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Two New Species of the Genus *Dicyema* (Mesozoa) from Octopuses of Japan with Notes on *D. misakiense* and *D. acuticephalum*

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ABSTRACT—We examined dicyemid mesozoans from the renal sacs of both *Octopus vulgaris* and *Octopus minor*, obtained off the coast of Japan, and found two new species that belong to the genus *Dicyema*.

Dicyema japonicum sp. nov. from *O. vulgaris*, is a medium sized dicyemid, rarely exceeding 1500 μm in length. The number of peripheral cells in the vermiform phases is usually 22. The disc-shaped calotte and parapolar cells form the cephalic enlargement. The axial cell is cylindrical but is rounded anteriorly, and it extends forward to the base of propolar cells. Infusoriform embryos consist of 37 cells. In each of the four urn cells, there are the cell's own nucleus and one germinal cell with its own nucleus.

Dicyema clavatum sp. nov. is a relatively small sized dicyemid, infrequently reaching 1000 μm in length, and it is the first mesozoan species described from *O. minor*. The number of peripheral cells in the vermiform phases is usually 22. The calotte is cap-shaped and smoothly rounded. The axial cell is enlarged and rounded in the calotte region, and it extends anteriorly to the base of the propolar cells. Uropolar cells occasionally become verruciform. Infusoriform embryos are composed of 39 cells. Each of the urn cells contains two nuclei of its own and one germinal cell with its own uncleus.

Further details relevant to the description of infusoriform embryos of *Dicyema misakiense* Nouvel et Nakao are provided and a note to *Dicyema acuticephalum* Nouvel is given. The dicyemid fauna in the two species of octopuses is briefly discussed.

INTRODUCTION

The first record of dicyemid mesozoans in Japan was published in 1938 by Nouvel and Nakao [1]. They found *Dicyema misakiense* Nouvel et Nakao, 1938, in the renal sac of *Octopus vulgaris*, and also *Dicyema orientale* Nouvel et Nakao, 1938, in *Sepioteuthis lessoniana*. Later, Nouvel [2] described *Dicyema acuticephalum* Nouvel, 1947, which was also obtained from *Octopus vulgaris*, and identified another dicyemid from *Sepia esculenta* as *Pseudicyema truncatum* Whitman, 1883, which had been already described in Europe. All these host cephalopods were collected in the waters close to the Misaki Marine Biological Station of the University of Tokyo. Since then no reports on the dicyemid species from Japan have been published.

We examined the dicyemids in the renal sacs of octopuses caught off the coast of Japan, and we found at least two new dicyemids that are distinctly different from each of the four species mentioned above and from the other species so far described in various regions outside Japan.

The present paper deals with these two new dicyemid species: one obtained from *Octopus vulgaris* and the other from *Octopus minor*. In addition, we give a detailed description of the infusoriform embryos of *Dicyema misakiense* Nouvel et Nakao and further give an account of *Dicyema acuticephalum* Nouvel.

MATERIALS AND METHODS

From April of 1989 to May of 1991, 23 individuals of *Octopus vulgaris* and 19 of *Octopus minor* were obtained for detection of dicyemids. Most of them were obtained from fish markets and fishermen, but two were caught by the authors. The size, sex, and source of each of these octopuses are given in Tables 1 and 2.

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From every octopus, which was brought alive to the laboratory, the head and tentacles were cut off just behind the eyes, without anesthetic. Then the visceral hump was placed, ventral side up, in a tray, and the mantle was opened to expose the paired renal sacs. Pieces of renal tissues were smeared with the fluid from the renal sac on slide glasses. Some preparations were used to confirm the occurrence of living dicyemids under the phase-contrast microscope, while others were

Table 1. *Octopus vulgaris* and parasite dicyemids

Hosts						Dicyemids
No.	Dorsal mantle length (cm)	Body weight (g)	Sex	Source*	Date of examination	
VU12	7.2	350	M	1	06.21.1989	*D. acuticephalum* *D. japonicum* *D. misakiense*
VU98	9.5	865	F	1	07.14.1990	*D. japonicum* *D. misakiense* *D.* sp. (A)
VU5	7.4	270	F	1	06.14.1989	
VU16	5.4	78	M	2	07.06.1989	
VU17	5.7	123	M	2	07.06.1989	
VU18	6.5	148	M	2	07.06.1989	
VU20	7.5	—	F	5	07.24.1989	
VU28	9.5	—	M	3	08.04.1989	
VU32	15.2	—	M	4	08.10.1989	*D. japonicum*
VU41	12.5	740	F	2	10.20.1989	*D. misakiense*
VU43	8.2	528	M	6	10.27.1989	
VU44	9.7	738	M	6	10.27.1989	
VU45	6.9	460	M	1	12.01.1989	
VU83	13.5	465	M	1	05.11.1990	
VU101	5.3	96	M	2	07.21.1990	
VU102	6.6	218	F	2	07.21.1990	
VU103	7.9	—	F	5	07.30.1990	
VU104	7.8	508	M	2	05.08.1991	
VU3	8.2	260	M	1	04.26.1989	
VU4	11.4	770	F	1	05.30.1989	*D. acuticephalum*
VU40	9.7	750	F	2	09.06.1989	
VU105	9.2	550	M	1	05.27.1991	*D. misakiense*
VU15	4.0	70	F	2	07.06.1989	None

* Source numbers indicate the following:
(1) Commercially supplied from a fish market at Akashi (Hyogo Pref.) where fish dealers receive octopuses caught mainly inside Osaka Bay and/or in the Sea of Harima (the eastern area of the Inland Sea).
(2) Commercially supplied from a fish market at Shounai (Toyonaka, Osaka Pref.). Locations of these octopuses are presumably similar to those of octopuses bought at Akashi.
(3) Commercially supplied from a fish market at Shirahama (Wakayama Pref.). The octopus was probably caught inside Tanabe Bay where it faces the Pacific Ocean.
(4) Commercially supplied from a fish market at Sakaiminato (Tottori Pref.). The octopus was possibly obtained from the coast of the Sea of Japan near Sakaiminato.
(5) Caught by fishermen in Dozen, Oki Islands (Shimane Pref.), located in the Sea of Japan about 50 km to the north of Sakaiminato.
(6) Collected by the authors in Maizuru Bay (Kyoto Pref.) where it is open to the Sea of Japan.

TABLE 2. *Octopus minor* and parasite dicyemids

Hosts						Dicyemids
No.	Dorsal mantle length (cm)	Body weight (g)	Sex	Source*	Date of examination	
MI89	6.5	78	M	2	07.11.1990	*D. clavatum* / *D.* sp. (B)
MI90	7.9	168	M	2	07.14.1990	
MI91	8.3	204	M	2	07.14.1990	
MI92	8.8	239	M	2	07.14.1990	*D. clavatum*
MI97	5.2	68	F	2	07.14.1990	
MI93	8.2	126	F	2	07.14.1990	
MI94	8.3	207	F	2	07.14.1990	
MI95	8.3	194	F	2	07.14.1990	
MI96	7.3	145	F	2	07.14.1990	
MI63	7.2	105	F	1	04.10.1990	*D.* sp. (B)
MI64	8.7	175	M	1	04.10.1990	
MI65	7.2	142	F	1	04.10.1990	
MI69	7.3	110	F	1	04.10.1990	
MI71	6.5	231	M	1	04.10.1990	
MI84	6.4	124	M	2	07.11.1990	*D.* sp. (C)
MI85	7.4	155	M	2	07.11.1990	
MI86	6.5	192	M	2	07.11.1990	
MI87	6.9	111	M	2	07.11.1990	
MI88	6.7	118	M	2	07.11.1990	

* See explanations for Table 1.

promptly fixed in Carnoy's fluid or in alcoholic Bouin's fluid (a 15 : 5 : 1 mixture of saturated picric acid in absolute ethanol, formalin, and acetic acid). Carnoy-fixed preparations were subjected to Feulgen or McMannus's periodic-acid Schiff (PAS) procedures, and then they were stained with Ehrlich's acid hematoxylin and light green. Alcoholic Bouin-fixed preparations were subjected to the last two staining procedures only. After the staining, the preparations were dehydrated and mounted in the usual fashion for observation of dicyemids under the light microscope.

