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On a New Marine Species of the Genus *Diphyllobothrium* (Cestoda: Pseudophyllidea) Found from a Man in Japan

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Introduction

During the course of reviewing strobilae expelled from some cases of human diphyllobothriasis in Japan, Kamo *et al.* (1977) suggested the occurrence of human infection with a certain kind of marine species of *Diphyllobothrium*. Their specimens exhibited several distinguishable characters in some morphology of reproductive organs and in the feature of egg-shell surface through the scanning electron microscopy. However, they withheld from the species determination due to the lack of scoleces in their specimens.

Recently, a complete specimen of the cestode expelled from a man, 41-year-old, who have never eaten any kind of salmonfish, suspected carrier of the broad tapeworm, was kindly sent to us for identification from Dr. J. Inoue, Yonago City (Yamane *et al.*, 1977). As a result of our careful examination it was recognized as a new marine species belonging to the genus *Diphyllobothrium* Cobbold, 1858.

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Materials and Methods

The specimen expelled from a man, 41-year-old, after the treatment with bithionol, was well relaxed, preserving in 5% formalin solution. Series of segments taken from different levels of the strobila were stained in Semichon's acetic carmine, dehydrated in ethanol, and amounted in Eukit, a mounting reagent, after they were rinsed in xylene. Transverse and sagital sections were prepared at different levels of the strobila. They were stained in trichrome stain solution. Some segments were cut off, being rinsed with phosphate buffer, and some eggs were taken off from the uteri of gravid proglottides, being rinsed in tap water. They were put into 5% glutalaldehyde with phosphate buffer, being fixed in 1% osmium tetroxide for 2 hours at 4 C. Then they were dehydrated by the passage through graded series of ethanol, being transfered into amylacetate, and dried by the critical point method using crushed dry ice. Finally they were coated with gold paradium alloy, being examined with the Hitachi MSM-V type scanning electron microscope. Some eggs were cultured in the fresh water and the sea water at 20 C.

Diphyllobothrium yonagoensis sp. nov.

Diagnosis—Strobila rather stout, with parallel, slightly serrate margins. Strobila 4,650 mm long, comprised of about 1,700 segments, maximum width 13 mm, attained about 500–900 mm posterior to scolex. Segments wider than long, with relative length increasing near posterior end of strobila. Length/width ratio of pregravid segments about 1:10, of terminal gravid segments as much as 1:3. Maximum thickness of strobila 0.6 mm. Innermost layer of longitudinal muscle fibre well developed. Excretory system situated bilaterally in medullary parenchyma as undulating longitudinal ducts. Calcareous corpuscles abundant.

Scolex 1.2 mm long ×1.1 mm wide ×0.7 mm thick, nearly hammer-shaped in lateral view. Bothria deep, extending full length of scolex, with broad bothrial margins folded (Fig. 3). Neck short, 3 mm long. Genital anlagen visible within about 250 mm posterior to scolex. Two sets of genital organs in one segment presented rather frequently, i.e. about 180 segments have two sets of genital organs among 630 mature segments (Figs. 8–13, 16).

Genital pore situated ventrally on midline in anterior fourth of segment. Cirrus sac piriform, 0.432–0.515 mm long by 0.278–0.360 mm in diameter, opening obliquely into genital atrium. Seminal vesicle large, spherical to subspherical, 0.206–0.463 mm long by 0.206–0.360 mm in diameter, with very thick wall (0.062–0.103 mm) connected with cirrus sac almost right behindly (Figs. 23–25). Subspherical testes, 0.082–0.111 mm in greatest diameter, arranging in single layer in medullary parenchyma, not continuous from segment to segment. Testes and vitelline follicles abundant, forming two lateral fields, only slightly overlapping ends of uterine loops. Uterine loops extending laterad and anterolaterad (usually 8–9 loops in each side), reaching level of anterior margin of genital atrium, forming elliptic uterine field in the center of segment (Figs. 18–20). Uterus opening into through uterine pore, about 15–20% of segment-length posterior to genital pore.

Eggs ellipsoidal with apical knob (Fig. 4), measuring 56.5±1.6 μ by 43.4±1.3μ in diameter on average. Eggshell (2.0±0.3 μ thick) exhibit small deep pits distributed densely on the surface (Fig. 6).

Embroys develop within a week at 20 C. Coracidia hatch 80% only in the sea-water.

Host—Homo sapiens. Habitat—Intestine.

Type locality—Yonago City, Tottori Prefecture, Japan.

