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AKKESHI, HOKKAIDO, JAPAN

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Notes on the Morphology of an Apodous Holothurian, *Scoliodotella uchidai* [1) 2)]

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The present study concerns the internal morphology and histology of an apodous holothurian, *Scoliodotella uchidai*. Although the followings are noticeable features in this species, the general organization is along the line of the other apodous species; unique combination of the alimentary canal and the mesenteries, non-winding small intestine, lack of outer connective tissue layer in the alimentary canal, and only one polian vesicle and stone canal.

In recent years works were only fragmental in the field of holothurian morphology (Filice, 1950; Millott, 1950, 1953; Smith, 1962), though considerable number of inclusive works were carried out on this subject several decades ago (cf. Hyman, 1955; Ludwig, 1889/1892). Recently the author described an apodous holothurian, *Scoliodotella uchidai*, as a new species belonging to a new genus in Chiridotinae, based mainly on endoskeletal ossicles and oral podia (Oguro, 1961). It seemed, however, that more detailed description on the species is desirable. Since that the author has had opportunities to collect the specimens enough to study. The following is a brief account of the morphological studies on *Scoliodotella uchidai*, done in order to give a supplementary information on this species.

MATERIAL AND METHODS

The material used in this study was collected at Cape Aikappu and Daikoku Island both in Akkesh Bay during 1956-1963. Some were fixed in 70% ethanol or 10% neutralized formol for observations by means of a binocular dissecting microscope. The others were fixed in Bouin's solution and cut into serial sections at 8-15 μ by routine paraffin method. They were stained with Ehrlich's hematoxylin-eosin or Heidenhain's azan stain and served for histological examination. Before the specimens were fixed, they had been kept in petri-dishes containing filtered sea-water to expel intestinal contents, mainly sand grains, and anesthetized by magnesium sulphate. All measuring were performed on fixed material.

OBSERVATIONS

Skeletal system The calcareous ring is composed of ten pieces, five radial and

1) This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in hornor of his sixtieth birthday, June 21, 1966.

2) Contributions from the Akkeshi Marine Biological Station, No. 127.

five inter-radial pieces, each being arranged alternately (Fig. 1). The mid-ventral (A) radial piece is the largest and the mid-dorsal (CD) inter-radial piece is the smallest one among them. The other eight pieces are similar in size and form; somewhat twisted rectangular and concaved in inner side. Notches or holes, which often exist in the radial piece of the other apodous species (Clark, 1907), are missing either in the radial or inter-radial pieces. The mid-ventral radial piece is also rectangular but provided with two anterior projections. These pieces are firmly bound each other forming a ring encircling the esophagus. Each radial piece is provided with longitudinal muscle bands. Description on the endoskeletal ossicles was given in the preceding paper (Oguro, 1961).

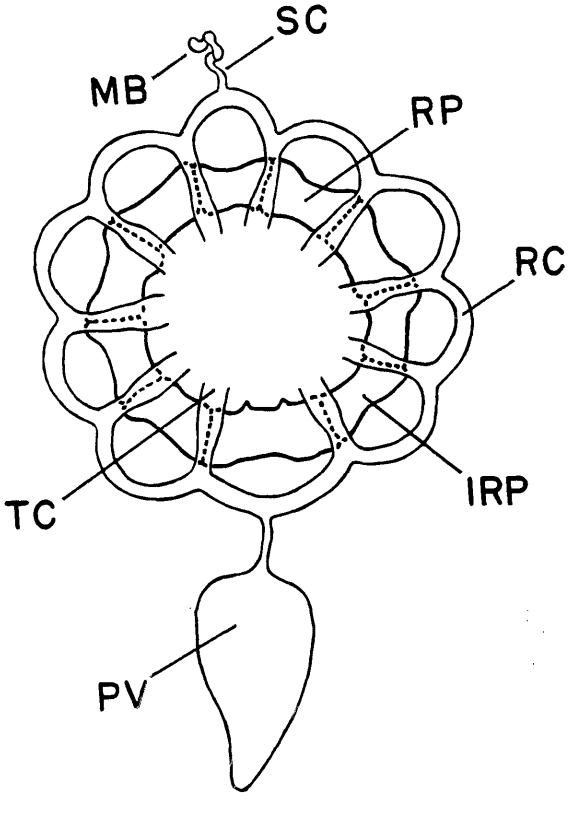


Fig. 1. Schematic illustration of water-vascular system with calcareous ring. View from posterior. IRP, inter-radial piece of calcareous ring; MB, madreporic body; PV, polian vesicle; RC, radial canal; RP, radial piece of calcareous ring; SC, stone canal; TC, tentacular canal.

Body wall Body wall is composed of five components (Fig. 2). The outermost is a very thin layer of homogeneous cuticle. Next is a thick layer known as epidermis which is mainly composed of tall columnar epithelial cells, being interspersed with gland cells. This layer tends to make clumps though not so markedly

as reported in *Synapta inhaerens* by Woodland (1907). It is from 15 μ to 70 μ in thickness according to absence or presence of clumps. The third layer of the body wall is the dermis, thick layer of connective tissue, in which endoskeletal ossicles are embedded. The tissue is considerably loose in outer part and tends to be rather dense toward the fourth layer, though there is no conspicuous boundary between the loose and the dense portions as shown in *Synapta inhaerens* (Woodland, 1907). Inner to the dermis is a thin layer of circular muscles, about 15 μ in thickness. This circular muscle layer is not interrupted with longitudinal muscles of single strip which exists on ambulacra, unlike in the members of Molpadonia (Gerould, 1896). The inner-most surface of the body wall is lined with coelomic epithelium which is thin and uniform in thickness, about 9 μ.

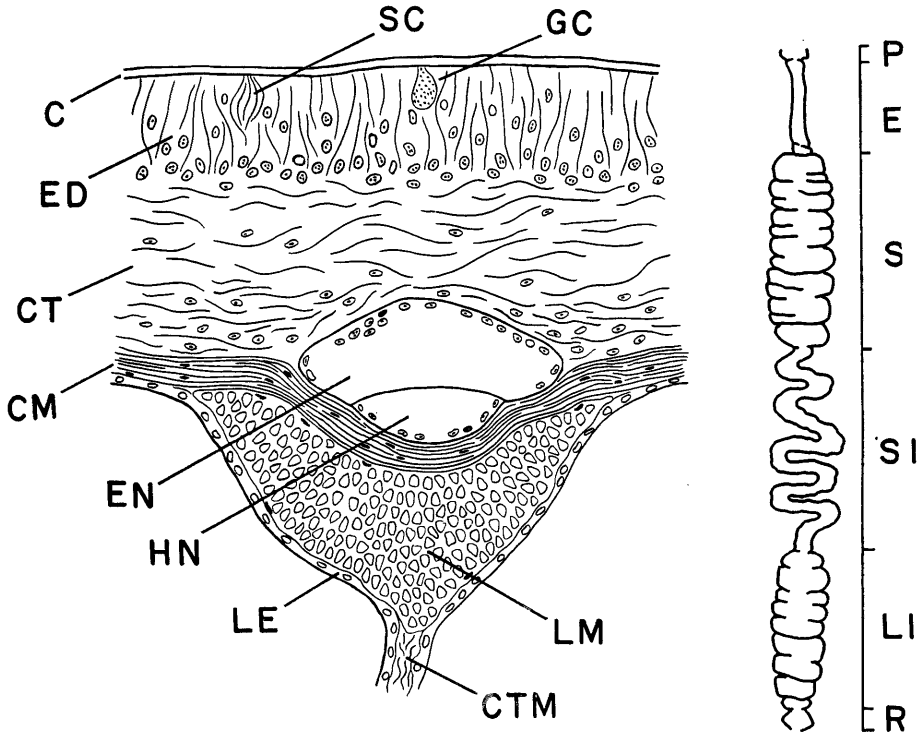


Fig. 2 (left). Schematic illustration of cross section of the body wall at the level of the large intestine. C, cuticle; CM, circular muscle; CT, connective tissue; CTM, connective tissue of mesentery; ED, epidermis; EN, ectoneural nerve; GC, gland cell; HN, hyponeural nerve; LE, lining epithelium; LM, longitudinal muscle; SC, sensory cell.

Fig. 3 (right). External view of the alimentary canal. E, esophagus; LI, large intestine; P, pharynx; R, rectum; S, stomach; SI, small intestine.

Water-vascular system As in the other apodous holothurians, the water-vascular system of this species is very simple, lacking in radial canal and their derivatives. It consists of a ring canal and ten tentacular canals which issue branches into digits (Fig. 1). Unlike the general tendency to multiplication in number of

the polian vesicle and stone canal in Apoda (Hyman, 1955), this species possesses one polian vesicle and a single stone canal. At the mid-ventral radius (A) the ring canal gives out the polian vesicle which is of cayenne-shape with a narrow and short neck. The size of the polian vesicle of this species shows wide variation. The stone canal is issued from the ring canal at the mid-dorsal inter-radius (CD), and supported by the mid-dorsal mesentery except the tip forming a madreporic body. No tentacular ampulla is found. The wall of the ring canal and polian vesicle is histologically composed of four layers; lining epithelium (ciliated in the ring canal),

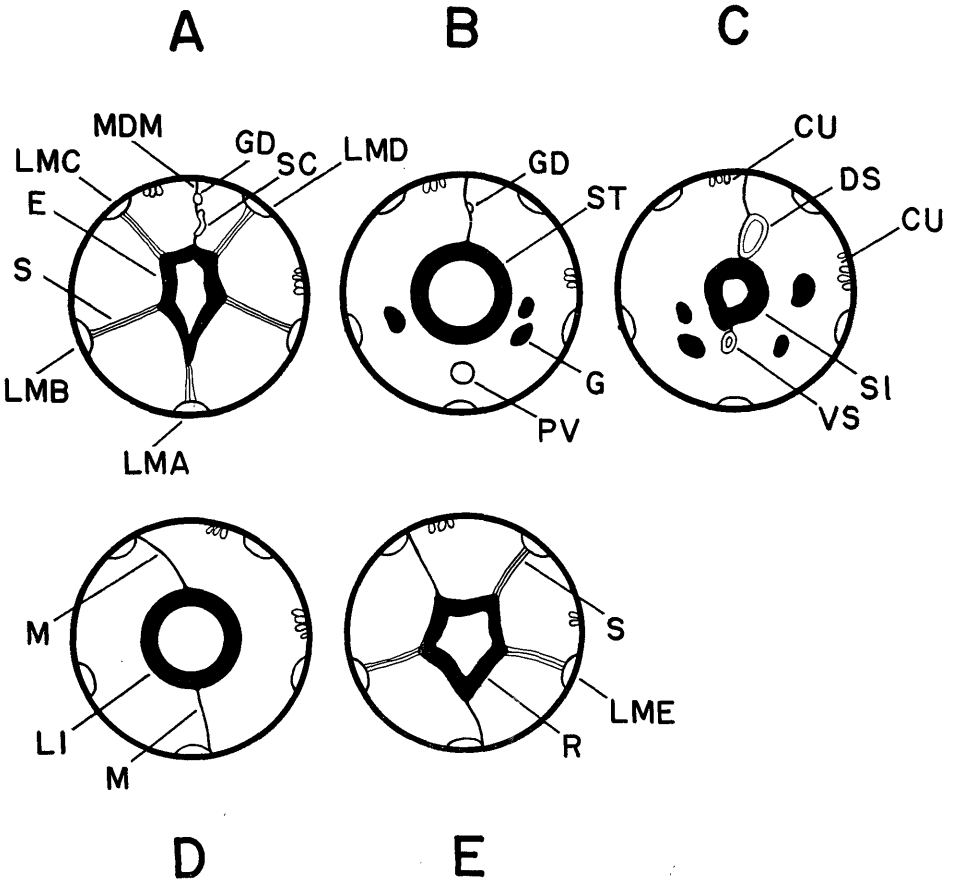


Fig. 4. Schematic illustrations of cross section of the body showing the arrangement of the organs. **A**, **B**, **C**, **D**, and **E** show sections at the level of the esophagus, stomach, small intestine, large intestine and the rectum, respectively. CU, ciliated urns; DS, dorsal sinus; E, esophagus; G, gonad; GD, gonoduct; LI, large intestine; LMA, longitudinal muscle on radius A; LMB, longitudinal muscle on radius B; LMC, longitudinal muscle on radius C; LMD, longitudinal muscle on radius D; LME, longitudinal muscle on radius E; M, mesentery; MDM, mid-dorsal mesentery; PV, polian vesicle; R, rectum; S, suspensor; SC, stone canal; SI, small intestine; ST, stomach; VS, ventral sinus.

circular muscle layer of connective tissue and coelomic epithelium. The wall is very thin, usually about 8 μ in total thickness.

Alimentary canal Although the small intestine does not wind (Fig. 3), general organization of the digestive system is similar to that of the other apodous species hitherto studied. The mouth opens at the center of the oral disk without armature. A very short pharynx, which is of trumpet-shape and connected by tiny connective tissue strands with longitudinal muscle bands, follows the mouth. Posterior to the pharynx is the esophagus. At the border of the esophagus and the stomach there is a deep constriction. The stomach is traversed by small constrictions. Next to the stomach is the small intestine which runs merely undulantly and does not show winding. This is different from the usual manner found in holothurian small intestines. Following the small intestine, the large intestine is marked off. It is relatively large tube with small constrictions as seen in the stomach. At the end of the large intestine there is a small portion which may be called a rectum where the intestinal wall becomes thin. It ends as an anus situated at the center of the posterior end of the body.