Measurements and drawings were made with the aid of a micrometer and an image tracer, respectively.

Dicyemidae van Beneden, 1882
Dicyema von Kölliker, 1849
Dicyema japonicum sp. nov. Furuya et Tsuneki
[New Japanese name: Yamato-nihaityu]
(Figs. 1–7, Tables 1, 3, and 4)

Host: *Octopus vulgaris* Lamarck, Octopodidae.
Locality: Western Honshu, Japan. See "Source" in Table 1.
Syntypes: A slide registered as NSMT-Me-1 was deposited at the National Science Museum, Tokyo, Japan. This slide was prepared from No. VU44 octopus (Table 1) and contains both nematogens and rhombogens. It includes *D. misakiense* as well, but *D. japonicum* can be clearly distinguished from *D. misakiense* in the shape of the head as described below. Other slides were numbered according to the host number in Table 1 and are in the authors' collection.
Etymology: The specific name "*japonicum*" was given, because this species is common in *Octopus vulgaris* caught off the coast of Japan.

DESCRIPTION

Diagnosis: Body length up to 1800 μm. Peripheral cell number of vermiform phases (vermiform embryo, nematogen, and rhombogen) usually 22:4 propolars, 4 metapolars, 14 trunk cells. Propolar and metapolar cells form a disc-like head together with parapolar cells. Infusoriform embryos consist of 37 cells. Nucleus number of urn cell, 1. Host, *Octopus vulgaris*.

Nematogens (Figs. 1–3): Body slender, 300 to 1800 μm long; 40–75 μm wide. Peripheral cell number usually 22 (Table 3): 4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, 2 uropolars. Calotte and parapolar cells form cephalic enlargement. Calotte becomes disc-shaped as individuals grow. Cilia covering calotte about 4.7 μm long, oriented forward, slightly shorter but denser than cilia of trunk cells. Cytoplasm of both propolar and metapolar cells, stained by hematoxylin more conspicuously than that of other cells; PAS-positive, but negative after saliva test. Cells and nuclei of propolars, smaller, respectively, than those of metapolars. Trunk mostly uniform in width. Trunk cells, arranged in opposed pairs. No verruciform cells. Axial cell, cylindrical and rounded anteriorly, extends forward to base of propolar cells. Usually two sizes of axoblasts, standard and large; the latter often twice the size of the former. In large individuals, about 80 vermiform embryos at most found in the axial cell, calotte occasionally flower-like in shape, and a few accessory nuclei evident in both peripheral and axial cell. In Carnoy-fixed large individuals, many fine granules found in peripheral cells.

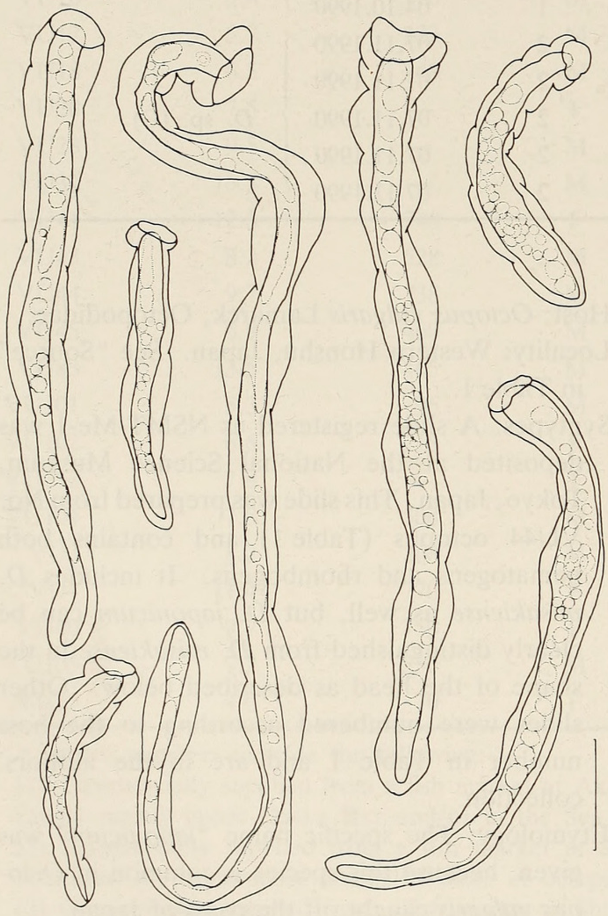


FIG. 1. *Dicyema japonicum* sp. nov. Four entire nematogens of various sizes are shown on the left and three rhombogens on the right. Note the characteristic shape of the head. Bar represents 100 μm. Drawn from specimens prepared from No. VU44 octopus.

Transitional individuals from nematogens to rhombogens enclose degenerating vermiform embryos, proliferating infusorigens, and developing or full-grown infusoriform embryos, simultaneously, in the axial cell. Standard axoblasts only, no large ones in these individuals.

Vermiform embryos (Fig. 4): Full-grown vermiform embryos, 40 to 70 μm long, 8 to 12 μm wide; peripheral cell number fixed at constantly 22 (Table 3). The ratio of total body length to calotte length, 1:0.24 to 0.28. Anterior end of calotte tapering slightly and pointed bluntly. Trunk cells, arranged in opposed pairs. Axial cell tapering anteriorly, sometimes pointed, extending forward to base of propolar cells, as seen in nematogens. Axial cell nucleus, usually located in center or in anterior half of axial cell, always anterior to one or two standard-sized axoblasts.

Stem nematogens (Fig. 5): Two stem nematogens were obtained. One of them, 221 μm long, with three axial cells. Peripheral cell number: 23 (4 propolars, 4 metapolars, 2 parapolars, 11 diapolars, 2 uropolars). Vermiform embryos found in both second and third axial cell but not in the first. The other stem nematogen, 223 μm long, also with three axial cells. Peripheral cell number: 26 (4 propolars, 4 metapolars, 3 parapolars, 14 diapolars, 1 uropolar). Vermiform embryos found in all three axial cells. Only standard-sized axoblasts in both stem nematogens.

Rhombogens (Figs. 1–3): Slightly shorter and stockier than nematogens, otherwise generally similar in shape and proportion; body 300 to 1000 μm long; 40 to 75 μm wide. Peripheral cell num-