Type—A strobila fixed and preserved in 5% formalin solution, prepared slides bearing short series of segments and serial sections of several portions of strobila have been deposited in Department of Medical Zoology, Tottori University School of Medicine, Yonago City, 683 Japan. No. HLS7703.

Discussion

In his studies on eggshell formation of diphyllobothriid cestodes, Hilliard (1972) demonstrated that "in those having marine intermediate and final hosts, the shell of the fully developed egg is deeply pitted, and hatching of eggs from such cestodes depends upon salinity rather than light, while in those having the aquatic phase of their life cycle in fresh water, the eggshell are only superficially pitted, and hatching usually mediated by light".

According to Hilliard's criteria eggs from our specimen showed typical characters of the cestode associated with a marine environment in structure of the shell and in hatching behavior.

Besides *Diphyllobothrium latum*, the common species in man, not a few species of the genus including some synonyms of *D. latum* have been reported from human cases. Although taxonomical situations are

not always definite in all of them, some authentic cases have been published recently as follows: *D. lanceolatum* (Krabbe, 1865) from Alaska by Rausch and Hilliard (1970); *D. pacificum* (Nybelin, 1931) from Peru by Baer *et al.* (1967); *D. cameroni* Rausch, 1969 from Japan by Kamo *et al.* (1978); *D. cordatum* (Leuckart, 1863) from Iceland by Leuckart (1863); *D. alascense* Rausch and Williamson, 1958 from Alaska by Rausch and Hilliard (1970); *D. dendriticum* (Nitzsch, 1824), *D. dalliae* Rausch, 1956, both from Alaska by Rausch and Hilliard (1970); *D. ursi* Rausch, 1954 from Alaska by Rausch and Hilliard (1970), from Canada by Margolis *et al.* (1973).

Of the above mentioned 8 species the latter three were regarded as the group having the aquatic phase of the life cycle in freshwater, being distinguishable from our specimen in their characters of eggshell surface and the hatching behavior as well as some morphological features of the strobila. The former 5 species were recognized as the group having marine intermediate and final hosts, showing remarkable specific characters different from our specimen as follows: *D. lanceolatum* in its typical lanceolate shape of the body; *D. pacificum* in the position of the genital pore (about in the middle of the segment-length), a series of transverse pits situated between the anterior border of the proglottid and the genital pore; *D. cameroni* in its small, stout body and the relative position of the uterine pore, within the genital atrium; *D. cordatum* in its small, thick body and the arrangement of testes in 2 or 3 layers; *D. alascense* in its large cordate scolex, the relative position of the cirrus sac and seminal vesicle and the arrangement of testes in 2 or 3 deep.

From various marine mammals there have been known numerous species as follows: *D. elegans* (Krabbe, 1865), *D. hians* (Diesing, 1850), *D. macrocephalus* Linstow, 1905, *D. phocarum* Deljamure, Kurotschkin et Skrjabin, 1964, *D. ponticum* Deljamure, 1971, *D. pterocephalum* Deljamure et Skrjabin, 1965, *D. romeri* (Zschokke, 1903), *D. schistochilum* (Germanos, 1895)—from the Arctic and Northern Sea; *D. lashley* (Leiper et Atkinson, 1914), *D. mobile* (Rennie et Reid, 1912), *D. quadratum* (Linstow, 1892), *D. scoticum* (Rennie et Reid, 1912), *D. ventropapillatum* Deljamure, 1955 and *Glandicephalus antarcticus* (Baird, 1853), *G. perfoliatus* (Railliet et Henry, 1912)—from the Arctic (Markowski, 1952 a,b; Deljamure, 1955; Yamaguti, 1959).

Most of them are distinct in their small but stout bodies or vermiform bodies, and in other characters of inner structure, being quite different from our specimen. *D. ponticum* is a species having exceptionally larger body among them, but it exhibits some distinctive characters such as the small, oval scolex, the short neck, the narrow gravid proglottid, the elongated uterus with numerous loops, etc.

Finally a few species of the genus have been known from some marine mammals in the surrounding area of Japan. According to a brief review by Kamo *et al.* (1976) they are tentatively recognized as the following 5 species: *D. stemmacephalum* from *Delphinus dussimierri* (now *D. capensis*) by Yamaguti (1935); *D. gondo* Yamaguti, 1942 (?=*D. subtile* Yamaguti, 1942) from *Globicephalus scammoni*; *D. pacificum* (Nybelin, 1929) from *Callotaria ursina* (now *Callorhinus ursinus*) by Yamaguti (1951) and others; *D. macroovatum* Jurachno, 1973 from *Balaenoptera acutorostrata* by Kamo *et al.* (1976); *D. fuhrmanni* Hsü, 1935 from *Lagenorhynchus obliquidens* by Kamo *et al.* (1976).