The alimentary canal from the esophagus to the posterior end of the small intestine is suspended by a mid-dorsal mesentery on the inter-radius CD but the mesentery disappears abruptly at the end of the small intestine. The large intestine is connected to the body wall by two mesenteries, one on the radius A and the other on the radius C. Finally the rectum is supported by three suspensors in addition to the two mesenteries. At the very end, it is supported by numerous tiny connective tissue strands as seen in the pharynx (Fig. 4).

The wall of the alimentary canal consists of four layers; lining epithelium, connective tissue layer, muscle layer composed of both longitudinal and circular muscles, and covering epithelium (Hamann, 1883; Siviks and Domantay, 1928). Total and relative thickness of these layers vary in different parts of the canal (Fig. 5). As described before, the wall of the alimentary canal lacks outer layer of connective tissue that is present between covering epithelium and the muscle layer in the majority of holothurians (Hamann, 1883). At the mouth the longitudinal muscle layer is inner to the circular muscle, while the directional relation of the muscle layers is reversed in the other parts of the canal even in the anal portion. In the lining epithelium of the pharynx numerous gland cells are present but they are rare in the esophagus. They increase again in the small and large intestine. The esophagus and the pharynx are covered with cuticle. In the connective tissue layer of the small intestine there are lacunar spaces. The thickness of the covering epithelium and the muscle layers shows constant value everywhere of the canal, about 15 μ. That of the connective tissue layer, however, shows a great variation in parts of the canal; 50–60 μ in the pharynx and esophagus, 15–50 μ in the stomach, 30–150 μ in the small intestine, 150–180 μ in the large intestine, and 30–50 μ in the rectum. The variation of the thickness is mainly due to the folding or invaginating of the lining epithelium.

Nervous system The nervous system mainly consists of a nerve ring, ten tentacular nerves and five radial nerves. The nerve ring lies latero-anterior to the calcareous ring encircling the pharynx. In cross section, it is elliptical in form and neurons are restricted in the anterior half of it. In cross section the tentacular nerve does

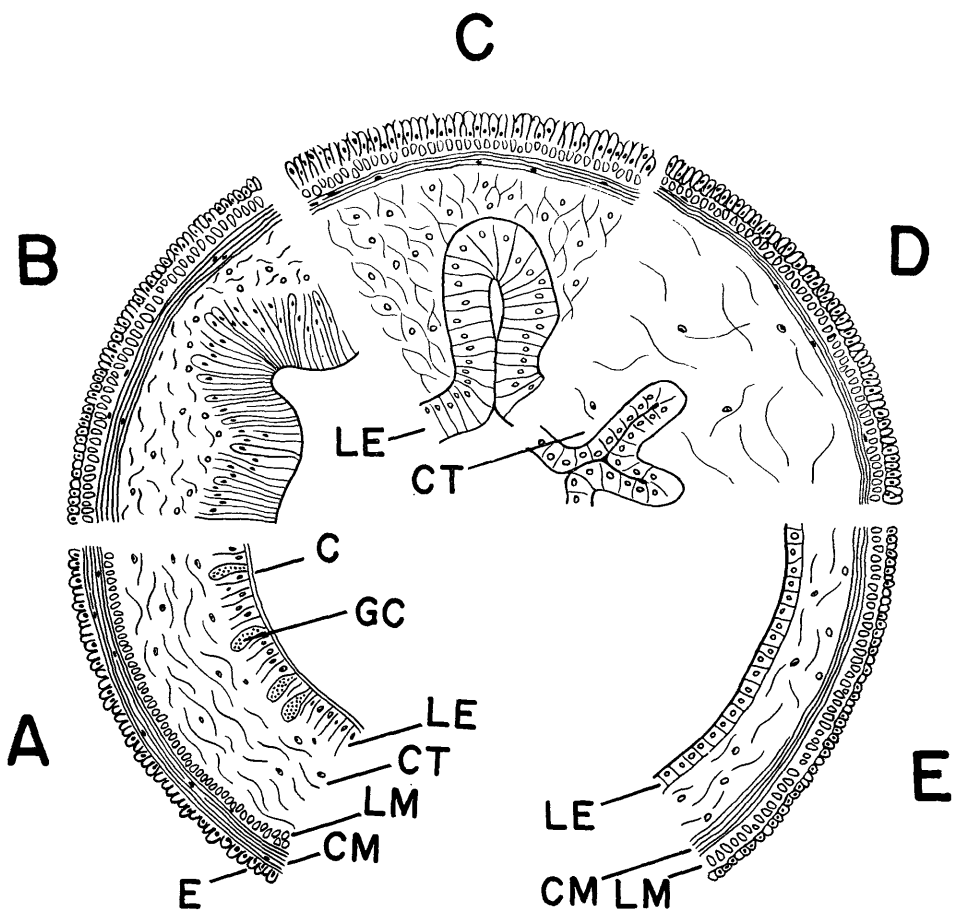


Fig. 5. Schematic illustration of cross section of the alimentary canal. **A**, **B**, **C**, **D**, and **E** show anterior portion of the pharynx, stomach, small intestine, large intestine, and the rectum, respectively. C, cuticle; CM, circular muscle; CT, connective tissue; E, epidermis; GC, gland cell; LE, lining epithelium; LM, longitudinal muscle.

not show a complete ring, as described in *Labdomolgus ruber* (Becher, 1906), but incomplete ring situating between the longitudinal muscle and the dermis. The radial nerve consists of two parts, outer ectoneural and inner hyponeural (Fig. 2).

Reproductive system This species is obviously dioecious but the both sexes are hardly distinguishable externally even in the breeding season. The gonad is of bi-lobed tube. The tubes are branched and suspended freely in the coelom, each lobe in either side of the mid-dorsal mesentery. Usually the ovarian lobes are thick, short and less branched than the testicular ones. Between the gonad and the genital opening is the gonoduct which is suspended by the mid-dorsal mesentery (Fig. 4). No difference in the structure of the gonoduct is found between the both sexes. The inner-most is ciliated epithelium. Next is loose connective tissue and the outer-most is peritoneum. The genital opening is situated at the mid-dorsal inter-radius

(CD) without special apparatus, near the base of the tentacles.

Coelomic organ *Scoliodotella uchidai* has ciliated urns which are known as a characteristic coelomic organ in Apoda (Hyman, 1955). The ciliated urns of this species are solitary, not mounted on common stems as seen in *Chiridota* (Semper, 1868[1]). Each urn is of cornucopia-shape, 50–70 μ high and 35–45 μ wide at the top, and the inner side of it is covered with cilia. The clumps of aggregated haemocytes are often found in the concaved parts of the urns (Cuénot, 1902). There are two rows of the urns on the coelomic side of the body wall, not mounted on the mesenteries, one on the inter-radius CD and the other on the inter-radius DE. The urns are many in the middle portion of the body and interspersed in the both extremities.

Remarks As described before, general organization of this species is along the line of the other apodous holothurians. The followings, however, are characteristic features of *Scoliodotella uchidai.*

The alimentary canal of holothurians including apodous species is in general suspended by three mesenteries; the esophagus and the stomach and the descending part of the small intestine, the ascending part of the small intesine, and the rest of the canal are suspended by the mid-dorsal mesentery, left mesentery and the ventral mesentery, respectively. In the present species, however, the alimentary canal from the esophagus to the posterior end of the small intestine is suspended by one mid-dorsal mesentery, and the rest of the canal, viz., large intestine and rectum, is suspended by two mesenteries. This may be due to non-winding nature of the alimentary canal of this species, which is another characteristic of the present species. Further, the alimentary canal of this species lacks outer connective tissue layer usually found between the epithelium and the muscle layer. Single polian vesicle and only one stone canal are also worth to notice among Apoda.

The author wishes to express his cordial thanks to Professor Yasuhiko Kanoh for reading the manuscript.

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Studies on the Development of *Tubularia radiata* and *Tubularia venusta* (Hydrozoa)[1),2)]

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The development from the egg to the young polyp of *Tubularia radiata* and *T. venusta* is described. The developmental processes of the two species coincide in principle with each other.

The cleavage is total and vertical until the fourth division, but some eggs of *T. venusta* cleave very irregularly and a syncytium is formed. The blastula is a modified coeloblastula. The gastrulation takes place, at first, by multipolar proliferation or by cell divisions like an inward proliferation and a solid mass of cells is formed, then the primary germ layers are differentiated by further cell divisions like a delamination. Finally the separation of the germ layers is completed by migration of the interstitial cells toward periphery, which have been produced by unequal divisions of the primary endoderm cells.

The embryo becomes flattened along the future body axis and conical rudiments of aboral tentacles protrude. Then the growth of body along the oral-aboral axis begins and the coelenteron is formed. Later, the embryo develops gradually into actinula by growth along the body axis and by elongation of the aboral tentacles. The internal changes such as simplify of future mouth area, development of fixing cells at aboral end, formation of endoderm cushion and others are also observed. Each characteristics of the actinulae of the two species seem to be adapted to their habitats.

The comparison with other species and the systematic discussion from the developmental view are made.

I. INTRODUCTION

Concerning the development of the germ cells and the gonophore of the genus *Tubularia* there have been a considerable number of works (Doflein, 1896; Labbé, 1899; Goette, 1907; Pérez, 1913; Broch, 1915; Benoit, 1925; Liu and Berrill, 1948; etc.). On the development from the egg to the actinula several works have been also published for some species of *Tubularia*. The development of *T. mesembryanthemum* has been well known (Ciamician, 1879; Tichomiroff, 1887; Brauer, 1891; Hargitt, 1904). In *T. larynx*, Allman (1871-2) reported a rough outline of the development and, later, Lowe (1926) gave a detailed description of it. In *T. crocea*, some knowledge on the development was given by Allen (1900) and Berrill (1952) and the cytological details in the early development were described by G. T.

1) Contributions from the Akkeshi Marine Biological Station, No. 128.

2) This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, June 21, 1966.

Hargitt (1909). Moreover brief observations on the development were made in *T. indivisa* by Allman (1871–2), and in *T. cristata* by Conn (1882). In a Japanese species, *T. radiata*, the author described an outline of its development based on living materials (Nagao, 1960). In the details of processes during the early development, however, there have been many disagreements among the different workers, not only between the different species but also within the same species.

On the other hand, contributions to detailed knowledge concerning the late development of *Tubularia* after the formation of the aboral tentacles are comparatively scanty except for the works of Ciamician (1879) on *T. mesembryanthemum* and Lowe (1926) on *T. larynx*. Moreover, there has been no detailed record of observation through the entire process of development from the egg to the young polyp based on both living and sectioned materials.

In the present paper a detailed description of the development from the egg to the young polyp in two Japanese species, *Tubularia radiata* and *T. venusta* is given from observations on living and sectioned materials, and some comparative considerations are dealt with.

Before going further the author wishes to express his sincere gratitude to Prof. Emer. Tohru Uchida and Prof. Mayumi Yamada for their kind guidance and encouragement constantly given to the author. The author is also very grateful to Prof. Paul L. Illg of the University of Washington, Seattle, for reading the manuscript.

II. MATERIALS AND METHODS

Tubularia radiata Uchida and *Tubularia venusta* Yamada are commonly found in Akkeshi Bay (Uchida, 1937; Yamada, 1950). The hydroids used were collected in their breeding season and kept in glass vessels filled with sea water after isolation of both sexes. For two to five days, eggs already fertilized before the isolation were liberated from their gonophores or showed late developmental stages, and mature unfertilized eggs remained in the gonophores. For observations, a gonophore with a mature egg obtained in this way was cut off from the pedicel and inseminated by adding one or two drops of the sperm suspension in sea water. As the wall of the gonophore was nearly transparent, the observations on the living materials were readily carried out through the gonophore wall. The materials were fixed in Bouin's solution, and serially sectioned at 10 μ in thickness, and stained with Delafield's hematoxylin and eosin or Heidenhain's azan triple method.

III. THE DEVELOPMENT OF *TUBULARIA RADIATA*

From July to November *Tubularia radiata* is commonly found on eel-grass at the inmost part of Akkeshi Bay and in Akkeshi Lake which is a lagoon and is directly connected with the bay (cf. Uchida *et al.*, 1963). The hydroids breed from September to early November. During these months the water temperature in their habitat gradually falls from 20° to 10°C. After breeding the hydranths gradually fall off. The gonophores develop on the hydranth just above the aboral tentacle circlet,

forming 10–12 or more racemose groups. They are born on short sparsely branched pedicels. Male gonophores are oval or elongated oval in form, 0.8–1.0 mm in the long diameter and 0.6–0.7 mm in the short diameter. Female gonophores have 2–4 short tentacular processes at the tip. In young gonophores the germ product assumes an ellipsoid shape surrounding a spadix which is scarlet in color. When completely matured, the egg is separated from the spadix and lies free in the gonophore cavity taking nearly a spherical form, pale yellow in color. The size of the female gonophores is variable in different developmental stages; for example, gonophores with a mature egg are 0.6–0.9 mm in the long diameter and 0.6–0.8 mm in the short diameter, gonophores with an embryo in preactinula stage are 1.0–1.7 mm × 0.6–0.8 mm in size. In a female gonophore usually there is one embryo, but sometimes there are two embryos in different developmental stages.

The spermatozoon (Fig. 1) is about 45 μ in total length. The head is banana-shaped, 3.0–3.8 μ in length and 1.1–1.3 μ in width. The middle piece is cap-like, covering the posterior end of the head, 0.8–1.1 μ in thickness and 1.2–2.0 μ in width.