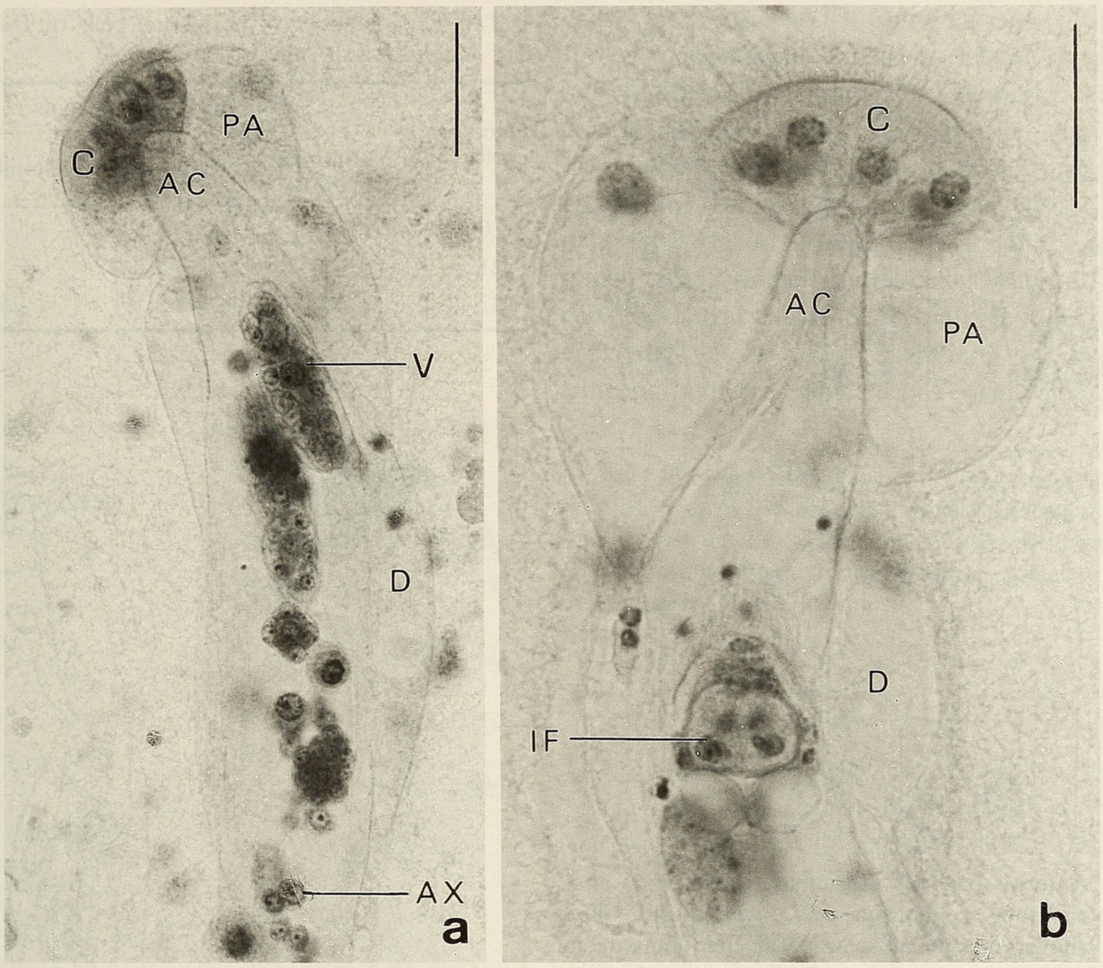


FIG. 2. Anterior part of a nematogen (a) and a rhombogen (b) of *D. japonicum*. Photographs were taken after alcoholic Bouin fixation, which was followed by Ehrlich's acid hematoxylin and light-green staining. Note that the calotte (C) is conspicuously stained and is covered with a dense array of cilia. AX, axoblast; AC, axial cell; D, diapolar cell; IF, infusoriform embryo; PA, parapolar cell; V, vermiform embryo. Bars represent 20 μm. (a) Prepared from No. VU103 octopus, (b) prepared from No. VU44 octopus.

ber, usually 22, sometimes lower (Table 3). Cephalic enlargement, composed of calotte and parapolar cells as in nematogens. Calotte becomes disc-shaped as individuals grow. Shape and tip position of axial cell, similar to those in nematogens. Axial cell sometimes expanded at the region where infusorigens are included. One, sometimes two, and very rarely three infusorigens in the axial cell. Some accessory nuclei observed occasionally in both peripheral and axial cell. No verruciform cells.

Infusorigens (Fig. 6): Medium-sized, sometimes relatively large. Axial cell usually irregular in shape. In 25 mature infusorigens examined: number of external cells including oocytes, 8 to 58 (mode, 15); number of internal cells including spermatocytes, 3 to 19 (mode, 3). Fertilized eggs, 12.3 μm in diameter.

Infusoriform embryos (Fig. 7): Ovoid, rounded bluntly to pointed posteriorly. Based on 100 full-grown embryos, length (excluding cilia), 23.68 $\pm$1.98 μm (mean$\pm$S.D.); length-width-height ratio, 1 : 0.80 : 0.73. Cilia at the posterior end, 6 to 7 μm long. Refringent bodies, smaller than total mass of four urn cells, occupy anterior one-third or so of embryo, when viewed from lateral side. Nuclei of second ventral cells, small and pycnotic. Ventral internal cells project cilia to urn cavity. Capsule cells with many large granules on side adjacent to urn. Granules, intensely stained by PAS procedure and staining-resistant in saliva test. Full-grown infusoriform embryos consist of 37

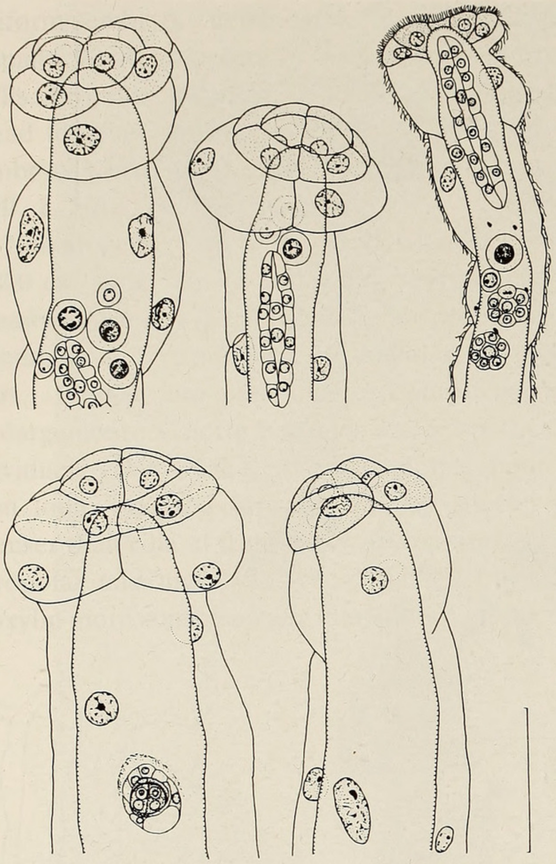


Fig. 3. Anterior part of three nematogens (top) and two rhombogens (bottom) of *D. japonicum*. Cilia are shown in optical section on the nematogen depicted on the far right. Bar represents 50 μm. Drawn from specimens prepared from No. VU103 octopus.

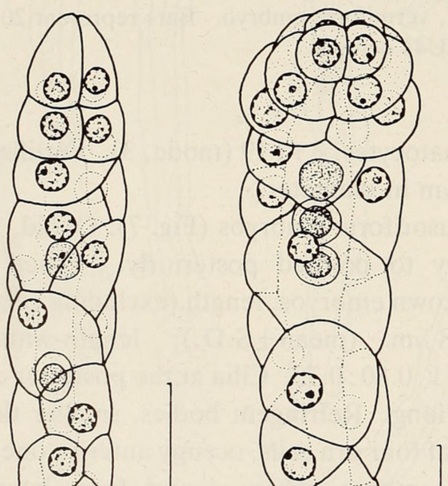


Fig. 4. A vermiform embryo within the axial cell (the cell outline is omitted) of a nematogen (on the left) and a free-living vermiform larva (on the right) of *D. japonicum*. Bars represent 10 μm. Drawn from specimens prepared from no. VU103 octopus.

Table 3. Number of peripheral cells of *Dicyema japonicum* sp. nov.

No. of cells	Number of individuals		
	Vermiform embryos	Nematogens	Rhombogens
20	0	2	1
21	0	2	8
22	54	52	54

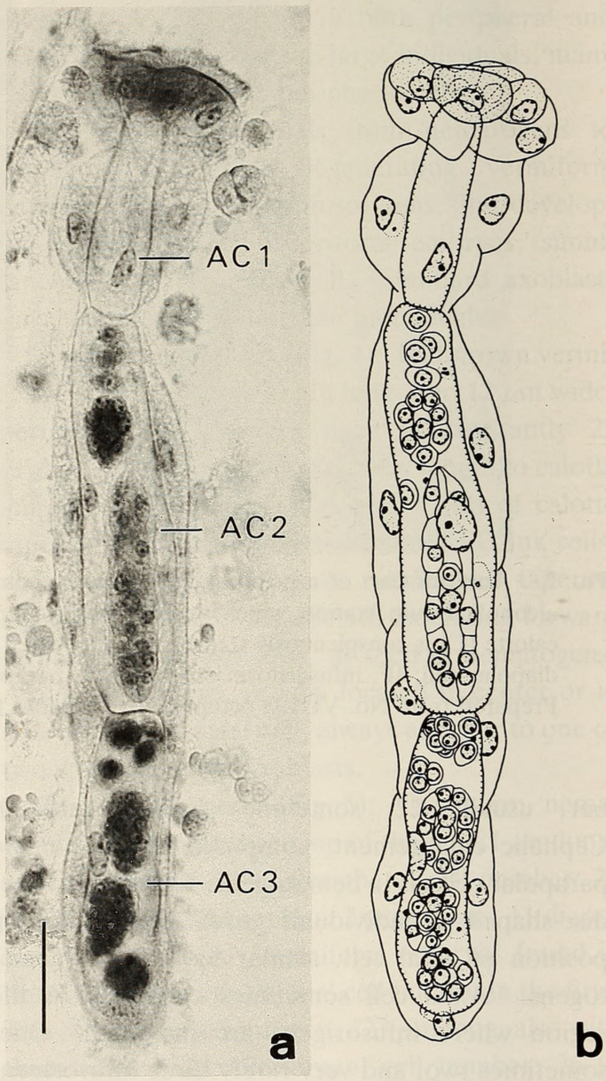
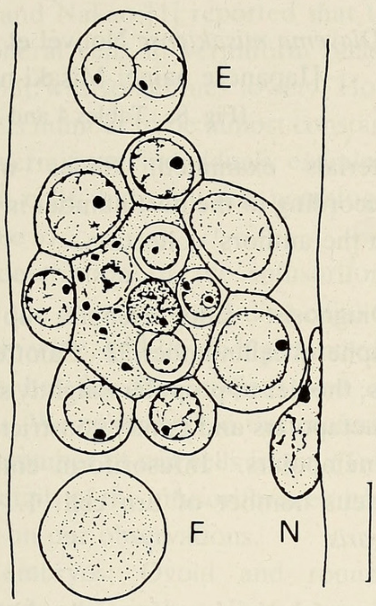