D. gondo (=*D. subtile*) is distinguished from our specimen in the funnelshaped scolex and in larger sizes of body and eggs. *D. macroovatum* is also different from our specimen in its remarkably larger size of eggs, larger number of uterine loops, and in relative position of the cirrus sac and

the seminal vesicle. *D. stemmacephalum* (Cobbold, 1858) is rather unsettled, but referring to some redescriptions (Deljamure, 1968; Stunkard, 1949; Cohn, 1912) it differs from our specimen in the number of excretory canals, in the number of uterine loops.

D. stemmacephalum by Yamaguti (1935), however, which has been recognized as junior synonym of *D. fuhrmanni* Hsü, 1935 by many authors, is very similar morphologically to our specimen in many characters such as the hammer-shaped scolex, the wider proglottids, the smaller number of uterine loops, and the thick-walled seminal vesicle connected right behind with the cirrus sac, differing from *D. fuhrmanni* in these characters.

Our specimen is also well consisted with the specimen No. 3 of "Koga-Okamura type" by Kamo *et al.* (1977), which has been found from a man living in the neighbour town—Nawa-cho.

Incidentally some species have been known from birds besides *D. dendriticum* as follows: *D. ditremum* (Creplin, 1825); *D. vogeli* Kuhlow, 1953; *D. sebago* (Ward, 1910); *D. arctomarinum* Serdyukov, 1969; *D. colymbi* Yamaguti, 1951; and *D. polyrugosum* Deljamure et Skrjabin, 1966.

Of these the former 3 species have been recognized as those associated with freshwater environment, being easily distinguished from our specimen in their morphological characters (Andersen, 1972; Bylund, 1975; Meyer and Robinson, 1963). The formerly mentioned characters of our specimen separate it also from the latter 3 species.

Some other uncertain species such as *D. didelphydis* (Ariola, 1900), *D. glaciale* (Cholodkowsky, 1915), *D. grañaia* Bacigalupo, 1948, *D. minus* (Cholodkowsky, 1916) and *D. podicipedis* (Diesing, 1854) were excluded from the objects for comparison.

The morphological features used as differential characters for these marine species here are not necessarily considered to be well established, but it seems to be unavoidable under the present situations lacking the knowledge of life cycle for marine species of diphyllobothriid cestodes.

In conclusion our specimen is regarded as a distinct species of the genus *Diphyllobothrium*, and future investigations may show that its natural hosts are some marine mammals such as *Delphinus capensis*, and so on.

Summary

A new species of cestode *Diphyllobothrium yonagoensis* n. sp. was described on the basis of a specimen expelled from a man, 41-year-old, Yonago City, after the treatment with bithionol (40 mg per kg body weight).

The new species is differentiated by the hammer-shaped scolex with folded bothrial margins, frequent occurrence of two sets of genital organs in one segment, and the thick-walled seminal vesicle connected almost right behind with the cirrus sac.

It is very similar morphologically to *D. stemmacephalum* by Yamaguti (1935), which has been recognized as junior synonym of *D. fuhrmanni* Hsü, 1935. Both of them, however, are distinguishable from *D. fuhrmanni* Hsü, 1935 as well as *D. stemmacephalum* (Cobbold, 1858).

According to Hilliard's criteria eggs of the new species exhibit the typical characters for the cestode associated the marine environment, and future investigations may show that its natural hosts are some marine mammals.

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Dept. of Zoology, British Museum (Nat. Hist.), for providing with facilities for studying cetsode materials collected by Markowski.

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人から見出された海洋性裂頭条虫の一新種（条虫綱：擬葉目）

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鳥取県米子市在住の男性（年齢41歳）からビチオノール（40 mg/kg）頓用により駆出された虫体に基づき，新種 *Diphyllobothrium yanogaensis* n. sp. 米子裂頭条虫（ヨナゴ裂頭条虫，新称）を記載した．

新種は，そのハンマー形の頭節，交互に巻き込まれた深い吸溝縁，２組の生殖器を備える片節の多発傾向，陰茎嚢に殊んど真後から接続する壁の厚い貯精嚢，などの形態的特徴によって他種と区別される．

本種は，山口（1935）がハセイルカから得て *D. stemmacephalumm* として報告したものに近似しているが，*D. stemmacephalumm* (Cobbold, 1858) や，*D. fuhrmanni* Hsü, 1935 とは区別される．さらに，虫卵の卵殻表面（走査電顕像）に深い点刻が密に分布し，海水中でコラシジゥムが孵化するなど，Hilliard (1970) のいう海洋性裂頭条虫の特徴を示しており，将来の研究により海産哺乳類の中に自然宿主が見出されるものと考えられる．

Explanation of Figures

Fig. 1 Whole worm preserved in 5% formalin solution.