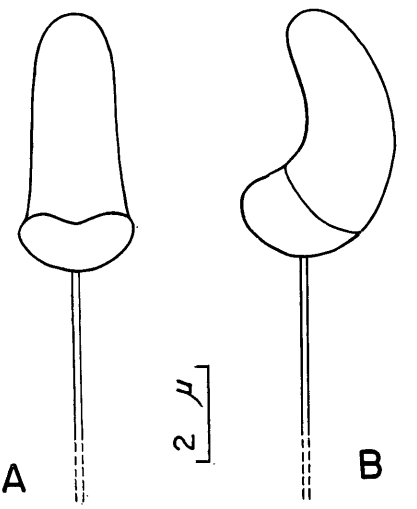


Fig. 1. Spermatozoon of *Tubularia radiata*. A: View from above. B: Side view.

The mature egg (Figs. 7, 29) is nearly spherical or ellipsoidal in shape, 0.5–0.6 mm in diameter, and is closely covered with an extremely delicate membrane. A very small polar body is observed on the egg surface for a short time (Fig. 30). Near the animal pole there is a small nucleus which contains a little chromatin and no nucleolus (Fig. 31). The yolk plasma is almost homogeneous and the cytoplasm is rather compactly gathered around the nucleus and at the cortical region. In the egg there are many small bodies deeply stained with hematoxylin. They are the degenerated nuclei of the oocytes which have been ingested by the growing oocyte, and were named 'Pseudozellen' by the early German workers. The so-called 'pseudocells' are almost evenly distributed in the egg.

The time for the process of development from insemination was as follows. The temperature ranged from 11° to 13°C.

Stage	Time
Insemination	
2-cell stage	3–4 hours
4-cell stage	4–5 hours
8-cell stage	5–6 hours
16-cell stage	6–7 hours
Solid mass of cells	24 hours
Beginning of aboral tentacle protrusion	2 days

Actinula formation	2–4 days
Actinula liberation	4–5 days
Settlement of young polyp	6–10 days

Cleavage and blastula

Three to four hours after insemination the first cleavage takes place by the formation of a vertical furrow which gradually becomes deeper (Figs. 8, 32). Prior to the cleavage, division of the egg nucleus which is located near the animal pole takes place, then the cytoplasmic furrow begins to become deeper. Subsequent to this the second furrow which is meridional and perpendicular to the first appears (Fig. 9). The nuclei are also located near the animal pole, and have already finished division. While the second furrow is deepening, the first one arrives at the vegetal pole and the egg is completely divided into two equal blastomeres. The cavity of the gonophore is so limited that each blastomere begins to show a slight dislocation by pressure of the gonophore wall, and the furrows do not always exactly cross at the pole. Meanwhile, the third division begins vertically crossing the second furrow. During these processes the second division is almost completely finished; thus two equal blastomeres are gradually divided into four. When the third division is about half completed the fourth cleavage occurs also vertically at right angles to the third, but the furrows do not always deepen simultaneously and exactly vertically, and occasionally become deeper obliquely to the vegetal end. Accordingly, intermediate stages from eight (Figs. 10, 33) to sixteen cells (Fig. 11) are frequently observed. Nuclei of the blastomeres still lie near the animal pole. Up to the fourth division the cleavage shows fundamentally the isobilateral symmetrical type, but it is modified by the pressure of the gonophore wall in more or lesser degree. In the genus *Tubularia* it is generally observed that the nuclei keep their position near the animal pole during the cleavage, namely in *T. mesembryanthemum* reported by Brauer (1891), *T. crocea* by G. T. Hargitt (1909) and *T. venusta* described in the following section.

In further cleavages, equatorial divisions take place rather at the animal half of the embryo along with the vertical ones, then the cleavage becomes gradually irregular and unequal. The blastomeres at the animal side of the embryo increase more rapidly in number than those at the vegetal side. When the blastomeres increase in number to about thirty, several spaces are found among the blastomeres (Fig. 34). Soon after, small irregular blastocoels appear in the mass of the blastomeres, then a modified coeloblastula with extremely reduced blastocoels is formed (Fig. 35). The blastomeres of the animal half are small, irregular round in shape and show a tendency to be arranged in a layer. Both radial and tangential mitotic figures are often observed. In the vegetal half the blastomeres are large and nearly rectangular in form, and arranged toward the animal side. Their nuclei are located near the animal pole of each blastomere. At this time inward proliferations of blastomeres begin. The pseudocells are almost evenly distributed in the embryo. The blastulae of the present species have more reduced blastocoels than those of the typical examples in *T. mesembryanthemum* observed by Brauer (1891) or those of *T. crocea* reported by G. T. Hargitt (1909).

Gastrulation

The first stage: Meanwhile, the inward multipolar proliferations of the outer blastomeres take place actively and the blastocoels are gradually filled with cells leaving some small irregular spaces (Fig. 36). The multipolar proliferation proceeds more actively at the animal side. Then the size difference of cells between the animal side and the vegetal side is being gradually dimini shed. The nucleus which has been located at the animal side of each blastomere becomes situated at the center. At this time, some of the nuclei begin to assume the characteristic double forms (Fig. 39) which were termed 'double nuclei' by G. T. Hargitt (1909) in *T. crocea*. They are found throughout the gastrula stage.

About ten hours after insemination the surface of the embryo becomes smooth and the embryo is often divided almost into two halves by a temporary furrow. The outside view of this stage resembles the form of the beginning of the two-cell stage (Fig. 12). At this stage, the outer cells at the animal half become gradually small and cuboid in shape and arranged in a layer. On the other hand the outer cells of the vegetal half are still large and located irregularly, and later they also become smaller and begin to be arranged in a layer (Fig. 37).

At the outer cells tangential divisions continue along with the inward proliferations by radial divisions. The inner cells are also divided rapidly and become gradually smaller. In this process, sometimes, the outer large cells at the animal side are unequally divided each into an inner large one and an outer small one, and those at the vegetal side into inner small and outer large cells. By this time the nuclei have become gradually larger than those at the stage of the cleavage.

This form like a two-cell stage is a peculiar feature in the development of *T. radiata*. The appearance of this peculiar temporary furrow may be attributed to the following reason. The inward proliferation of cells is so active at the polar region where the temporary furrow is formed that the cells of the surface tend to invaginate. This temporary furrow finally disappear in consequence of the multiplication of cells and the repletion of inner cells (Fig. 38). G. T. Hargitt (1909) observed double blastulae in *T. crocea*, and considered them as the result of an incomplete first cleavage.

The second stage: One day after insemination the embryo becomes spherical in form and the remnants of the blastocoels are almost filled with cells, so that a solid mass of cells is formed (Figs. 13, 40). In the beginning of this stage, the radial divisions of outer cells are still observed, and some unequal divisions by which inner large clear cells and outer small compact cells are produced are often seen (Fig. 41). Thus the outer cells become smaller assuming a nearly cuboid form. They are more deeply stained with hematoxylin than the inner cells. On the other hand, the inner cells become composed of considerably large, irregular round or square shaped cells. Meanwhile, the differentiation of the primary ectoderm cells and the primary endoderm cells has been almost completed.

In the primary endoderm the multiplication of cells is also actively taking place. Among equal divisions of cells unequal divisions become frequently occurred. By these unequal divisions small dark cells 'interstitial cells' are budded off from the large primary endoderm cells (Fig. 42), and gradually increase in number, being scat-

tered among the large primary endoderm cells. Occurrence of these small dark cells is also observed by Brauer (1891) in *T. mesembryanthemum* and Lowe (1926) in *T. larynx*, but these two authors considered that these cells do not remain long in the endoderm and finally degenerate. On the contrary to their view, in the present species, these cells divide actively (Fig. 43), and later migrate toward the periphery of the embryo described as follows.

The third stage: With the progress of cell divisions the size difference of cells between the animal side and the vegetal side is much diminished, and then the polarity of the embryo becomes obscure (Fig. 44). By this stage the unequal divisions of the primary endoderm cells have taken place, and one or two nucleoli appear in each nucleus. The interstitial cells begin to migrate toward the periphery and crowd in closely contact with the primary ectoderm cell layer (Fig. 45). Then these cells form the interstitial cell zone closely inside the primary ectoderm cell layer. The interstitial cells are small and irregular in form, each with rich cytoplasm and a slightly smaller nucleus than that of other cells. These interstitial cells continue to divide even after they have been settled, and decrease in size. Later, this interstitial cell zone, with the primary ectoderm cells, becomes distinctly separated from the endoderm. Thus the final differentiation of the germ layers is completely established (Fig. 56). The pseudocells are scattered mostly within the endoderm as also in *T. mesembryanthemum* (Brauer, 1891), *T. larynx* (Lowe, 1926) and *T. crocea* (Allen, 1900).

Formation of the aboral tentacles

When the migration of the interstitial cells toward the periphery has been almost completed, the spherical embryo begins to flatten along the oral-aboral axis and local differentiations at the future oral and aboral sides become recognizable (Fig. 46). At the future oral side the interstitial cells crowd more compactly and the ectoderm cells are modified by these interstitial cells. On the other hand, at the future aboral side there are fewer interstitial cells and the ectoderm cells in the middle area are rather clear and columnar in shape where the differentiation of future fixing cells of the pedal disk of the actinula begins. While these differentiations are taking place in the ectoderm, the endoderm cells near the ectoderm layer begin to elongate and, later, to be arranged side by side toward the ectoderm layer. By this time the nuclei which assume the characteristic double forms no longer appear.

At this time the rudiments of the aboral tentacles appear around the margin of the flattening embryo (Fig. 47). In these rudiments the ectoderm shows a slight evagination, and the ectoderm cells become cuboid in form and the interstitial cells become few in number. The endoderm cells come to be arranged side by side perpendicularly to the growing axis. The evagination of the ectoderm at the rudiments is caused, at first, by the outgrowth of the endoderm cells as observed in *T. mesembryanthemum* (Brauer, 1891). However, the case of the present species is not so marked as that of the latter species.

One to two days after insemination the embryo becomes more flattened and takes on a disk-like shape, and 8–11 conical protrusions of the future aboral tentacles appear around the margin of the embryo, so that the embryo assumes a star-like

shape (Figs. 14, 15). Later, the young aboral tentacles gradually elongate and begin to curve toward the future aboral side of the embryo. Further, the central area of the aboral surface of the embryo begins to protrude outward.

Meanwhile, the local differentiations in the oral, the aboral and the aboral tentacle areas are clearly recognizable (Fig. 48). In the middle area of the oral side, the separation between the ectoderm and the endoderm is indistinct; later, the ectodermal interstitial cells begin to remigrate into the endoderm (Fig. 49). In the central area of the aboral ectoderm the future fixing cells become taller, and their cytoplasm becomes more dense at the distal half. Along with the elongation of the future aboral tentacles, their endoderm cells become flattened. They are arranged side by side in two rows in the early phase, and later gradually form one row (Fig. 50).

At this stage cnidoblasts begin to appear by the gradual modifications of the ectodermal interstitial cells. On the other hand, the interstitial cells which are still left in the endoderm assume a spindle shape. They may be developing into nerve cells or muscle cells.

Meanwhile, the endoderm cells in the central region also assume a columnar form and are arranged side by side in parallel with those of the periphery. When the young aboral tentacles begin to curve toward the aboral side, the connection of the endoderm cells between the oral and the aboral side becomes loose, then several irregular spaces appear. These are the first appearance of the coelenteron. Later these irregular spaces become larger and form a single coelenteron at the center of the endoderm (Fig. 51). The coelenteron gradually increases in size as development proceeds. Concerning the origin of the coelenteron Brauer (1891) and Hargitt (1904), in *T. mesembryanthemum*, interpreted it as the result of the dissolution of some endoderm cells. However, Lowe (1926) stated, in *T. larynx*, that the formation of the coelenteron is probably caused by the shrinkage of some endoderm cells. In the present species, the formation of the coelenteron is chiefly caused by the separation of the connection of the endoderm cells between the oral and the aboral side in consequence of the rapid outgrowth of the aboral side of the embryo and the subsequent rearrangement of the endoderm cells.

Formation of actinula

In further development the young aboral tentacles gradually elongate along the growing aboral protrusion of the embryo (Fig. 16). The aboral part of the embryo becomes gradually tubular in form being encircled by the growing aboral tentacles, and the oral surface of the embryo becomes also gradually protruded outward (Fig. 52).

While these external changes proceed internal changes are also taking place. At the oral tip the remigration of the interstitial cells into the endoderm has almost completed and the ectoderm becomes a simple layer of columnar or cuboid cells, usually 6–11 cells in a sagittal section (Fig. 53). The separation between the ectoderm and the endoderm becomes again distinct, and a mass of endoderm cells is growing upward pushing up the single ectoderm cell layer. This mass is mainly composed of the interstitial cells remigrated from the ectoderm and the undifferentiated endo-

derm cells which are actively growing, both being deeply stained with hematoxylin. In *T. larynx*, Lowe (1926) attributed these cells to the result of the rapid divisions of endoderm cells. The interstitial cells are found compactly mainly at the oral wall except the tip and at the lateral wall of the aboral portion (Fig. 52). In these areas the ectoderm cells are greatly modified by the invasion of the interstitial cells. Among these interstitial cells many cnidoblasts are actively developing, especially near the base of the aboral tentacles.

