Explanation of Figures

Figs. 1–4 Type A plerocercoid.
Fig. 1 General appearance, lateral view. (×112)
Fig. 2 Cross section of bothrial part. (×202)
Fig. 3 Cross section of midbody. (×202)
Fig. 4 Cross section of posterior extremity. (×381)
Figs. 5–8 Type B plerocercoid.
Fig. 5 General appearance.
Fig. 6 Cross section of bothrial part. (×25)
Fig. 7 Cross section of midbody. (×25)
Fig. 8 Cross section of posterior extremity. (×112)
Figs. 9–16 Adult developed from type A plerocercoid in hamster.
Fig. 9 Total strobila.
Fig. 10 Scolex, lateral view. (×11)
Fig. 11 Scolex, dorsoventral view. (×11)
Fig. 12 Cross section of scolex. (×19)
Fig. 13 Schematic illustration of genital organs of mature proglottid, ventral view.
Fig. 14 Midsagittal section of mature proglottid. (×23)
Fig. 15 Parasagittal section of mature proglottid. (×23)
Fig. 16 Eggs. (×560)
Abbreviations used in figures.
b: bothrium, c: cirrus, ci: central invagination, cs: cirrus sac, elm: epidermal longitudinal musculature, fg: frontal gland, ga: genital atrium, mg: Mehlis' gland, o: ovary, oi: ovarian isthmus, od: oviduct, ot: ootype, plm: parenchymal longitudinal musculature, ptm: parenchymal transverse musculature, sr: seminal receptacle, sv: seminal vesicle, t: testes, u: uterus, up: uterine pore, v: vagina, vd: vas deferens, vg: vitelline glands, vr: vitelline reservoir.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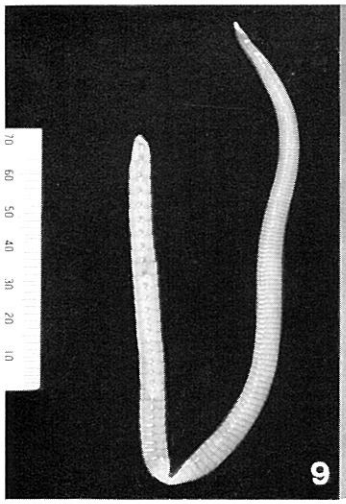
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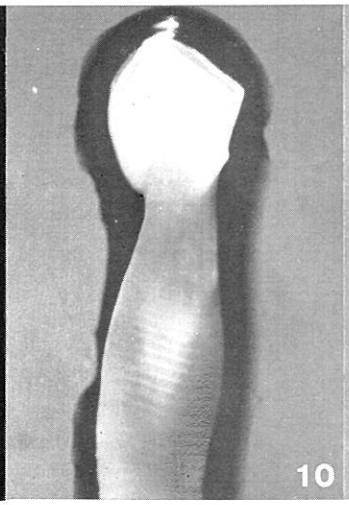
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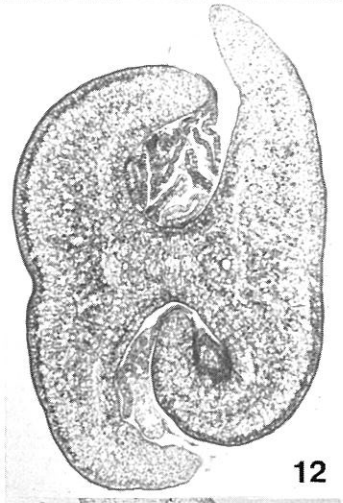
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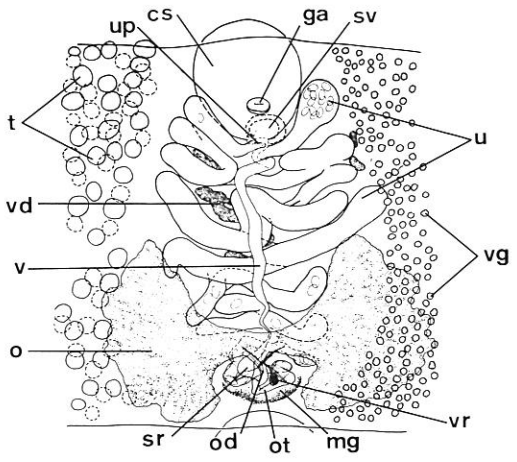
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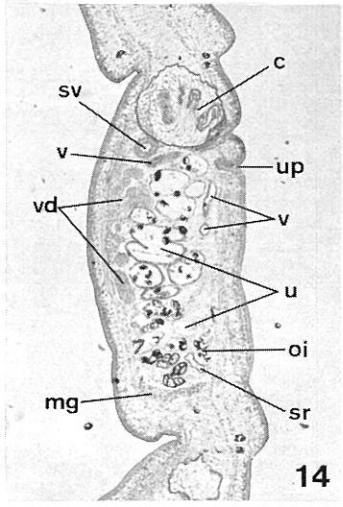
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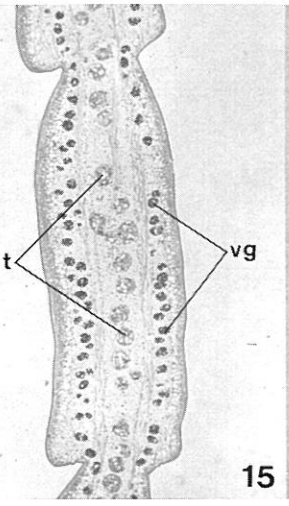
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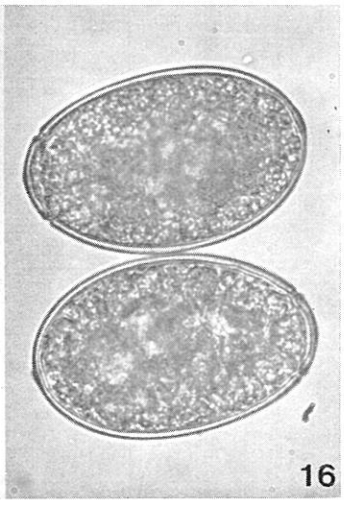
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