Fig. 2 Anterior part of strobila.

Fig. 3 Scolex, neck and immature segments. (scale=1 mm)

Fig. 4 Egg. (scale=10 μ)

Fig. 5 Coracidium hatched in sea-water.

Fig. 6 Eggshell surface by scanning electron microscopy. (×5,000)

Fig. 7 Immature segments at ca. 100 mm from anterior end (no staining).

Fig. 8 Mature segments at ca. 500 mm from anterior end (no staining).

Fig. 9 Mature segments at ca. 1,000 mm from anterior end (no staining).

Fig. 10 Mature segments at ca. 2,000 mm from anterior end (no staining).

Fig. 11 Gravid segments at ca. 3,000 mm from anterior end (no staining).

Fig. 12 Mature segments at ca. 2,500 mm from anterior end, showing abnormal segmentation (no staining).

Fig. 13 Gravid segments at ca. 3,400 mm from anterior end. Eggs scarecely seen in uteri (no staining).

Fig. 14 Terminal segments (no staining).

Fig. 15 Area of genital atrium by scanning electron microscopy. (×50).

Fig. 16 Area of genital atrium by SEM, showing double sets of genital organs. (×50) c=cirrus, u=uterine opening.

Fig. 17 Cross cracking of mature segment by freeze-fructure method of scanning electron microscopy. (×50)

Fig. 18 Mature segments stained with carmine, showing double sets of genital organs in one segments. (scale=2 mm)

Fig. 19 Uterine loops in mature segment.

Fig. 20 Uterine loops in two mature segments, showing single set and double sets of genital organs, respectively.

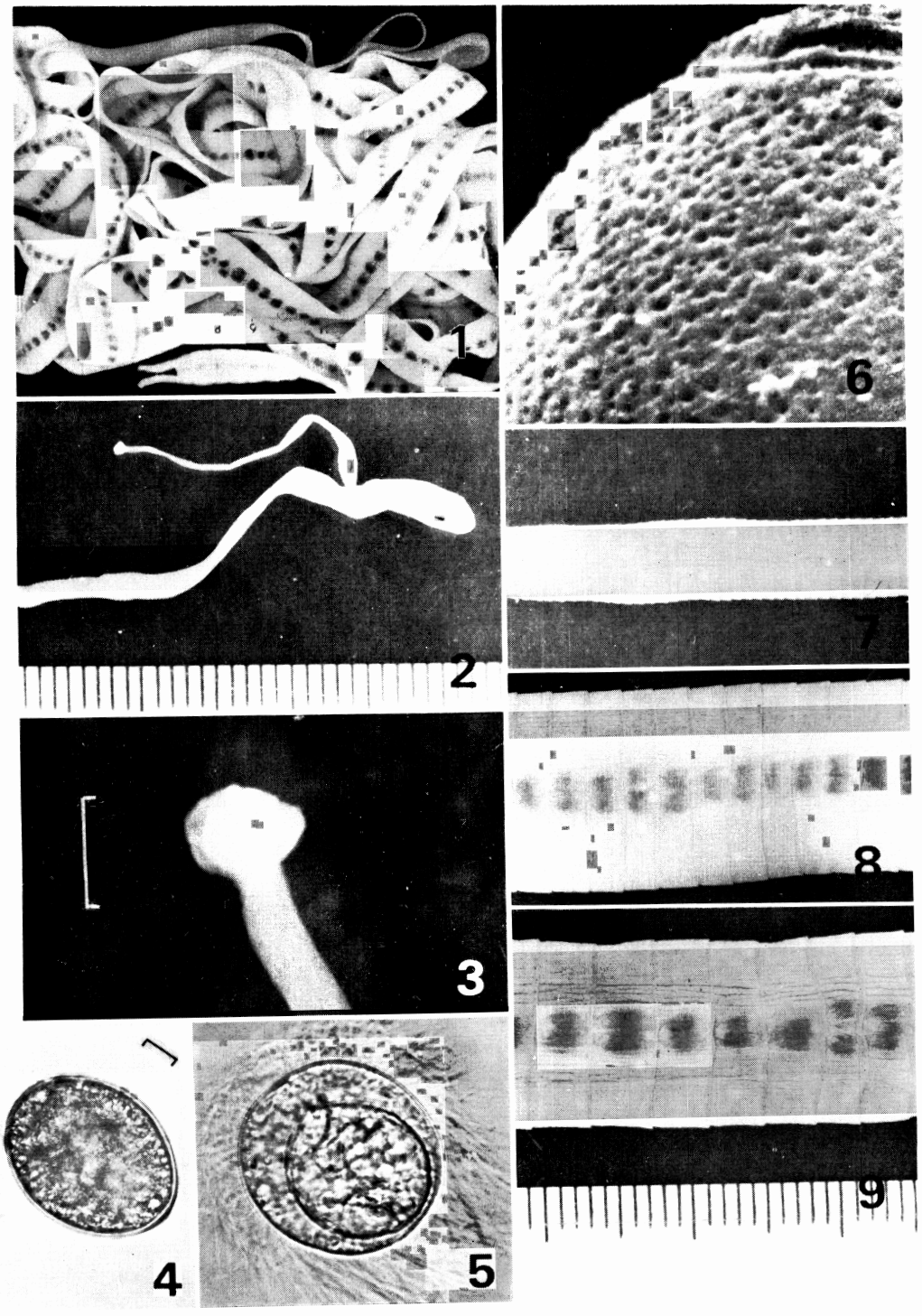
Fig. 21 Cross section of mature segment, showing double sets of genital organs. (scale=0.5 mm)