Meanwhile, the area of the future fixing cells of the actinula continues to extend and becomes composed of about 25–30 columnar ectoderm cells with rich cytoplasm in a longitudinal section. Then some eosinophilic secretory granules appear at the free ends of these cells and gradually increase in number (Fig. 54). In *T. larynx* Lowe observed that the elongation of the future fixing cells does not take place regularly over the whole surface but it is delayed in the middle area, and a peculiar evagination of the ectoderm cells with a solid core of endoderm is seen. In the present species and *T. venusta* described in the following section, however, the author could not detect any sign of this peculiar evagination. Judging from Lowe's figure (Lowe, 1926, fig. 27) his observation seems to be a mistake caused by an oblique section of the embryo.

While the elongation of the embryo along the oral-aboral axis is taking place the coelenteron becomes larger in size and some pieces of degenerating endoderm cells are often found in the cavity. The endoderm cells are columnar in form and regularly arranged side by side toward the ectoderm layer. Meanwhile, the development of a mesolamella between the ectoderm and the endoderm becomes clearly recognizable.

Preactinula stage

While the embryo continues to elongate along the body axis by the outgrowth of the oral half and the tubular growth of the aboral half, it gradually assumes an actinula form within the gonophore in three to four days after insemination (Figs. 17, 55).

By this time the area of the single cuboid ectoderm cells at the oral tip develops to its full extent occupying 20–25 cells in a longitudinal section, and becomes gradually taller toward the lateral wall. The endoderm cells of this area become gradually narrowly columnar in form, placed with their axes perpendicular to the ectoderm layer. Among these endoderm cells many gland cells with secretory substance which is deeply stained with hematoxylin at the distal half develop. Later, at the tip where the mouth will break through these columnar endoderm cells become invaginated; however, there still remain many small undifferentiated cells stained deeply with hematoxylin.

At this stage the oral tentacles appear around the area of single ectoderm cells as conical evaginations of the ectoderm in consequence of rapid outgrowth of endoderm cells (Fig. 56). Next, the endoderm cells become disk-like in shape and arranged side by side perpendicular to the growing axis in one row, and they increase in number. As the result of this process, the oral tentacles gradually elongate (Fig. 57). The ectoderm cells of the lateral side of the tentacle are nearly cuboid in shape.

Those of the tip where a terminal knob will be formed just before liberation are columnar in shape. At the beginning of the formation of the oral tentacles the boundary between the endoderm of the oral tentacles and that of the hypostome is obscure, but soon after, it becomes distinct by the development of a mesolamella. In *T. larynx* Lowe (1926) described that the first outgrowth of the oral tentacles is formed by ectoderm only, but in the present species, it appears as the result of the rapid outgrowth of the endoderm cells. Meanwhile, the endoderm cells of the wall of the hypostome between the oral tentacle circlet and the aboral one become distinguishable from those of the anterior part encircled by the oral tentacles by their shorter form and irregularly located nuclei.

While these changes proceed at the oral half, the formation of the peculiar endoderm cushion is taking place at the base of the aboral tentacles. At first, the endoderm core of each aboral tentacle is separated from the endoderm of the body by the development of a mesolamella. At the same time the endoderm cells just beneath the aboral tentacle base increase rapidly in size and become extremely taller and wider than others, and highly vacuolated (Fig. 58). They continue to grow until a circular cushion of the endoderm cells is formed. On the other hand, endoderm cells surrounding the growing endoderm cushion retreat as the result of the rapid enlargement of the cushion and finally a part of them becomes small and flattened covering the cushion. After that, they are demarcated from the cushion by a mesolamella newly formed. Then the formation of the peculiar endoderm cushion is completed. In the present species as well as in *T. venusta*, the base of the endoderm core of the aboral tentacles never directly contacts the coelenteron, in contrast with the case of *T. larynx* reported by Lowe (1926). The cushion is composed of 5–7 very large long vacuolated endoderm cells in a sagittal section, which are set side by side almost parallel with the body axis. Later, the cushion gradually encroaches on the coelenteron.

During these processes the aboral tentacles continue to elongate almost to their full length within the gonophore cavity. By this time their ectoderm cells become very flattened, but are thicker at the aboral side than at the oral side. The endoderm cells are arranged almost in one row, and later begin to form two rows again. At the terminal end a knob is formed with taller ectoderm cells with several nematocysts and terminal endoderm cells.

In the early phase of this stage, just posterior to the aboral tentacle circlet, a short constricted circular part appears, which is composed of 7–13 short columnar ectoderm cells in a longitudinal section. In this region the interstitial cells are almost absent and the endoderm cells become shorter than those of other aboral parts. At the lateral wall of the aboral tube posterior to this circular band both the ectoderm cells and the endoderm cells are the tallest of the embryo. The ectoderm is greatly modified by being packed with many interstitial cells and several cnidoblasts. The endoderm cells are narrowly columnar in shape early, then they become typical gastrodermis. At this stage Lowe observed in *T. larynx* that the aboral part of the coelenteron is packed with the greater mass of the endoderm. But such a case is never seen in the present species. At the basal part, the future fixing cells become more attenuated. Their secretory substance increases in volume

at the distal part to occupy about one third of the cell. Between the fixing cells and the lateral wall of the aboral tube a slight constriction which is composed of a few narrowly columnar cells with few secretory granules appears. By this time the endoderm cells of this area become short and wide. Later, at the central part of the endoderm, there appears a large space with 5–6 small dark cells at the base in a sagittal section (Fig. 59). These dark cells seem to play an important part in future metamorphosis. Meanwhile, the periderm begins to develop surrounding the aboral tube. The pseudocells are usually observed decreasing in number in the aboral endoderm.

Then the embryo becomes a fully developed actinula and, 4–5 days after insemination, it is liberated from the gonophore, usually oral part first as in the cases reported by Allman (1871–2) in *T. indivisa* and by Ciamician (1879) in *T. mesembryanthemum*. On the other hand, the aboral end of the actinula was reported as first emerged in *T. larynx* by Pyefinch and Downing (1949) and *T. crocea* by Berrill (1952).

Actinula

The actinula just after liberation (Fig. 2) is 0.7–1.0 mm in height and 0.5–0.8 mm in width at the aboral tentacle circlet. It has 4–7 short oral tentacles, and 11–15 long, slender aboral tentacles which are 2.5–4.0 mm in length and run upward and downward alternately, each terminating in a somewhat swollen terminal knob. The

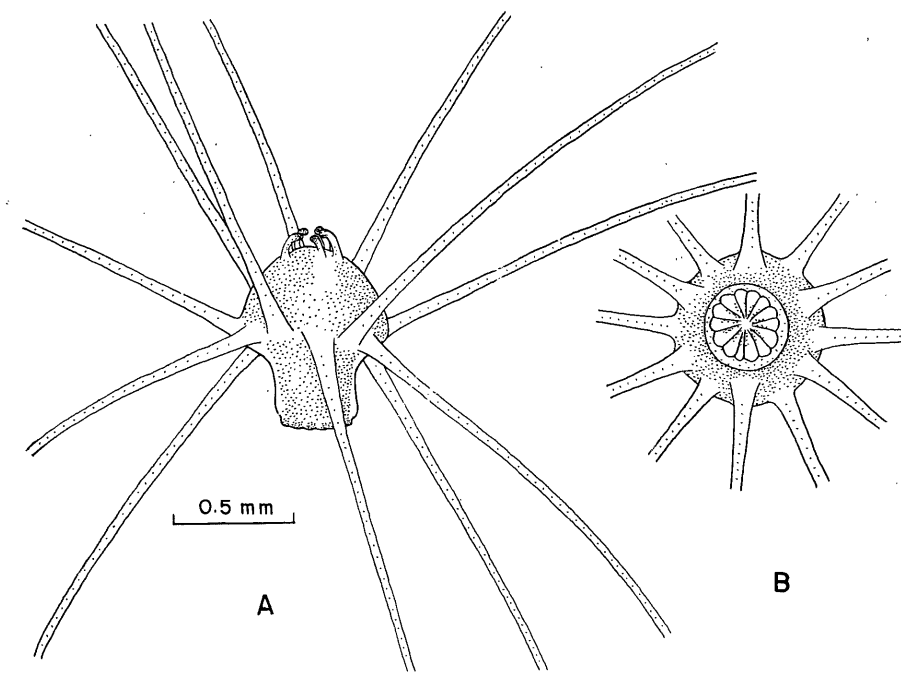


Fig. 2. Actinula of *Tubularia radiata*. A: Side view. B: Aboral view.

oral half of the actinula is hemispherical in form and the mouth is not yet opened. The aboral half is rather short cylindrical in form, provided with a pedal disk with a basis of radial stripes which are white in color.

The general internal structure of the actinula is similar to that in the preactinula stage, but further development takes place in some regions (Fig. 60). The ectoderm and the endoderm of the oral half become thinner than those in the former stage. At the oral tip some of the ectoderm cells often become rounded in shape and protrude outwardly. The mesolamella of this area becomes obscure and the endoderm is composed of spindle shaped cells, and invaginated toward the tip. Both the ectoderm cells and the endoderm cells are deeply stained with hematoxylin.

In the region of the aboral tentacle circlet the endoderm cushion forms a narrow extension encroaching on the coelenteron. At the basal part the fixing cells become rapidly taller and are demarcated as a pedal disk (Fig. 61). The secretory substance increases extremely in volume, and seems to take a chief part in settlement of the actinula. Later, surrounding the pedal disk, a circular outgrowth of the ectoderm which is composed of several long columnar cells is often seen.

Soon after liberation the endoderm cells of the circular band and those of the lateral wall of the aboral tube become thinner and transparent assuming flat or cuboid forms. The constriction between the lateral wall of the aboral tube and the pedal disk becomes more conspicuous.

Development of polyp

One to five days after liberation, the aboral part of the actinula gradually elongates. The radial stripes of the pedal disk become more distinctly recognizable, and the hypostome becomes more slender; thus the actinula settles to the substratum.

The young polyp newly settled to the substratum has a slender hypostome with the mouth just opened, and the oral tentacles are now upwardly erected. All of the aboral tentacles come to run upward in a single circlet. The hydrocaulus elongates and becomes thicker at the basal part, and it is soft and tubular just below the hydranth. The pedal disk spreads around itself forming a hydrorhiza. In the culture dish, the mode of formation of the hydrorhiza is variable. About two or three days after settlement the thick basal part of the hydrocaulus elongates rapidly and becomes slender. At that time it occupies about two-thirds or a half of the whole length of the hydrocaulus and grades smoothly to the upper tubular naked portion. During the five to eight days after settlement the lower part of the hydrocaulus is gradually hardened by the deposition of a periderm, and slight annulations appear (Fig. 3). Meanwhile the terminal knobs of the aboral tentacles become gradually indistinct as in *T. larynx* reported by Pyefinch and Downing (1949).

During these processes great differentiations are also taking place in the internal structure. Six days after liberation, the oral half of the hypostome is composed of small cuboid or flat ectoderm cells and long columnar endoderm cells among which there are many gland cells with secretory substance in the distal half of cells. The posterior half of the hypostome is composed of columnar ectoderm cells together with many interstitial cells and some nematocysts, and short columnar endoderm cells (Fig. 62). The ectoderm cells of the oral tentacles are flat or nearly cuboid in form

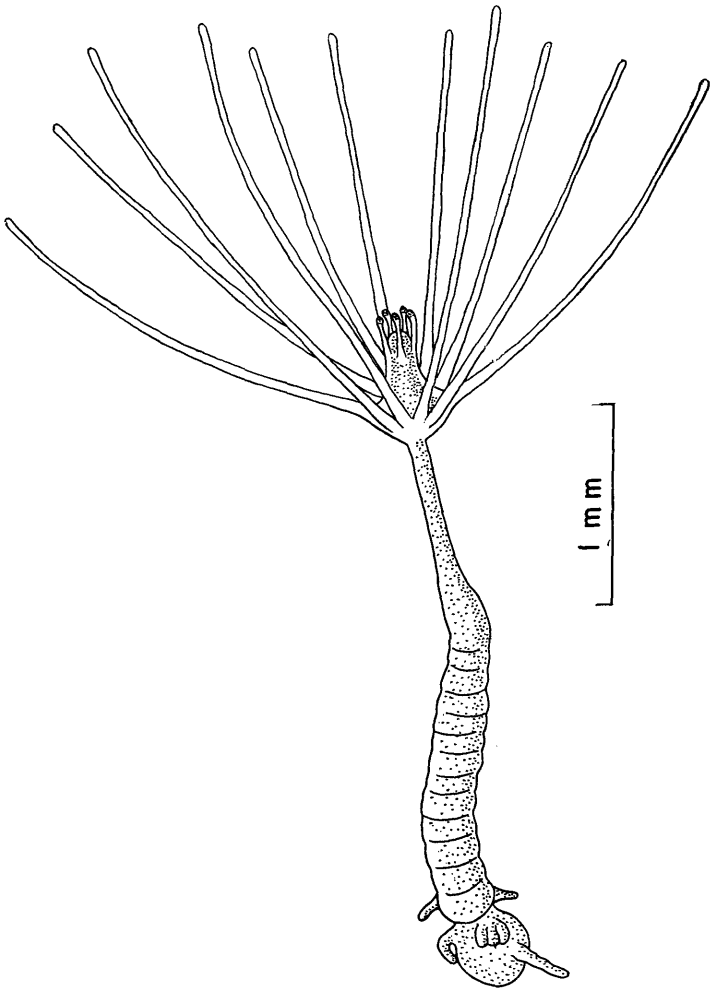


Fig. 3. Young polyp of *Tubularia radiata*, 8 days after liberation.

and those of the oral side are more flat than those of the aboral side as is also seen in the aboral tentacles. The endoderm cells now begin to be arranged in several rows in some parts.