Fig. 5. A stem nematogen of *D. japonicum* with three axial cells. This individual has vermiform embryos in both the second and the third axial cell; in the first axial cell its nucleus is visible but no embryos are seen. Photograph (a) was taken after alcoholic Bouin fixation, Ehrlich's acid hematoxylin and light-green staining. The tracing (b) was drawn from the photograph. AC1, first axial cell; AC2, second axial cell; AC3, third axial cell. Bar represents 20 μm. Prepared from No. VU103 octopus.



cells: 33 somatic and 4 germinal cells (Table 4). Cell terminology used here is that of Nouvel [3] and Short and Damian [4]. Somatic cells are composed of peripheral cells that cover a large part of anterior and lateral surfaces of embryo (2 enveloping cells), peripheral cells with cilia on external surfaces (2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral caudal cell, 2 lateral cells, 2 posteroventral lateral cells), peripheral cells with refringent bodies (2 apical cells - cilia not clearly

FIG. 6. Infusorigen of *D. japonicum*. Around the infusorigen, a two-pronuclei stage egg (E), a fertilized egg (F), and an axial cell nucleus (N) are found. Bar represents 10 μm. Drawn from a specimen prepared from No. VU103 octopus.

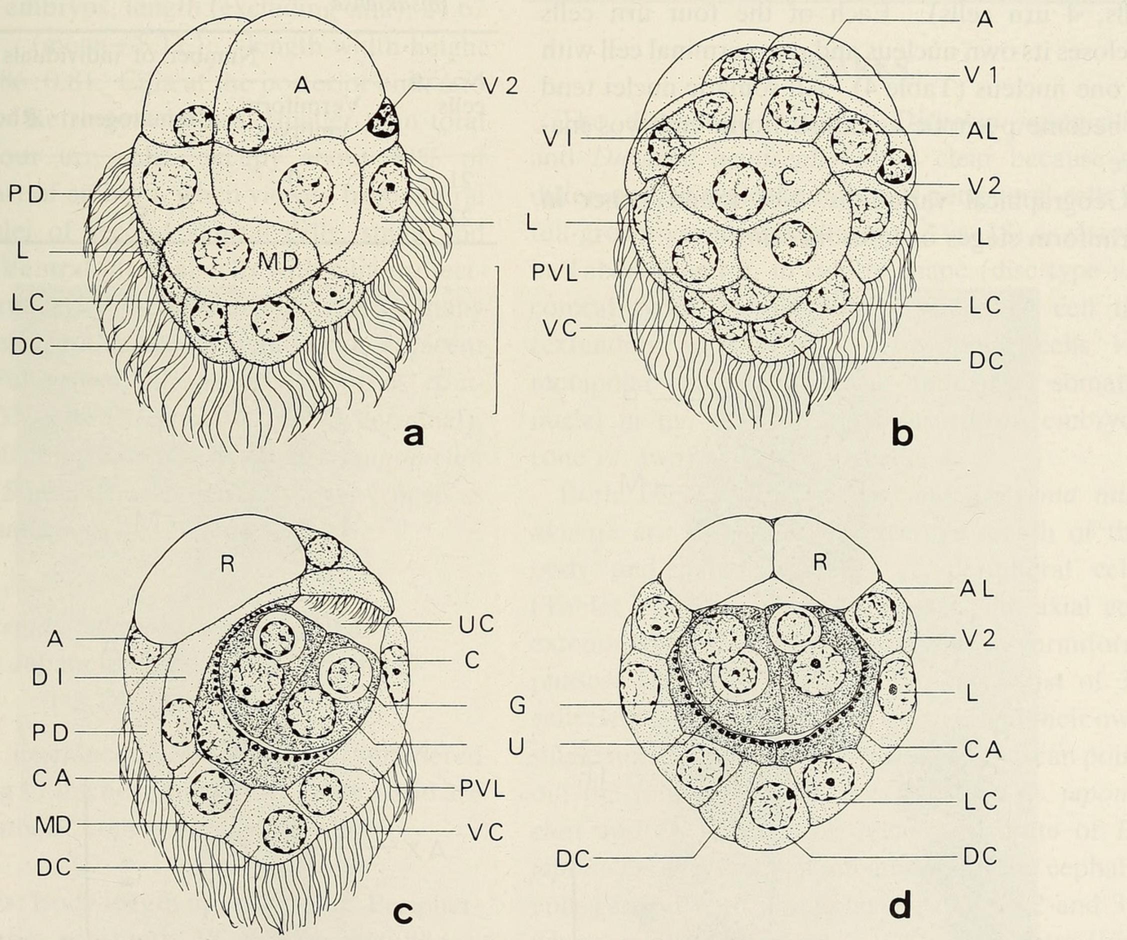


FIG. 7. Infusoriform embryo of .*D. japonicum*. Dorsal view (a), ventral view (b), sagittal section (c), and horizontal section (d, cilia oimtted) are shown. Enveloping cells are ommitted. A, apical cell; AL, anterior lateral cell; C, couvercle cell; CA, capsule cell; DC, dorsal caudal cell; G, germinal cell; L, lateral cell; LC, lateral caudal cell; MD, median dorsal cell; PD, paired dorsal cell; PVL, posteroventral lateral cell; R, refringent body; U, urn cell; UC, urn cavity; VC, ventral caudal cell; VI, ventral internal cell, V1, first ventral cell; V2, second ventral cell. Bar represents 10 μm. Drawn from specimens prepared from No. VU103 octopus.

TABLE 4. Number of cells in infusoriform embryos and the nucleus number of urn cells in four dicyemid species

Species	No. of cells*	No. of nucleus of urn cells*
D. japonicum	37	1
D. misakiense	37	1
D. acuticephalum	37	2
D. clavatum	39	2

* These numbers were very constant in four species examined.

visible on these cells), peripheral cells without cilia (2 first ventral cells, 2 second ventral cells, 2 anterior lateral cells, 1 couvercle cell), internal cells with cilia (2 ventral internal cells), internal cells without cilia (2 dorsal internal cells, 2 capsule cells, 4 urn cells). Each of the four urn cells encloses its own nucleus and one germinal cell with its one nucleus (Table 4). All somatic nuclei tend to become pycnotic as infusoriform embryos mature.

Geographical variations: Not found either in vermiform stages or infusoriforms.

Dicyema misakiense Nouvel et Nakao, 1938
[Japanese name: Misaki-nihaityu]
(Fig. 8a, Tables 4 and 5)

Materials examined: Slides were numbered according to the host number in Table 1 and are in the authors' collection.

Diagnosis: Body length up to 1700 μm. Peripheral cell number 22. Calotte consists of two tiers; the tier of propolars slightly smaller than that of metapolars and faintly constricted from the tier of metapolars. Infusoriform embryos, 37 cells. Nucleus number of urn cell, 1. Host, *Octopus vulgaris*.