Fig. 22 Cross section of mature segment. (scale=0.5 mm)
lm=longitudinal muscle layer, n=nerve cord, t=testes, tm=transverse muscle layer, y=yolk glands (vitellaria).

Fig. 23 Sagittal section of mature segment. (scale=0.5 mm).

Fig. 24 Larger magnification of Fig. 23. (scale=0.5)
c=cirrus sac, g=genital atrium, s=seminal vesicle, u=uterus, uo=uterine opening, v=vagina

Fig. 25 Sagittal section of another mature segment. (scale=0.5 mm)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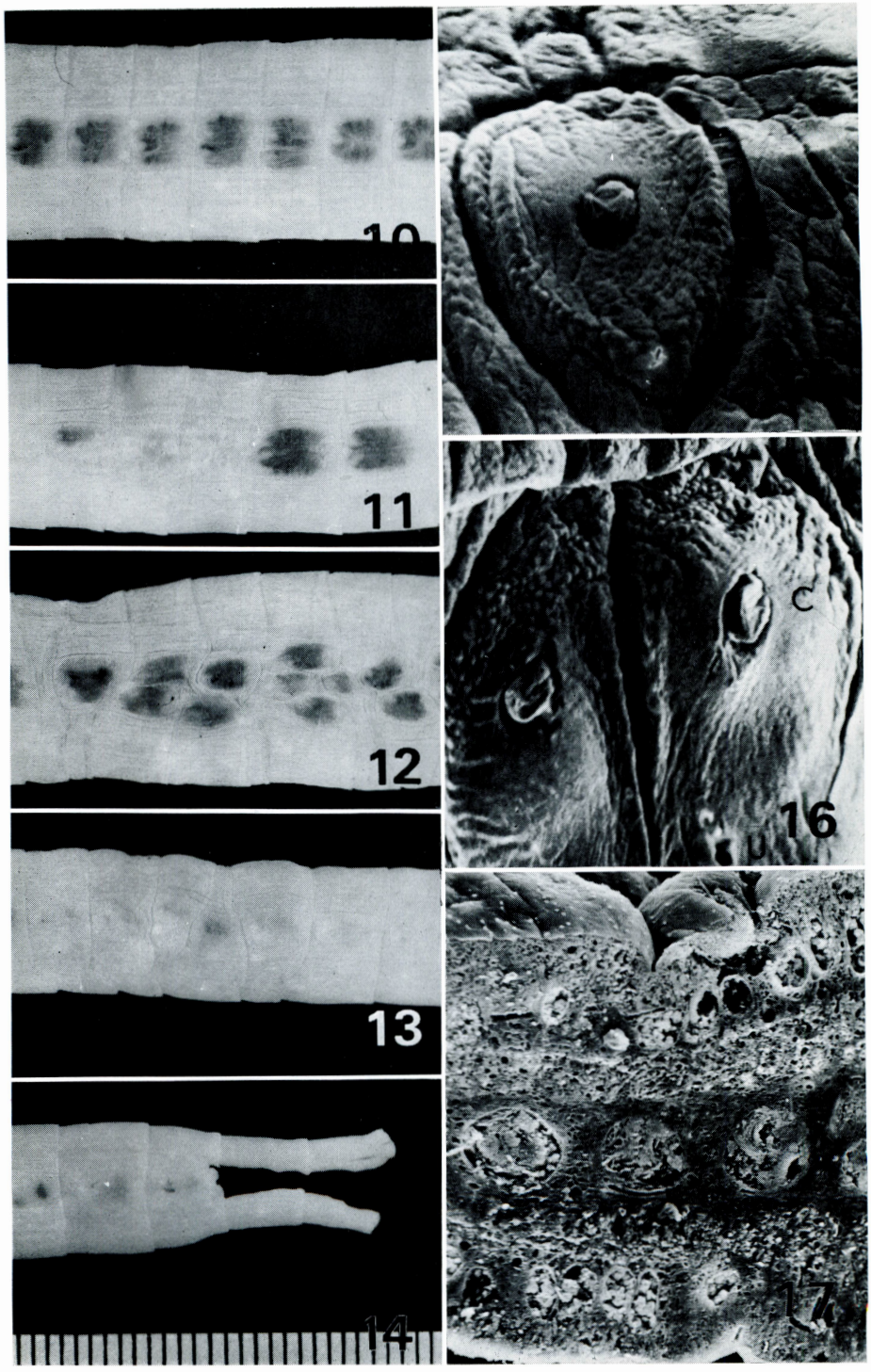