At the basal part of the aboral tentacle circlet the endoderm cushion becomes extremely large, so that the coelenteron of this area is greatly narrowed, and becomes tubular (Fig. 63). The endoderm cells covering the cushion increase both in size and number, encroaching on the coelenteron. Next, they become arranged again in a layer surrounding the tubular coelenteron. In the hydrocaulus the following three parts are distinguishable. At the first part just below the hydranth which derives from the circular band of the actinula the ectoderm cells are composed of columnar or cuboid cells and are more transparent than those of the actinula. The endoderm

cells become wider than those in the actinula stage and assume short columnar or cuboid forms. The periderm is absent at this part (Fig. 63). From the oral tip to this part many circular muscles are developed between the endoderm and the mesolamella. In the second part next above, the ectoderm cells are cuboid in form and become smaller than those of the first part. The endoderm cells become also wider, flattening toward the basal part. Later, both the ectoderm and the endoderm become flattened like those of the next part. From this part toward the base a periderm develops. The first and the second part occupy one half to one third the length of the hydrocaulus and have a well developed mesolamella. In the last part (Fig. 64) both the ectoderm and the endoderm are very thin. At the basal end which is developing into the hydrorhiza the ectoderm and the endoderm become very thin and there are often observed several rather large undifferentiated cells. At this time most of the pseudocells have been almost assimilated and only a few of them still remain at the basal part of the hypostome.

IV. The Development of *Tubularia venusta*

In Akkeshi Bay colonies of *Tubularia venusta* are abundantly found on the rocky shore such as at Aikappu, Aininkappu and Daikokujima where the sea is more or less powerful (cf. Uchida *et al.*, 1963). They grow actively from May to October, and breed usually from July to September. In this season the water temperature ranges from 12° to 21°C (Average, 17°C). From November to April most of hydranths fall off and there remain only empty stems and resting stolons. The decapitation tends to take place gradually from the high tidal line to the low tidal line along with the succession of the tide.

The gonophores are born on about 10 peduncles which form racemous clusters on the hydranth just above the aboral tentacle circlet. Male gonophores are ellipsoidal or oval in shape, about 0.5 mm in long diameter, about 0.4 mm in short diameter, with no tentaculiform process. Female gonophores have 4–8 tentaculiform processes at the tip and each contains the germ product surrounding a spadix in early time. When completely matured, the egg becomes free from the spadix and becomes oval in form, and translucent pale yellowish grey in color. The size of the female gonophores of this species is also variable in different developmental stages as in *T. radiata*; for example, gonophores with a mature egg are 0.5–0.6 mm in the long diameter and 0.4–0.5 mm in the short diameter, gonophores with an embryo in preactinula stage are 0.6–1.0 mm × 0.4–0.6 mm in size.

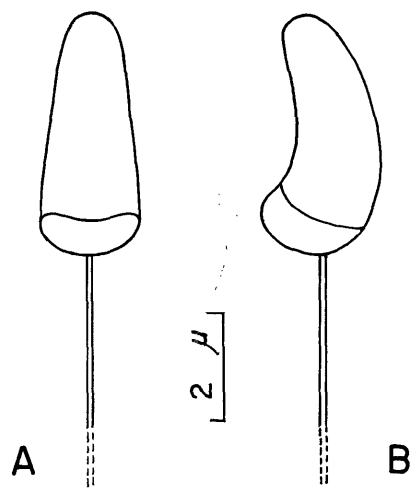


Fig. 4. Spermatozoon of *Tubularia venusta*. A: View from above. B: Side view.

The spermatozoon (Fig. 4) is closely similar to that of *T. radiata*, but the head and the middle piece of the present species are slightly smaller than those of *T. radiata*. It is about 45 μ in total length. The head is 2.8–3.1 μ long and 1.2–1.4 μ wide. The middle piece is 1.0–1.2 μ in thickness and 1.5–1.8 μ in width. The tail is 35–43 μ long.

The cavity of the gonophore is so small that the mature egg is more or less pressed, and assumes an ellipsoidal form, about 0.5 mm in the long diameter, about 0.4 mm in the short diameter (Fig. 18). Usually a nucleus without nucleolus is located near the animal pole. The cytoplasm is distributed rather compactly at the periphery. 'Pseudocells' are scattered evenly. In some eggs, however, many pseudocells lie in the animal side (Fig. 65), and such eggs seem to show the cleavage of the irregular type described later on.

The time for the process of development from insemination was as follows. The water temperature ranged from 20°–23°C.

Stage	Time
Insemination	
2-cell stage	2 hours
4-cell stage	2.5 hours
8-cell stage	3.5 hours
16-cell stage	4 hours
Solid mass of cells	10–16 hours
Beginning of aboral tentacle protrusion	1–2 days
Actinula formation	2–3 days
Settlement of young polyp	4–6 days

Cleavage and blastula

The cleavage of *T. venusta* shows two different types as seen in *T. mesembryanthemum* as reported by Brauer (1891). The first is a regular type in which the egg develops to the 16-cell stage by total, equal and vertical divisions (Figs. 19–22) as seen in *T. radiata*. The second type shows extremely irregular unequal divisions (Figs. 66, 67).

The first type: About two hours after insemination a vertical division begins (Fig. 19). When the first cleavage is about half completed the second furrow appears. It is also vertical and perpeudicular to the first (Fig. 20). The dislocation of blastomeres is already seen and some embryos assume a T-shape arrangement of the two original blastomeres. When the second furrow arrives at the vegetal pole, or just prior to this, the third furrows begin to deepen vertically, crossing the second one, but the dislocation of blastomeres by the pressure of the gonophore wall is so remarkable that each furrow does not precisely cross the other (Fig. 21). When the third division is almost finished the fourth furrows begin to deepen perpendicular to the third ones. The furrows, however, are greatly modified by the dislocation (Fig. 22) and they do not always deepen synchronously in each blastomere. During these processes the nuclei lie constantly near the animal pole in each blastomere. In the present species the dislocation of blastomeres is more remarkable than that seen in *T. radiata*.

The second type: The cleavage pattern of this type is seen in the eggs in which the nuclear divisions proceed without cytoplasmic divisions resulting in a syncytium in a greater or lesser degree. The cleavage is very irregular and unequal. At first a few small blastomeres are cut off at the animal side of the embryo (Fig. 66). Subsequent to this further divisions take place rapidly only at the animal side leaving large blastomeres at the vegetal side (Fig. 67). These processes resemble closely the second mode in *T. mesembryanthemum* observed by Brauer (1891) and the usual manner of development in *T. crocea* by Allen (1900) and in *T. larynx* by Lowe (1926). Such irregular cleavage is frequently observed in the materials collected at the end of the breeding season or in those from Daikokujima. On the other hand, those from Aikappu usually show the first or regular cleavage.

Following the successive divisions, in the first type, some equatorial divisions take place at the animal side along with the vertical divisions, then the cleavage becomes gradually irregular and unequal (Fig. 23). Rather small blastomeres are located at the animal side and larger ones lie at the vegetal side. Several small spaces appear among these blastomeres. The nuclei are located near the center in the small blastomeres and near the animal side in the large blastomeres. Then the embryo develops into a blastula of which blastocoels are reduced to a greater or lesser degree. In some cases a coeloblastula with greatly reduced blastocoels is formed (Fig. 68). An extreme case shows a loose sterroblastula in which the blastocoel is completely reduced. On the other hand, some embryos have a comparatively large blastocoel (Fig. 69).

The embryo formed through the cleavage of the second, irregular type also comes to a coeloblastula with greatly reduced blastocoels which are more irregular and the size difference of blastomeres is very large (Fig. 70).

Gastrulation

The first stage: In the further development active radial divisions of outer cells are seen and the blastocoels become gradually filled with cells as the result of cell divisions like an inward proliferation of the outer blastomeres (Figs. 69–71) or the multipolar proliferation (Fig. 69) as seen in *T. radiata*. While these proliferations continue, the cells become smaller and a loose solid mass of cells is formed. The surface of the embryo becomes smooth and, as a rule, the embryos come to be spherical in 10–16 hours after insemination, however, the embryo often tends to assume a rather ellipsoidal form facing the spadix (Fig. 24). This may be caused by the pressure of the gonophore wall. Early in this stage the size difference of cells between the animal side and the vegetal side is still large and the remnants of the blastocoels remain in some places (Fig. 72). In the present species the form like a two-cell stage as seen in *T. radiata* does not occur. At this stage the nuclei which assume the characteristic double forms as in *T. radiata* appear in some cells but are less in number than those in *T. radiata*.

The second stage: By continued divisions the spaces among the cells are almost completely filled with cells and the size difference of cells between the animal side and the vegetal side becomes smaller and is finally eliminated. Meanwhile a compact mass of cells is formed from the loose mass of cells, and primary ectoderm and the

primary endoderm differentiate as the result of the continued radial divisions and the layered arrangement of the outer cells (Fig. 73). The primary ectoderm cells assume a cuboid form, taking a layered arrangement, and the cytoplasm is more dense than that of the primary endoderm cells. The primary endoderm cells are large and irregular in form. In this stage the unequal divisions of some of the primary endoderm cells, as seen in *T. radiata*, by which the interstitial cells are produced, are actively taking place and the small interstitial cells are increasing in number among the primary endoderm cells (Fig. 74). The interstitial cells are of small irregular form with a rather small nucleus, and are deeply stained with hematoxylin. The mitotic figures of these cells are frequently found. Most of the pseudocells lie in the endoderm.

In the embryo derived from the irregular cleavage type a solid mass of cells is also brought about by a process similar to that of the first type, but there remains a conspicuous size difference of cells between the animal side and the vegetal side of the embryo (Fig. 75).

The third stage: Later, the interstitial cells begin to migrate toward the periphery and gradually settle in closely contact with the primary ectoderm cell layer. This migration takes place more rapidly at the animal side than at the opposite side. Moreover, more interstitial cells settle at the animal side than at the opposite side (Fig. 76). These local differences are distinctly recognizable unlike in the case of *T. radiata*. Contrary to this, Brauer (1891) reported that, in *T. mesembryanthemum*, the interstitial cells are more rapidly formed at the aboral pole. The mitotic divisions of the interstitial cells often take place even after their settlement. At this time the nucleoli appear in the nuclei and bcome gradually distinct. On the other hand, the characteristic double formed nuclei have not been seen up to this stage.

In the embryo which is brought about by the second irregular type of cleavage the remarkable difference is seen in the size of cells and the number of the interstitial cells settled between the animal side and the vegetal side (Fig. 77). In the extreme examples the interstitial cells have settled closely inside the primary ectoderm cells at the animal side, while the opposite side consists of large cells without any trace of layer arrangement, as seen in *T. larynx* by Lowe (1926).

Meanwhile, most of the interstitial cells have migrated toward the periphery and the interstitial cell zone is formed just inside the primary ectoderm cell layer; then it is gradually separated from the endoderm (Fig. 78). Next, a mesolamella begins to develop between the ectoderm with the accompanying interstitial cell zone and the endoderm. Now the final separation of the germ layers is completed. The inner surface of the ectoderm cells is modified by the settlement of the interstitial cells. The interstitial cell zone at the animal side is thicker and more compact than that of the opposite side. Near the vegetal pole where the fixing cells of the actinula will eventually develop only a few interstitial cells are located. While these changes are taking place in the ectoderm, the endoderm cells near the periphery become gradually elongated and arranged side by side toward the periphery. This change proceeds more rapidly at the animal side.

Formation of the aboral tentacles

Meanwhile, the embryo becomes flattened along the embryo axis, then the rudiments of the aboral tentacles begin to develop in a closely similar way to the case of *T. radiata.*

One to two days after insemination 6–10 conical outgrowths of the future aboral tentacles appear around the margin of the flattened embryo, then the embryo gradually assumes a star-like form (Figs. 25, 26). In the present species, the embryo of this stage is more thick and stocky than that of *T. radiata.* Later, the central area of the future aboral side of the embryo begins to protrude. During these changes go on, some local differentiations among the future oral and aboral sides and the aboral tentacle area become distinguishable (Fig. 79). At the middle of the future oral side the boundary between the ectoderm and the endoderm is somewhat obscure; later, the ectodermal interstitial cells show the first indications of the remigration into the endoderm as observed in *T. radiata* (Fig. 80). On the one hand, the ectoderm cells in the middle area of the future aboral side, about 25–30 cells in a sagittal section, become narrowly columnar in shape, with their nuclei near the center. In these cells the differentiation of the future fixing cells of the pedal disk of the actinula is now taking place. In the ectodermal area except for the oral tip, aboral end and the rudiments of the aboral tentacles there are many interstitial cells, most of which are still undifferentiated, but some of them begin to develop into nematocysts.

While these changes are taking place in the ectoderm most of the endoderm cells have elongated along the oral-aboral axis of the embryo leaving some irregular shaped cells at the center; next, at the middle between the oral and the aboral side the connection of the endoderm cells are split apart as the result of the outgrowth of the embryo along the oral-aboral axis, then a coelenteron is formed similar to the way in *T. radiata* (Fig. 79).