TABLE 5. Number of peripheral cells of *Dicyema misakiense*

No. of cells	Number of individuals		
	Vermiform embryos	Nematogens	Rhombogens
21	0	1	0
22	51	51	55

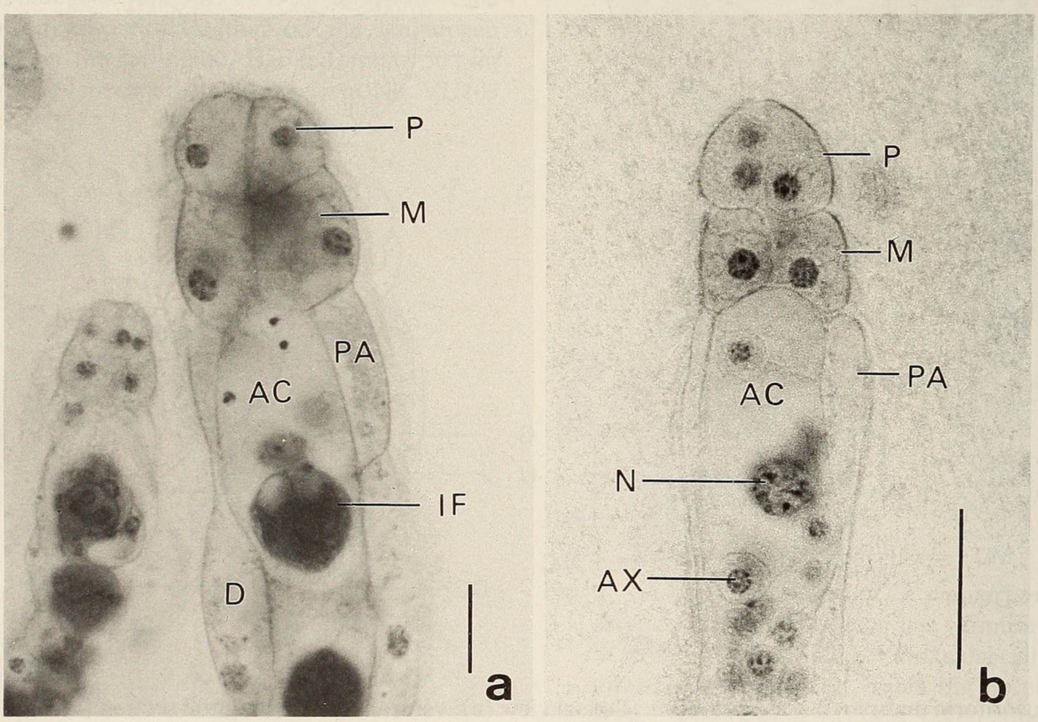


FIG. 8. Anterior part of two rhombogens of *D. misakiense* (a) and a nematogen of *D. acuticephalum* (b). Compare the head shape of these species with that of *D. japonicum* (shown in Fig. 2). Photographs were taken after alcoholic Bouin fixation, Ehrlich's acid hematoxylin and light-green staining. AC, axial cell; AX, axoblast; D, diapolar cell; IF, infusoriform embryo; M, metapolar cell; N, axial cell nucleus; P, propolar cell; PA, parapolar cell. Bar represents 20 μm. (a) Prepared from No. VU44 octopus, (b) prepared from No. VU40 octopus.

Note: Nouvel and Nakao [1] reported that the number of peripheral cells in vermiform phases was usually 22, but was sometimes lower. However, we found this number to be almost constant; 157 out of 158 vermiform individuals examined had 22 peripheral cells, but only one nematogen had 21 cells (Table 5).

The original description of the infusoriform embryos is brief: the embryos (excluding cilia) are 25 μm long, 20 μm wide, and 18 μm high; and each of the urn cells has its own nucleus and one germinal cell [1]. We confirmed in our materials that the nucleus number of urn cells is one (Table 4). Additional details of the infusoriform embryos are given, based on our observations.

Infusoriform embryos: Ovoid and rounded bluntly to pointed posteriorly. Based on 100 full-grown embryos, length (excluding cilia), 24.67 $\pm$1.28 μm (mean$\pm$S.D.); length-width-height ratio, 1 : 0.86 : 0.81. Cilia at the posterior end, 6 to 7 μm long. Refringent bodies, smaller than total mass of four urn cells, occupy about 40% of anterior part of embryo, when viewed from lateral side. Nuclei of second ventral cells, small and pycnotic. Ventral internal cells with cilia projecting into urn cavity. Capsule cells contain many large granules, which are located on side adjacent to urn. Full-grown infusoriform embryos composed of 37 cells (33 somatic and 4 germinal). Somatic cell composition same as in *D. japonicum* (Table 4). Somatic nuclei tend to show pycnosis as embryos mature.

Dicyema acuticephalum Nouvel, 1947
[New Japanese name: Togari-nihaityu]
(Fig. 8b, Tables 4 and 6)

Materials examined: Slides were numbered according to the host number in Table 1 and are in the authors' collection.

Diagnosis: Body length up to 800 μm. Peripheral cell number constantly 18. Calotte bell-like in shape. Infusoriform embryos consisting of 37 cells. Nucleus number of urn cell, 2. Host, *Octopus vulgaris*.

Note: Nouvel [2] reported that the number of peripheral cells in this species ranged from 16 to 19 (generally 18), and the nucleus number of urn cells was two. We confirmed his findings in our materials, but the number of peripheral cells in the vermiform embryos was constant and equal to 18 and no variation was detected (Table 6). We also revealed that fully mature infusoriform embryos consist of 37 cells (Table 4).

TABLE 6. Number of peripheral cells of *Dicyema acuticephalum*

No. of cells	Number of individuals		
	Vermiform embryos	Nematogens	Rhombogens
16	0	0	2
17	0	1	0
18	62	56	58
19	0	1	2

REMARKS

The distinction between *Dicyema japonicum* and *Dicyema acuticephalum* is clear because of differences in typical number of peripheral cells in full-grown vermiform phases (22 *vs.* 18) as shown in Tables 3 and 6, in calotte shape (disc type *vs.* conical type), in position of the axial cell tip (extending to the base of propolar cells *vs.* metapolar cells), and in the number of somatic nuclei in the urn cells of infusoriform embryos (one *vs.* two) as shown in Table 4.

Both *Dicyema japonicum* and *Dicyema misakiense* are similar in the average length of the body and have, typically, 22 peripheral cells (Tables 3 and 5). In both species, the axial cell extends to the base of propolar cells in vermiform phases, and infusoriform embryos consist of 37 cells, which include urn cells that contain their own single nuclei (Table 4). Nevertheless, we can point out the following differences between *D. japonicum* and *D. misakiense.* (1) The calotte of *D. japonicum* is a disc-shaped and forms the cephalic enlargement with parapolar cells (Figs. 2 and 3), whereas *D. misakiense* has a slender calotte (Fig. 8a). No individuals with a calotte that was intermediate in shape between those of the two species were found. (2) Vermiform embryos of *D. japonicum* often have two axoblasts within the axial cell, while those of *D. misakiense* consistently have only one axoblast [1]. (3) The length-width-height ratio of infusoriform embryos of *D. japonicum* is different from that of *D. misakiense* (1:0.80:0.73 *vs.* 1:0.86:0.81). These differences should be sufficient for establishing species in the dicyemids and therefore we have identified *D. japonicum* as a new species. In the paper by Nouvel and Nakao [1], the text in lines 33–36 on p. 74, in lines 1–5 on p. 75, and Figures 6 and 7 on p. 76 could be regarded as referring to *D. japonicum* and not to *D. misakiense*.

Dicyema aegira McConnaughey and Kritzler, 1952 [5], from *Octopus vulgaris,* has 22 peripheral cells as equal as *D. japonicum,* but the two species are easily distinguishable from the following differences. *D. aegira* has a calotte that is slightly longer than the breadth of the anterior end of the trunk, and the axial cell ends at the base of the metapolar cells, unlike the arrangement in *D. japonicum.*

Dicyema orientale Nouvel et Nakao, 1938 [1] has a calotte that is very similar in shape to that of *D. japonicum,* and it also has 22 peripheral cells. However, at full-grown vermiform stages, *D. orientale* is much larger than *D. japonicum*; the number of axoblasts within vermiform embryos in *D. orientale* is also larger than in *D. japonicum* (8 vs. 1 or 2). The host cephalopod of *D. orientale* is *Sepioteuthis lessoniana,* a decapod, not an octopod. It seems reasonable, therefore, that these two dicyemids should be considered members of different species.