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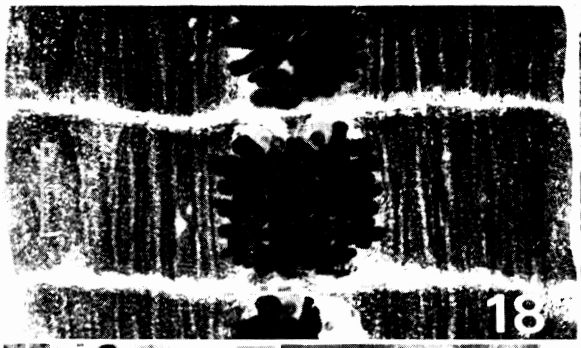
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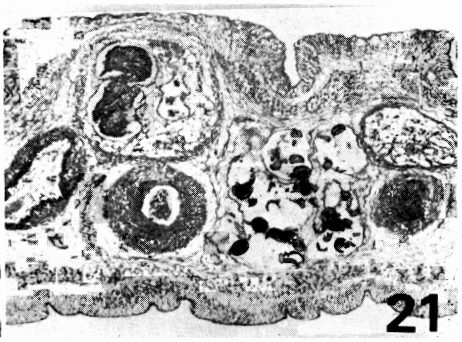
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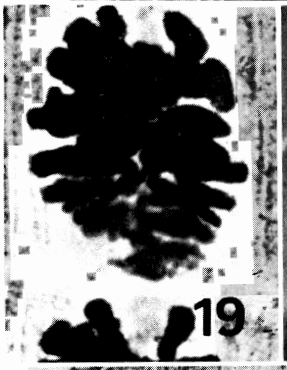
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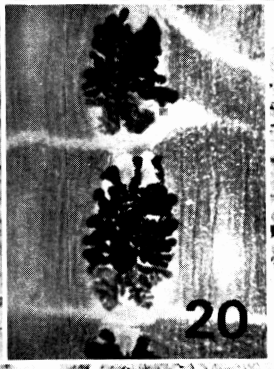
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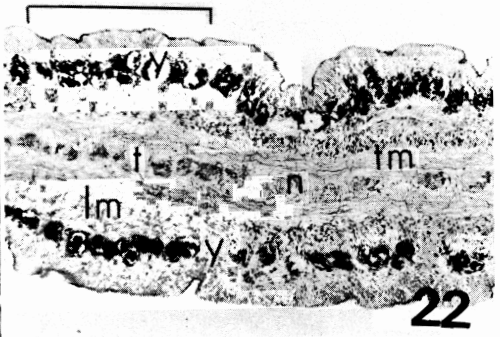
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