Formation of actinula

Meanwhile, the embryo begins to grow outwardly along the oral-aboral axis (Fig. 27). The young aboral tentacles are gradually elongating toward the aboral side of the embryo, which becomes convex outwardly and finally assumes a tubular form. The oral side of the embryo becomes convex also. Later, the terminal knobs of the aboral tentacles are formed by the thickening of the ectoderm.

At the oral tip the remigration of the interstitial cells has been taking place. Later, the ectoderm of this region becomes composed of a single layer of small cuboid cells, 6–8 cells in a longitudinal section, these being pushed outward by the conical outgrowth of the active endoderm cells, as seen in *T. radiata.* The interstitial cells are compactly located at the lateral wall of the oral and the aboral parts (Fig. 81). In these places the ectoderm cells are greatly modified by the intrusion of the interstitial cells and the cnidoblasts which have been rapidly formed by the modification of the interstitial cells. At the aboral end the future fixing cells are more attenuated and later the secretory granules become recognizable, but they are much more scanty than those in *T. radiata.*

On the other hand, the endoderm cells are now composed of columnar cells

arranged side by side toward the ectoderm. Among the endoderm cells several long and spindle-shaped cells deeply stained with hematoxylin are often observed. They may eventually develop into nerve cells or muscle fibers. The coelenteron is gradually enlarged along with the elongation of the body. In the coelenteron several pieces of degenerating endoderm cells are sometimes found but no prominent dissolution of cells is observed.

Preactinula stage

As the change of the body proceeds the embryo assumes gradually an actinula form in the gonophore (Fig. 28). The area of cuboid ectoderm cells at the oral tip is extended to its full extent (Fig. 82), and at the periphery of this area the oral tentacles begin to develop as in the similar process seen in *T. radiata* (Fig. 83). The process of further development of the oral tentacles is also similar to that of *T. radiata*. The endoderm cells near the oral tip are shorter than those of other regions and are narrowly columnar with the nucleus located rather toward the basal part. The oral wall posterior to the oral tentacle circlet is composed of columnar ectoderm cells containing many cnidoblasts and interstitial cells, and long columnar endoderm cells.

At this time the endoderm of the aboral tentacles becomes separated by a mesolamella from the endoderm of the body at the base of the tentacles, and the endoderm cushion begins to develop as the result of enlargement of the endoderm cells of the body located slightly toward the aboral side of the tentacle base in the same way as in *T. radiata* (Fig. 84). Later, the endoderm cushion covered with the cuboid or flat endoderm cells grows inwardly encroaching on the coelenteron. By this time the aboral tentacles have elongated to their full extent within the gonophore. Their structure is almost similar to that of *T. radiata*, however, in the present species, the endoderm cells are usually arranged in two rows throughout their development. The terminal knobs which have been formed by this stage are more prominent than those of *T. radiata*.

The aboral tube differentiates into the following three parts; the circular band, the lateral wall and the aboral end, as observed in *T. radiata*. These three parts coincide well with those of *T. radiata* in their constitution. The future fixing cells, however, show some characteristic modifications. Their nuclei become located near the proximal part and the cytoplasm is more compact proximal to the nucleus and the part distal to the nucleus shows a somewhat loose reticular appearance (Fig. 85). Later, when the oral tentacles appear, the middle secretory area and the peripheral inactive area become distinguishable. The coelenteron is more enlarged but it is comparatively limited by the highly elongated endoderm cells at the aboral tube.

Then the embryo becomes a fully developed actinula and, 3–4 days after insemination, it becomes free from the gonophore by the contraction of gonophore wall and the movement of the actinula. Usually the oral part of the actinula first appears from the opened oral tip of the gonophore, as in *T. radiata*.

Actinula

The actinula (Fig. 5) is 0.3–0.6 mm in height and 0.3–0.4 mm in diameter at its

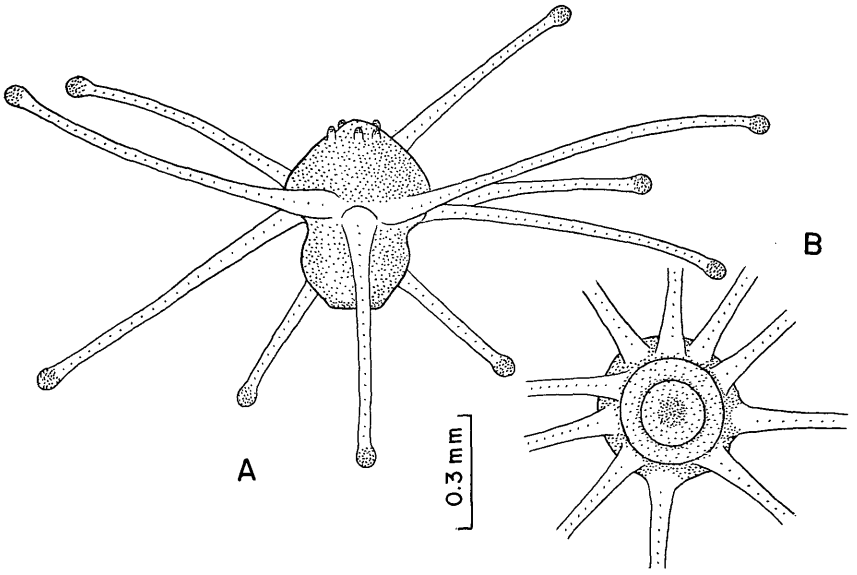


Fig. 5. Actinula of *Tubularia venusta*. A: Side view. B: Aboral view.

widest part. It has 5–10 oral tentacles near the oral tip, but they may show various degree of growth in each newly liberated actinula. Seven to thirteen rather rigid aboral tentacles, disposed in a circlet and 1.0–1.4 mm long, run upward and downward alternately, each terminating in a comparatively distinct terminal knob which is strongly adhesive. The oral half of the actinula is almost hemispherical in form and the mouth is not yet opened. The aboral half is nearly cylindrical in form, slightly tapering toward the base where the pedal disk is formed.

The general internal structure of the actinula is similar to that of the preactinula but some further differentiations occur (Fig. 86). In the future mouth area the mesolamella becomes obscure and there are a few small spindle shaped endoderm cells. Later the endoderm cells at the anterior half of the hypostome become composed of much elongated gland cells containing secretory substance at the distal half (Fig. 87). The well developed oral tentacles are composed of short columnar ectoderm cells and flat disk-like endoderm cells which are arranged side by side in one row perpendicular to the shaft At the terminal knob the ectoderm cells are taller than those of other parts, and they are provided with many nematocysts. Later, at the oral half of the embryo, circular muscle fibers develop between the mesolamella and the endoderm. In the aboral tentacle circlet region the endoderm cushion which has a similar constitution to that of *T. radiata* becomes large and grows tapering inwardly (Fig. 86). By this time the ectoderm of the aboral tentacles which consists of thin ectoderm cells has become thinner at the oral side of the shaft than at the opposite side, as seen in *T. radiata*. At the aboral tube the distinction among the three parts observed in the preactinula stage becomes more conspicuous. The differentiation of the fixing cells is completed and the cells are more elongated. The secretory substance becomes conspicuously increased in volume at the periphery, but

later it is released. Surrounding the active fixing cells area a circular outgrowth of the ectoderm cells is often observed (Fig. 86). The endoderm cells facing the fixing cells become extremely tall surrounding the central empty space. The pseudocells still remain mainly in the aboral endoderm.

Soon after liberation marked changes begin to take place in the actinula. The anterior region of the oral part becomes sharply protruded. The distinction of the three parts of the aboral tube becomes obscure and the fixing cells become extremely flattened together with the transformation of the endoderm cells. In these regions conspicuous cell organization and adjustment are taking place (Fig. 88). Moreover, the boundaries among cells and the mesolamella become obscure and the interstitial cells located at the lateral wall are migrating toward the aboral end. These cells seem to participate in the elongation of the hydrocaulus and the formation of the

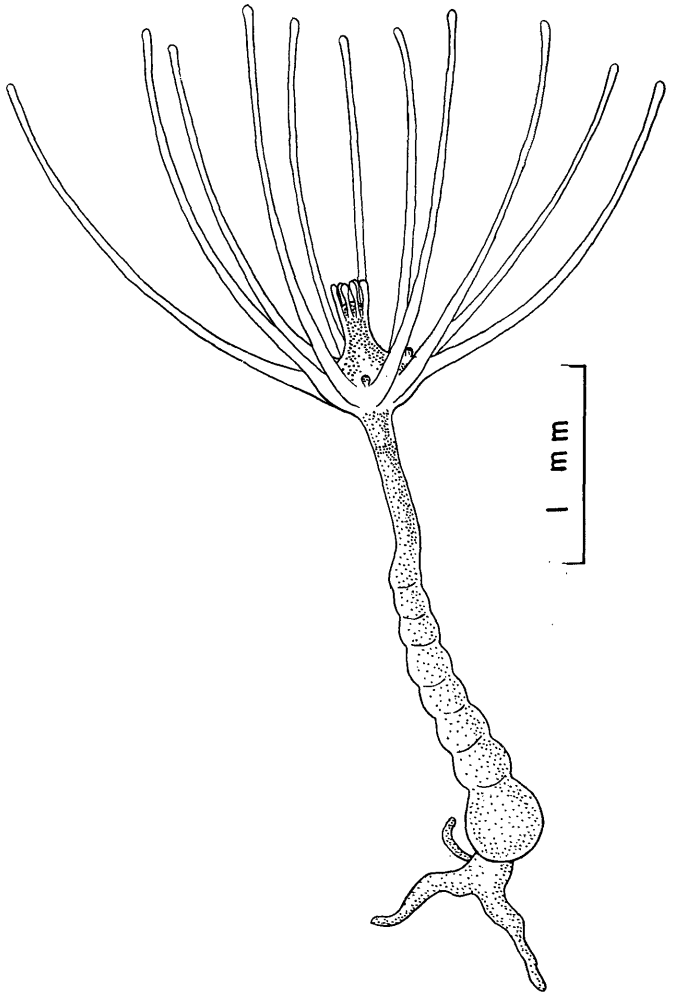


Fig. 6. Young polyp of *Tubularia venusta*, 3 days after liberation.

stolon.

Development of polyp

Shortly after liberation the aboral part of the actinula begins to elongate rapidly and the actinula settles to the substratum on its pedal disk in one to two days. All of the aboral tentacles run upward and the oral part assumes a slender hypostome which is furnished with a mouth just opened and oral tentacles which are now upwardly erected. After that, the aboral tube becomes rapidly longer, becoming metamorphosed to the hydrocaulus and the following two parts are differentiated; a narrow soft tubular part just posterior to the hydranth and the rigid rather big part below. By this time the aboral end of the young polyp gives rise to 1–3 stolons extending in different directions. Two to three days after settlement slight annulations appear on the lower rigid part of the hydrocaulus. Now 2–4 rudiments of the gonophore begin to develop on the hypostome just above the aboral tentacle circlet (Fig. 6). During these processes the terminal knobs of the oral and the aboral tentacles become gradually indistinct as observed in *T. radiata*. According to Pyefinch and Downing (1949) gonophore buds of *T. larynx* begin to appear about a week after settlement and some new actinulae are liberated about three weeks after that. In the present species the development of the polyp takes place more rapidly than in the case of *T. radiata*.

While the above changes are taking place internal changes are also undergone (Fig. 89). The ectoderm cells of the hypostome become much shorter than those of the actinula and the endoderm cells of this area are clearly differentiated into an anterior region with long columnar cells and a posterior region with short columnar cells, the latter gradually transforming to the endoderm covering the cushion. The oral tentacles bocome slightly elongated. Their ectoderm cells become thin and flat, and are more flat at the oral side. The endoderm cushion and the aboral tentacles show no marked change. As the hydrocaulus elongates the following two parts become distinguishable; the first part just below the aboral tentacle circlet, corresponding to the soft tubular part in external view, consists of simple rather rectangular ectoderm cells and thin, flat pavement endoderm cells, and is destitute of a periderm; and the next part below that described above, in which both the ectoderm and the endoderm become very thin (Fig. 90) and in the ectoderm of which there are often seen several interstitial cells migrating to the base and some nematocysts.

V. Consideration

Although the cleavage of *Tubularia* has been observed by several investigators in the following species, *T. crocea* by Allen (1900) and G. T. Hargitt (1909), *T. larynx* by Lowe (1926), *T. mesembryanthemum* by Ciamician (1879), Brauer (1891) and C. W. Hargitt (1904), and *T. radiata* by Nagao (1960), the results given by them have not always agreed with each other not only among the different species but also within the same species. Generally the embryo of *Tubularia* tends to show more or less irregular and unequal cleavage, and the nuclear divisions without the cytoplasmic divisions. The cleavage of *T. radiata* previously reported by the author (1960),

however, goes on in equal, regular and vertical pattern till the fourth division. Moreover, the present investigation reveals that, in *T. radiata*, the cytoplasmic divisions always take place soon after the nuclear divisions and no syncytium is formed during cleavage. In *T. venusta* the cleavage takes place in an almost similar way to that of *T. radiata*, but some embryos show very irregular, unequal divisions and a syncytium is observed in the early period. According to Brauer (1891) the cleavage of *T. mesembryanthemum* shows two distinct modes. In the first mode it takes place regularly and vertically until the third division, like that of *T. radiata*, but in the second mode only the nuclear divisions go on at first and the cleavage is very irregular. In *T. crocea* the cleavage also takes place more or less irregularly and unequally, but comparatively regular examples show vertical divisions until the 12-cell stage or later (G. T. Hargitt, 1909). In *T. larynx*, reported by Lowe (1926), the cleavage is irregular, unequal and multinuclear, but it also shows considerable variation from relatively regular patterns to extremely irregular ones.