Dicyema benthoctopi Hochberg et Short, 1970 [6] is very similar to *D. japonicum* in the calotte shape and in the size of vermiform stages, but the number of peripheral cells in *D. benthoctopi* is very variable. The host of *D. benthoctopi* is *Benthoctopus magellanicus,* a deep-sea octopod distributed in the Atlantic Ocean near the Falkland Island, at about 500 km east of the Strait of Magellan, the southern extremity of the South American Continent. It is hard to conclude, therefore, that *D. benthoctopi* and *D. japonicum* are the same species, even though infusoriforms of *D. benthoctopi* have not yet been described.

Dicyemidae van Beneden, 1882
Dicyema von Kölliker, 1849
Dicyema clavatum sp. nov. Furuya et Koshida
[New Japanese name: Konbou-nihaityu]
(Figs. 9–14, Tables 2, 4. and 7)

Host: *Octopus minor* (Sasaki), Octopodidae.

Locality: Western Honshu, Japan. See “Soruce” in Table 2.

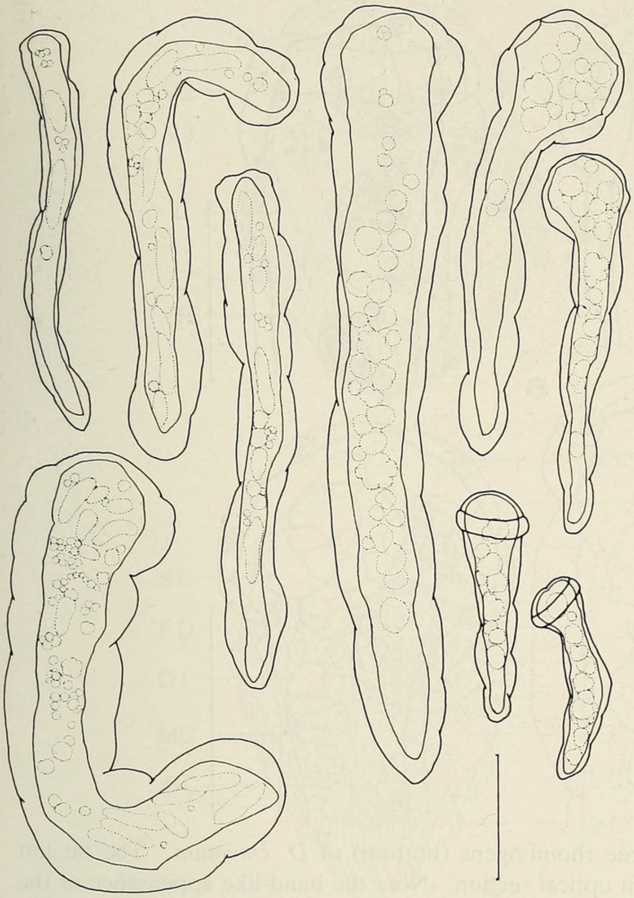
Syntypes: A slide registered as NSMT-Me-2 was deposited at the National Science Museum, Tokyo, Japan. This slide was prepared from No. MI95 octopus (Table 2) and contains nematogens and rhombogens of *D. clavatum* exclusively. Other slides were numbered according to the host number in Table 2 and are in the authors’ collection.

Etymology: The specific name “*clavatum*” is given, because of the shape of the body is characteristically clavate.

DESCRIPTION

Diagnosis: Body length up to 1000 μm. Peripheral cell number of vermiform phases usually 22: 4 propolars, 4 metapolars, 14 trunk cells. Propolar and metapolar cells flat and covering the enlarged end of the axial cell. The infusoriform embryos consist of 39 cells. Nucleus number of urn cell, 2. Host, *Octopus minor.*

Nematogens (Figs. 9–11): Body, pestle-like; 200 to 1000 μm long; 60 to 120 μm wide. Peripheral cell number usually 22; 4 propolars, 4 metapolars, 2 parapolars, 10 diapolars, 2 uropolars, diapolars sometimes lower (Table 7). Cephalic swelling distinct. Calotte, smoothly rounded and cap-shaped in most individuals, flattened and disc-shaped in a few individuals. Cilia covering calotte about 6 μm long, oriented forward, more densely distributed than those on trunk cells. Trunk cells arranged in opposed pairs. Uropolar cells occasionally verruciform; their nuclei, often enlarged; their cytoplasm, stained intensely by hematoxylin. Axial cell extends anteriorly to base of propolar cells, typically enlarged and rounded in calotte regions. Rounded tip of axial cell is covered by a cap of thin propolar cells and by metapolar cells often arranged as a band. Large individuals en-



close about 80 vermiform embryos, and peripheral cells often decrease to 21, sometimes to 20, in number.

Vermiform embryos (Fig. 12): Full-grown vermiform embryos, 70 to 100 μm long; 12 to 18 μm wide; peripheral cell number fixed at 22 (Table 7). Ratio of total body length to calotte length, 1:0.17 to 1:0.19. Anterior end of calotte, rounded.

TABLE 7. Number of peripheral cells of *Dicyema clavatum* sp. nov.

No. of cells	Number of individuals		
	Vermiform embryos	Nematogens	Rhombogens
20	0	3	0
21	0	10	1
22	59	44	20

FIG. 9. *D. clavatum* sp. nov. Four entire nematogens of various sizes are shown on the left and five rhombogens on the right. Note the characteristic ballooning of anterior part of the axial cell. Bar represents 100 μm. Drawn from specimens prepared from No. MI95 octopus.

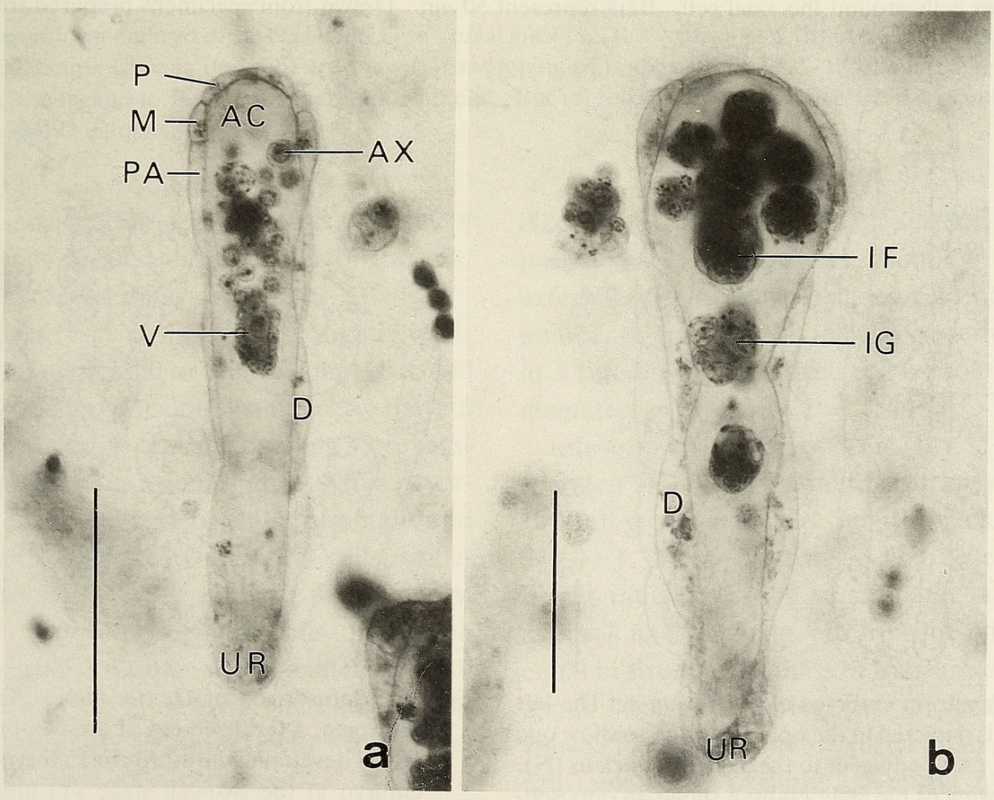


FIG. 10. Photographs of a nematogen (a) and a rhombogen (b) of *D. clavatum,* after the same procedure as used in the case of *D. japonicum* shown in Fig. 2. AX, axoblast; IG, infusorigen; M, metapolar cell; PA, parapolar cell; UR, uropolar cell. See the legend to Fig. 2 for other abbreviations. Bars represent 50 μm. Prepared from No. MI95 octopus.

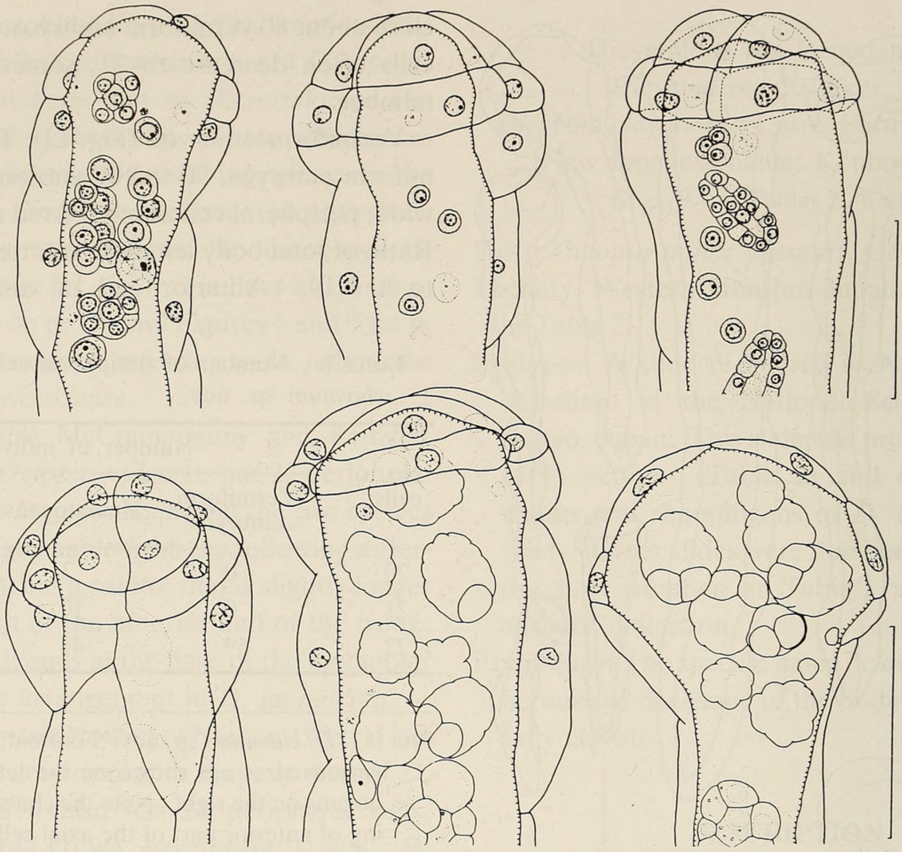


FIG. 11. Anterior part of three nematogens (top) and three rhombogens (bottom) of *D. clavatum*. The fat left nematogen and the far right rhombogen are depicted in optical section. Note the band-like appearance of the metapolar cells around the axial cell. Bars represent 50 μm. Drawn from specimens prepared from No. MI95 octopus.

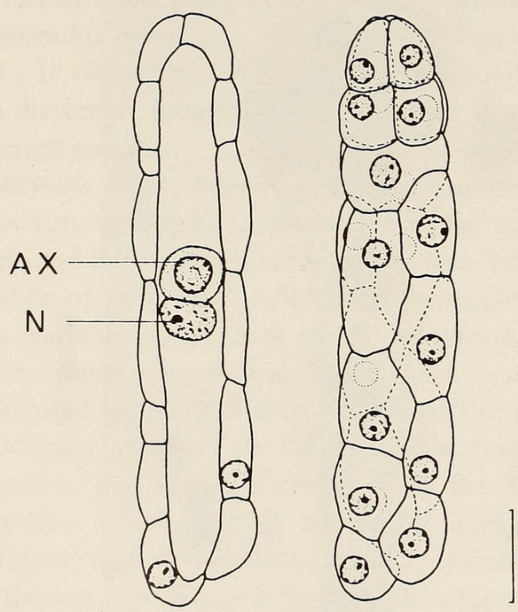


FIG. 12. Vermiform embryos of *D. clavatum*. The left embryo is depicted in the optical section to show one axoblast (AX) adjacent to the axial cell nucleus (N). The drawing of the right embryo shows an opposed-type arrangement of diapolar peripheral cells. Bar represents 10 μm. Drawn from specimens prepared from No. MI95 octopus.

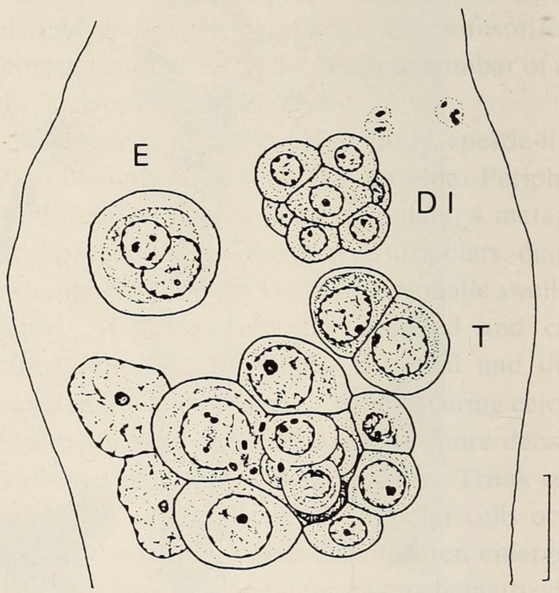


FIG. 13. Infusorigen of *D. clavatum*. Around the infusorigen, a fertilized egg at the two-pronuclei stage (E), a developing infusoriform embryo (DI), and an embryo at the two-cell stage (T) are found. Bar represents 10 μm. Drawn from a specimen prepared from No. MI95 octopus.