Judging from the facts above cited it is probable that the cleavage observed in *T. radiata* is the original pattern in *Tubularia* and that of the other species above noted may be derived from this original pattern by modifications which may be caused by several factors such as yolk density, pressure of gonophore wall, number of eggs in a gonophore and others. According to Hargitt (1906) the pattern of cleavage in *Clava leptophyra* is greatly affected by the difference of pressure of the gonophores. As reported by G. T. Hargitt (1909), the pattern of cleavage of *T. crocea* shows some variations by the localities and the seasons as seen in *T. venusta*. Such reports suggest that cleavage of *Tubularia* may be often modified by some environmental factors. In *T. larynx*, it seems that the cleavage is so greatly modified that it does not give any indication of the resemblance to the original pattern seen in *T. radiata*. On the other hand, the cleavage of *Ectopleura dumortieri* takes place in equal and vertical fashion until the third division (Aurich and Werner, 1955). Although the cleavage of *E. dumortieri* shows the bilateral symmetrical type, which may arise from the loose connection of blastomeres in consequence of free spawning. Therefore the cleavage of *Ectopleura* seems to be included in the original pattern represented by *T. radiata*. Fundamentally the early cleavage of *Tubularia* takes place vertically until the third or the fourth division and shows an isobilateral symmetry. It is noticeable that the cleavage of *Tubularia* gives a characteristic pattern differing from the usual pattern seen in the Hydroida, in which the first and the second division are vertical and the third one is equatorial, showing a radial type.

Blastulae of *Tubularia* also show considerable variation not only among the different species but also within the same species. In *T. mesembryanthemum* the blastocoel always appears in the embryos passing through regular divisions, but its size and form are irregular (Brauer, 1891). In *T. crocea* blastulae also show considerable variation and there is no blastocoel in extreme cases (G. T. Hargitt, 1909). The blastocoel of *T. larynx* is also very irregular and uneven as observed by Lowe (1926). In the two present species coeloblastulae with more or less reduced blastocoels are formed. The blastulae of *T. radiata* show less variation than those of the other species above cited. On the other hand, in *T. venusta*, some of the blastulae have extremely reduced blastocoels, but blastulae with a large cavity are observed too.

From the observations given by the earlier workers and the present author it may be said that the blastulae of *Tubularia* are principally of the coeloblastula type with more or less reduced blastocoels and they may be often modified by some external factors such as the pressure of the gonophore and others, as seen in the cleavage.

Concerning the formation of the germ layers the observations of the earlier workers roughly coincide with each other. In *T. mesembryanthemium* the blastocoel is obliterated by the radial divisions of the outer blastomeres of the blastula which gives rise to a solid mass of cells and the germ layers are formed (Brauer, 1891). On the other hand, Hargitt (1904) agreed also with Brauer's conclusion to some degree, though he pointed out that, in the cases forming a syncytium, the gastrula is brought about by a slow and gradual process of cell organization and adjustment as seen in *Eudendrium* and *Pennaria*. In *T. crocea* the primary endoderm cells are budded off by a multipolar proliferation and a solid mass of cells is brought about, but the definitive positions of the cells are assumed as the result of further divisions and rearrangement (G. T. Hargitt, 1909). According to Lowe (1926) the blastocoel of *T. larynx* is filled by the multipolar buddings and a solid mass of cells results. Later, the ectoderm is differentiated by a simple delamination and becomes clearly distinguishable from the endoderm by more deeply stained cytoplasm. On the other hand, in some very irregular embryos a partial epiboly is observed.

At all events the gastrulation of *Tubularia* is characterized by the formation of a solid mass of undifferentiated cells. Conn (1882), Allen (1900) and Hargitt (1904) considered that this mass of cells is a true morula. On the other hand, Brauer (1891) and G. T. Hargitt (1909) pointed out that this mass of cells is not a true morula but shows a phase of the germ layer formation. Lowe (1926) was also of the same opinion. In the two present species, *T. radiata* and *T. venusta*, the primary germ layer formation takes place in the way similar to the case of *T. crocea*. As the result of multipolar proliferation in *T. radiata*, and of cell divisions like an inward proliferation or multipolar proliferafion in *T. venusta*, the solid mass of cells is formed, but the final position of the cells is not yet settled. Then the primary germ layers are differentiated by further divisions like a delamination and by the arrangement in a layer of the outer cells. These processes take place more rapidly at the animal side as in *T. mesembryanthemum* and *T. larynx*. Later, the interstitial cells which are budded off by the unequal divisions of the primary endoderm cells migrate toward the periphery, then the final separation of the germ layers is completed. In the two present species it is also considered that the solid mass of cells does not arise as the result of the end of cleavage but as a phase of the germ layer formation. From the facts above mentioned the gastrulation of *Tubularia* may be characterized as follows. At first a solid mass of cells is brought about by modified multipolar proliferations. Next the primary germ layers are differentiated by further divisions like a delamination and by the arrangement in a layer of the outer cells, and the interstitial cells are budded off by the unequal divisions of the primary endoderm cells. Then the final differentiation of the germ layers is established by the migration and the attachment of the interstitial cells to the primary ectoderm.

Concerning the origin of the ectodermal interstitial cells Brauer (1891) in *T. mesembryanthemum* and Lowe (1926) in *T. larynx* stated that the interstitial cells are proliferated by the rapid divisions of ectoderm cells of gastrula. On the contrary, in the two present species, the interstitial cells are produced by the unequal divisions of the primary endoderm cells in the second stage of the gastrula, and later they migrate toward the periphery to form the ectodermal interstitial cell zone. At this time the author could not detect any cell divisions in the primary ectoderm for the formation of the interstitial cells as observed by Brauer and Lowe. Tichomiroff (1887) pointed out, however, the possibility of the transformation of the inner cells to the ectoderm in *T. mesembryanthemum*. According to Mergner (1957), in *Eudendrium racemosum*, many interstitial cells appear in the endoderm at the stage of young gastrula, and next they form the cnidoblast zone at the periphery of the endoderm.

The relation between the polarity of the egg and the future body axis in *Tubularia* has not been ascertained (Brauer, 1891; Lowe, 1926; etc.). In *T. radiata*, the polarity also becomes obscure at the gastrula stage. On the other hand, in *T. venusta*, the polar differentiation is so distinct that it is recognizable through the whole of development. In this species the animal side of the egg becomes the oral side of the polyp and the vegetal side differentiates into the aboral side.

It is noteworthy that, in the two present species, the ectoderm of the future mouth area becomes gradually a simple layer by the remigration of the ectodermal interstitial cells into the endoderm in the process of actinula formation.

There are some distinct differences between the actinula of *T. radiata* and that of *T. venusta*. The actinula of *T. radiata* is larger than that of *T. venusta* and has long and pliable aboral tentacles. On the other hand, the aboral tentacles of the actinula of *T. venusta* are short and rigid, and have well developed terminal nematocyst knobs which are very adhesive. Moreover, the pedal disks of the two species show a distinct difference. It may by considered that these characteristic differences show adaptation for their habitats: namely, *T. radiata* lives on eel-grass growing at the inmost part of the bay and in Akkeshi Lake where the sea is comparatively calm; on the other hand, *T. venusta* grows on rocks between tide marks where the sea is comparatively rough.

Next the author intends to discuss the systematics of the family Tubulariidae from the developmental point of view. As mentioned above the cleavage pattern in Tubulariidae shows the characteristic pattern and differs from other members of the Hydroida.

The following significant difference in the developmental processes is observed between the Tubulariidae and the Corymorphidae. The embryo of the Tubulariidae passes through the actinula stage, on the other hand, the developing eggs of *Corymorpha* sink to the substratum, and later the polyps grow directly from the egg cases as observed by Torrey (1907) and Rees (1937). Moreover, the pattern of the cleavage of *Corymorpha* reported by Torrey differs from that of *Tubularia*. On the other hand, the cleavage pattern of *Ectopleura* is closely similar to that of *Tubularia* as discussed above. Kramp (1949) pointed out that the morphological resemblance between the Tubulariidae and the Corymorphidae is derived from the result of con-

vergence, and these two families are not so closely related to each other but phylogenically they are separated from the Corynidae at an early period. Contrary to this, Rees (1957) thought that the Tubulariidae may be assumed to have arisen from the Corymorphidae. Moreover, he proposed a new super-family Tubularoidea including above two families and the Margelopsidae, and referred it to the most primitive form in the Capitata, based upon his opinion that the solitary forms are more primitive than the colonial forms.

Among the genera of the Tubulariidae the three genera, *Tubularia*, *Hybocodon* and *Ectopleura*, are closely related to each other. According to Aurich and Werner (1955) the embryo of *Ectopleura* carries on a true planktonic life through the whole of its development. On the other hand, *Hybocodon* has a rather short pelagic period in its development, and the embryo of *Tubularia* is incubated in the gonophore until the actinula stage. From these accounts of development Aurich and Werner (1955) concluded that the genus *Ectopleura* is more primitive than *Hybocodon* and *Tubularia*, and *Hybocodon* stands in the intermediate position between *Ectopleura* and *Tubularia*. The development of polyps of *Hybocodon prolifer* was observed by Uchida and Nagao (1960) and they also agreed with the conclusion of Aurich and Werner by considering medusan morphology too. On the other hand, the present investigation reveals that the pattern of cleavage as seen in *Tubularia radiata* is closely similar to that of *Ectopleura dumortieri*. Judging from the developmental process as noted above it is obvious that the three genera, *Tubularia*, *Hybocodon* and *Ectopleura*, are closely related to each other, though our knowledge of the early development of *Hybocodon* remains comparatively scanty.

Summary

The development from the egg to the young polyp of two Japanese hydroids, *Tubularia radiata* and *Tubularia venusta* is described.

1. The development of *Tubularia radiata*: The cleavage is total and vertical until the fourth division, later some equatorial divisions take place and the cleavage becomes gradually irregular. A syncytium is never observed. Then a coeloblastula with a few greatly reduced blastocoels is formed. The gastrulation is first achieved by multipolar proliferation and a solid mass of cells is formed, next the primary germ layers are differentiated by further divisions like a delamination and by rearrangement of cells. At that time the interstitial cells are produced by the unequal divisions of some of the primary endoderm cells, and later they migrate toward the periphery and form the ectodermal interstitial cell zone, then the final separation of the germ layers is established. Subsequently the embryo becomes flattened along the oral-aboral axis and 8–11 conical outgrowths of the future aboral tentacles appear. Next the aboral part of the embryo protrudes and the coelenteron is formed by the separation of the connections of the endoderm cells. After that the embryo develops gradually into the actinula by growth of the body along the oral-aboral axis and elongation of the aboral tentacles. During this process local differentiations in the future oral, aboral and aboral tentacle areas occur. In the oral area the interstitial cells remigrate into the endoderm, then the ectoderm forms a single cuboid cell

layer. At the aboral end the differentiation of the future fixing cells takes place. The endoderm cells become arranged side by side toward the ectoderm layer. At the base of the aboral tentacles the endoderm cushion is formed by the rapid enlargement of the endoderm cells located just below the base. Four to seven short oral tentacles are formed and the actinula is ready to be set free. After the settlement to the substratum the actinula metamorphoses to the young polyp by rapid elongation and great differentiations of the tissue of the aboral tube.

2. The development of *Tubularia venusta*: The cleavage shows two types; the first is regular and proceeds in a similar way to that of *T. radiata*, but the dislocation of blastomeres is more remarkable. The second type is very irregular, unequal and multinuclear. The blastula is a coeloblastula with more or less reduced blastocoels, but the size and form of the blastocoels show considerable variation. The gastrulation takes place, at first, by cell divisions like an inward proliferation or multipolar proliferation, and later proceeds in a similar way to the case in *T. radiata*, but the polar differentiation is more distinct. The gastrula derived from the second irregular cleavage type shows a remarkable difference between the animal side and the vegetal side. The animal pole of the egg becomes the oral side of the polyp and the vegetal side differentiates into the aboral tube. The process of formation of the actinula is almost similar to that of *T. radiata*. The actinula develops into the polyp more rapidly than in the case of *T. radiata*.

3. From comparison with the observations of earlier workers the cleavage pattern in *T. radiata* seems to be the original type in *Tubularia*. The gastrulation of *Tubularia* may be characterized by a multipolar proliferation, the further divisions like a delamination and the migration of the interstitial cells. The developmental process indicates close affinity among the three genera, *Tubularia*, *Hybocodon* and *Ectopleura*.

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Explanation of Plates

Plate I

Figs. 7–17. Successive processes in the development of *Tubularia radiata* from life. × ca. 50.

Fig. 7. One-cell stage. Fig. 8. Side view of 2-cell stage. Fig. 9. The second cleavage. Fig. 10. The third cleavage. Fig. 11. Polar view of 16-cell stage. Fig. 12. Early gastrula showing the form like a 2-cell stage. Fig. 13. Gastrula. Fig. 14. Beginning of aboral tentacle protrusion. Fig. 15. A phase in star-shaped form. Fig. 16. A stage of formation of actinula. Fig. 17. Preactinula.