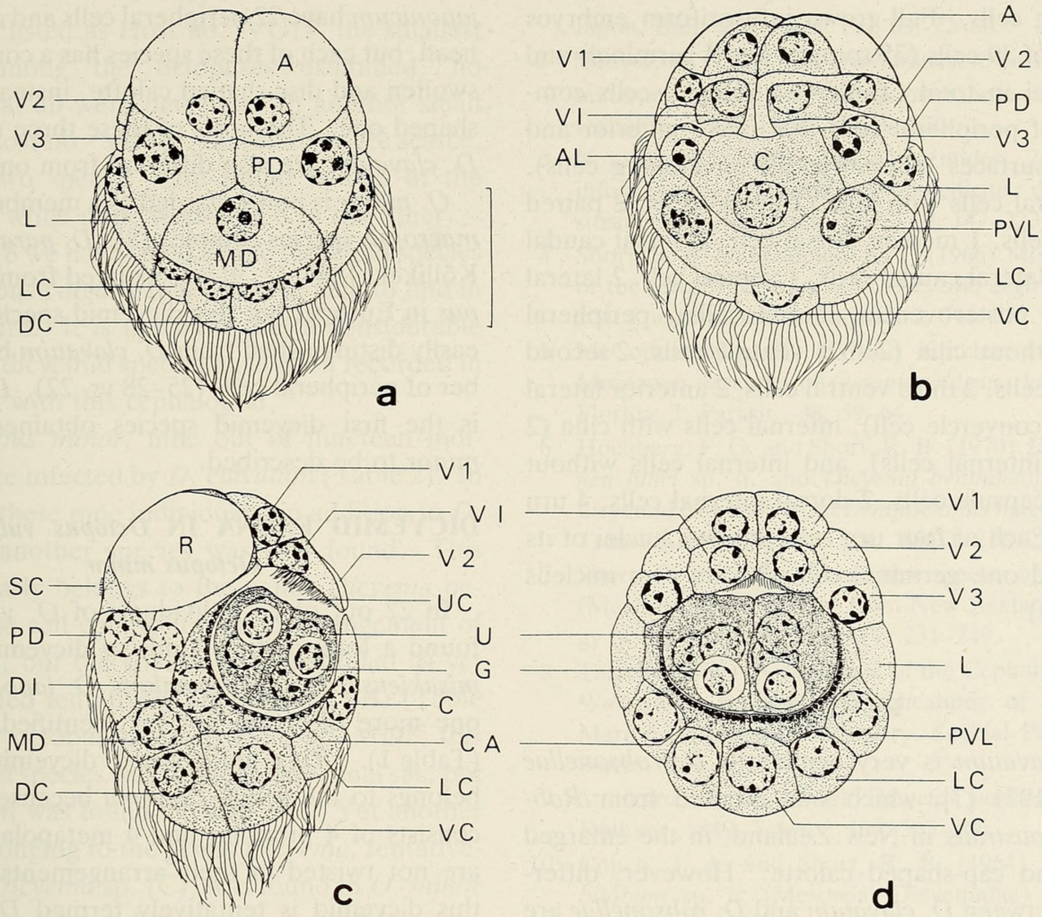


FIG. 14. Infusoriform embryo of *D. clavatum*. The dorsal view (a), the ventral view (b), a sagittal section (c), and a horizontal section (d, cilia omitted) are shown. Enveloping cells are omitted. SC, short cilia; V3, third ventral cell. See the legend to Fig. 7 for other abbreviations. Bar represents 10 μm. Drawn from specimens prepared from No. MI95 octopus.

Trunk cells, arranged in opposed pairs. Axial cell nucleus, usually located in the center of the cell, one or two axoblasts anteriorly.

Rhombogens (Figs. 9, 10 and 11): Slightly smaller than nematogens, 200 to 600 μm long; 60 to 140 μm wide. Shape of calotte similar to that in nematogens, although cephalic swelling more massive and broader as individuals grow. Cilia covering calotte, similar in length and in orientation to those of nematogens. Two uropolar cells, sometimes one additional cell adjacent to uropolars, occasionally verruciform; these cells often with large nuclei, with cytoplasm staining intensely with hematoxylin. Shape of axial cell and arrangment of both propolar and metapolar cells with respect to axial cell, similar to nematogens.

Infusorigens (Fig. 13): Relatively small. Axial cell usually ovoid, about 12 to 17 μm in long axis. About one-third of axial cell surface, always exposed. In 25 infusorigens examined: number of external cells including oocytes, 5 to 12 (mode, 6); number of internal cells including spermatocytes, 2 to 5 (mode, 3). Fertilized eggs, about 12.1 μm in diameter.

Infusoriform embryos (Fig. 14): Ovoid and rounded bluntly to pointed posteriorly. Based on 100 full-grown embryos, length (excluding cilia), 24.10 ± 1.35 μm (mean ± S.D.); length-width-height ratio, 1:0.86:0.80. Cilia at posterior end about 7 to 8 μm long. Refringent bodies, about equal in size to total mass of four urn cells, occupy about 40% of anterior part of embryo, when viewed from lateral side. Nuclei of anterior lateral cell, small and pycnotic. Ventral internal cells project cilia into urn cavity. Short cilia growing from apical cells through dorsal fenestrae of enveloping cells. Full-grown infusoriform embryos consist of 39 cells (35 somatic and 4 germinal) and 43 nuclei in total (Table 4). Somatic cells composed of peripheral cells that cover anterior and lateral surfaces of embryo (2 enveloping cells), peripheral cells with cilia (2 apical cells, 2 paired dorsal cells, 1 median dorsal cell, 2 dorsal caudal cells, 2 lateral caudal cells, 1 ventral cell, 2 lateral cells, 2 posteroventral lateral cells), peripheral cells without cilia (2 first ventral cells, 2 second ventral cells, 2 third ventral cells, 2 anterior lateral cells, 1 convercle cell), internal cells with cilia (2 ventral internal cells), and internal cells without cilia (2 capsule cells, 2 dorsal internal cells, 4 urn cells). Each of four urn cells has two nuclei of its own and one germinal cell with its own nucleus (Table 4).

REMARKS

D. clavatum is very similar to *D. robsonellae* Short, 1971 [7], which was isolated from *Robsonella australis* in New Zealand, in the enlarged head and cap-shaped calotte. However, differences between *D. clavatum* and *D. robsonellae* are distinct in terms of the peripheral cell number in vermiform phases (22 *vs.* 20), in the cell number of infusoriform embryos (39 *vs.* 37), and in the occurrence of cilia on ventral internal cells (present *vs.* absent), respectively.

D. benthoctopi [6], *D. orientale* [1], and *D. japonicum* have 22 peripheral cells and an enlarged head, but each of these species has a conspicuously swollen and disc-shaped calotte, instead of a cap-shaped one. The hosts of these three species and *D. clavatum* are also different from one another.

O. minor is now regarded as a member of the *O. macropus* species complex [8]. *D. paradoxum* von Kölliker, 1849, [2, 9] was isolated from *O. macropus* in Europe, but this dicyemid species could be easily distinguished from *D. clavatum* by the number of peripheral cells (25–28 *vs.* 22). *D. clavatum* is the first dicyemid species obtained from *O. minor* to be described.

DICYEMID FAUNA IN *Octopus vulgaris* AND *Octopus minor*

In 22 out of 23 individuals of *O. vulgaris,* we found a total of four kinds of dicyemid, i.e., *D. misakiense, D. acuticephalum, D. japonicum,* and one more dicyemid not yet identified to species (Table 1). This unidentified dicyemid certainly belongs to the genus *Dicyema* because its calotte consists of 4 propolar and 4 metapolar cells that are not twisted in their arrangements; therefore this dicyemid is tentatively termed *Dicyema* sp. (A). As shown in Table 1, three species of dicyemids, including *Dicyema* sp. (A), were detected together in two hosts; two species of dicyemids, *D. misakiense* and *D. japonicum* in all cases, were found in 16 hosts; and a single species of dicyemid was found in four hosts. Only in one

Table 8. Dicyemid species recorded as parasites of *Octopus vulgaris* in the literature

Species	Typical number of peripheral cells	Distribution	Reference
Dicyema acuticephalum	16–19	Japan	[2]
Dicyema aegira	22	Florida, U.S.A.	[5]
Dicyema bilobum	16–18	Florida, U.S.A.	[10]
Dicyema megalocephalum	16	West Africa	[12]
Dicyema misakiense	22	Japan	[1]
*Dicyema monodi**	16	West Africa	[12]
Dicyema paradoxum	28	Europe and West Africa	[9]
Dicyema typoides	18	Florida, U.S.A.	[11]
Dicyema typus	18–19	Europe	[2]
Dicyemennea lameerei	23	Europe	[13]
Conocyema polymorpha	12	Europe	[14]

* According to Bogolepova-Dobrokchotova [15], this species name is a synonym of *D. megalocephalum*.

individual, listed as Host no. VU15, the smallest in size among the octopuses examined, no dicyemids at all were detected. In another small octopus, Host no. VU101, dicyemids were scarce, although two species were found in one of the renal sacs, while none were found in the other.

In Table 8 we have listed all the dicyemid species from *Octopus vulgaris* that we were able to find in the literature. It is apparent that a considerable number of dicyemid species have been recorded in association with this cephalopod.

In *Octopus minor,* nine out of nineteen individuals were infected by *D. clavatum* (Table 2). In five out of these nine individuals, in addition to *D. clavatum,* another species was also found. This species clearly belongs to the genus *Dicyema* because of the cell composition and arrangement of the calotte, but the species is unidentified as yet and is named tentatively *Dicyema* sp. (B) (Table 2). In the octopus listed as Host no. MI92, this unidentified species was found in one renal sac, but *D. clavatum* was found in other one. Yet another species belonging to the genus *Dicyema,* tentatively named *Dicyema* sp. (C), was found in *O. minor* (Table 2), but it too has not yet been fully characterized.

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