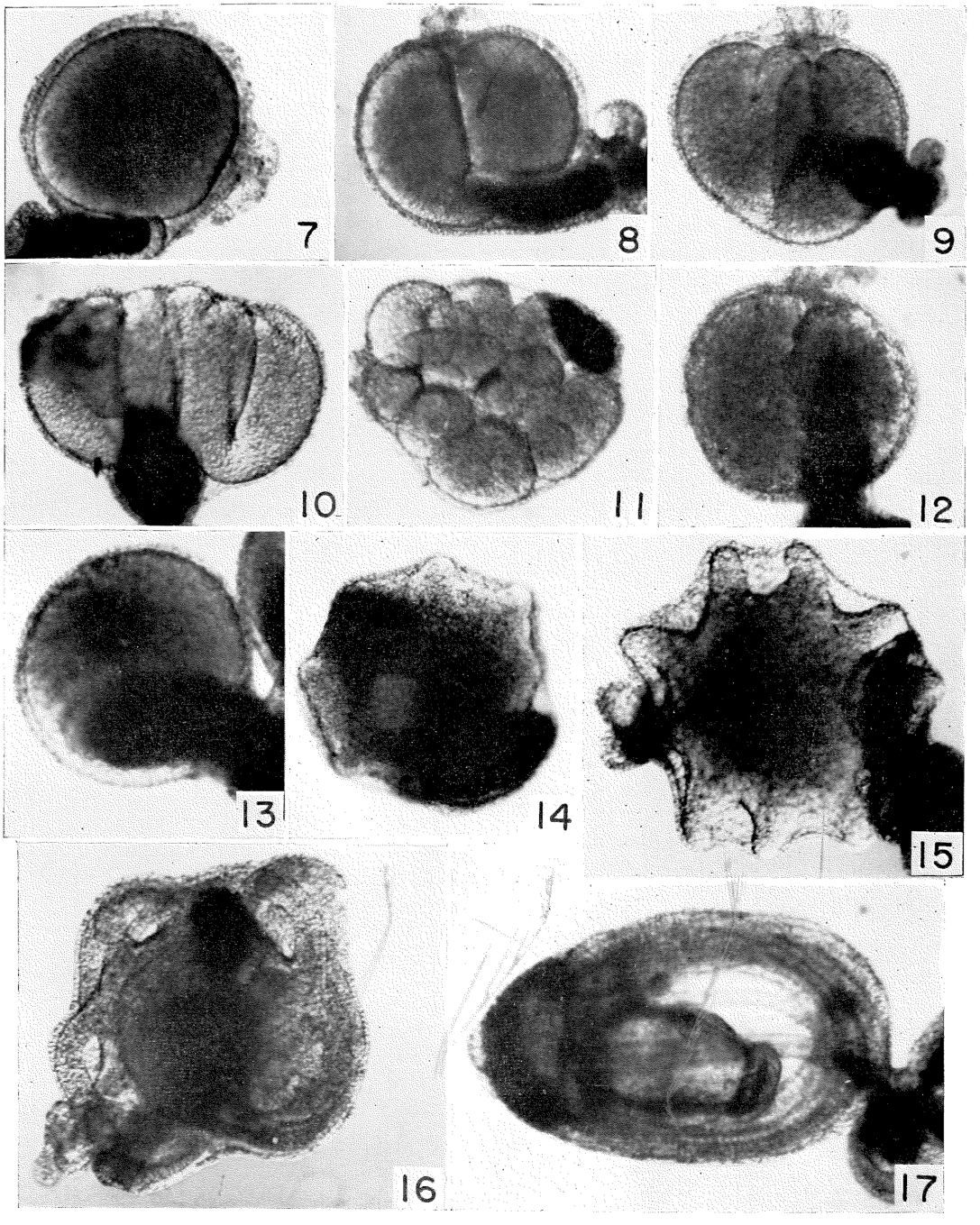

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PLATE II

Figs. 18–28. Successive processes in the development of *Tubularia venusta* from life. × ca. 70.

Fig. 18. One-cell stage. Fig. 19. Side view of 2-cell stage. Fig. 20. The second division. Fig. 21. Side view of 8-cell stage. Fig. 22. Polar view of 16-cell stage. Fig. 23. Later stage of cleavage, 7 hours after insemination. Fig. 24. Gastrula. Fig. 25. Beginning of aboral tentacle protrusion. Fig. 26. A phase in star-shaped form. Fig. 27. A stage of formation of actinula. Fig. 28. Preactinula.

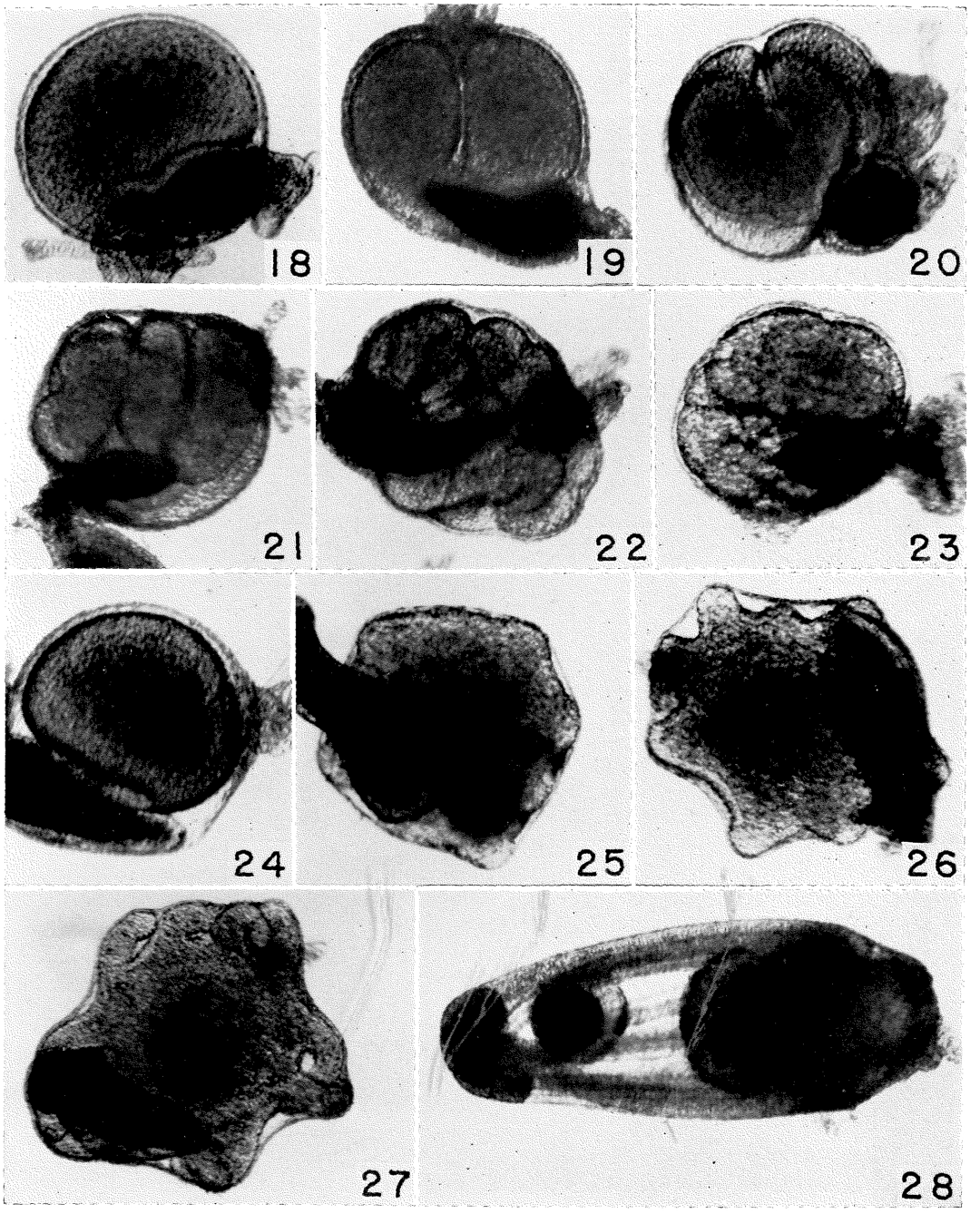

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PLATE III

Tubularia radiata

Fig. 29. A section of a mature egg. × 100. Fig. 30. Animal pole of the same showing a polar body. × 500. Fig. 31. Animal pole of a mature egg showing an egg nucleus. × 500. Fig. 32. Vertical section of 2-cell stage. × 100. Fig. 33. Vertical section of 8-cell stage. × 100. Fig. 34. Vertical section of an embryo with 37 blastomeres. × 100. Fig. 35. Vertical section of blastula. × 100. Fig. 36. Vertical section of early gastrula showing the multipolar proliferation. × 100. Fig. 37. Vertical section of early gastrula appearing like 2-cell stage. × 100. Fig. 38. Later stage of embryo appearing like 2-cell stage. × 100. Fig. 39. Outer cells of gastrula showing a 'double nucleus'. × 400. Fig. 40. Gastrula in the second stage. × 100.

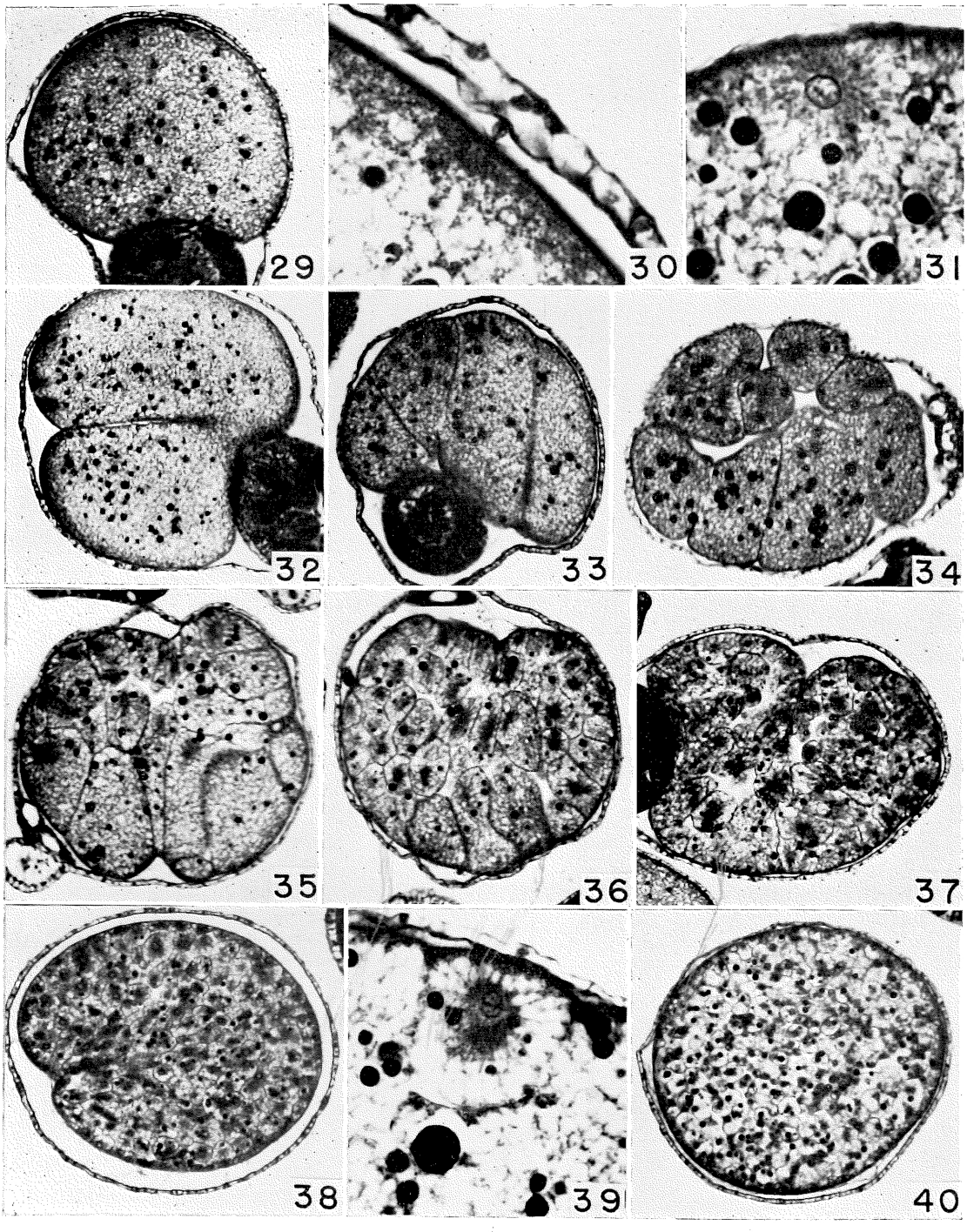

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PLATE IV

Tubularia radiata

Fig. 41. A part of gastrula, showing unequal division of primary ectoderm cell. × 480. Figs. 42 and 43. A part of primary endoderm of gastrula. × 480. Fig. 42. Two unequal divisions of primary endoderm cells marked by an arrow. Fig. 43. Interstitial cells and their divisions (arrows). Fig. 44. Gastrula with migrating interstitial cells. × 110. Fig. 45. Migration of interstitial cells toward periphery. × 350. Fig. 46. The end of gastrulation. × 110. Fig. 47. Sagittal section of a rudiment of aboral tentacle. × 350. Fig. 48. Sagittal section of a phase in a star-shaped form. × 110. Fig. 49. Sagittal section of oral part of the same stage, showing remigration of interstitial cells (arrows). × 350.

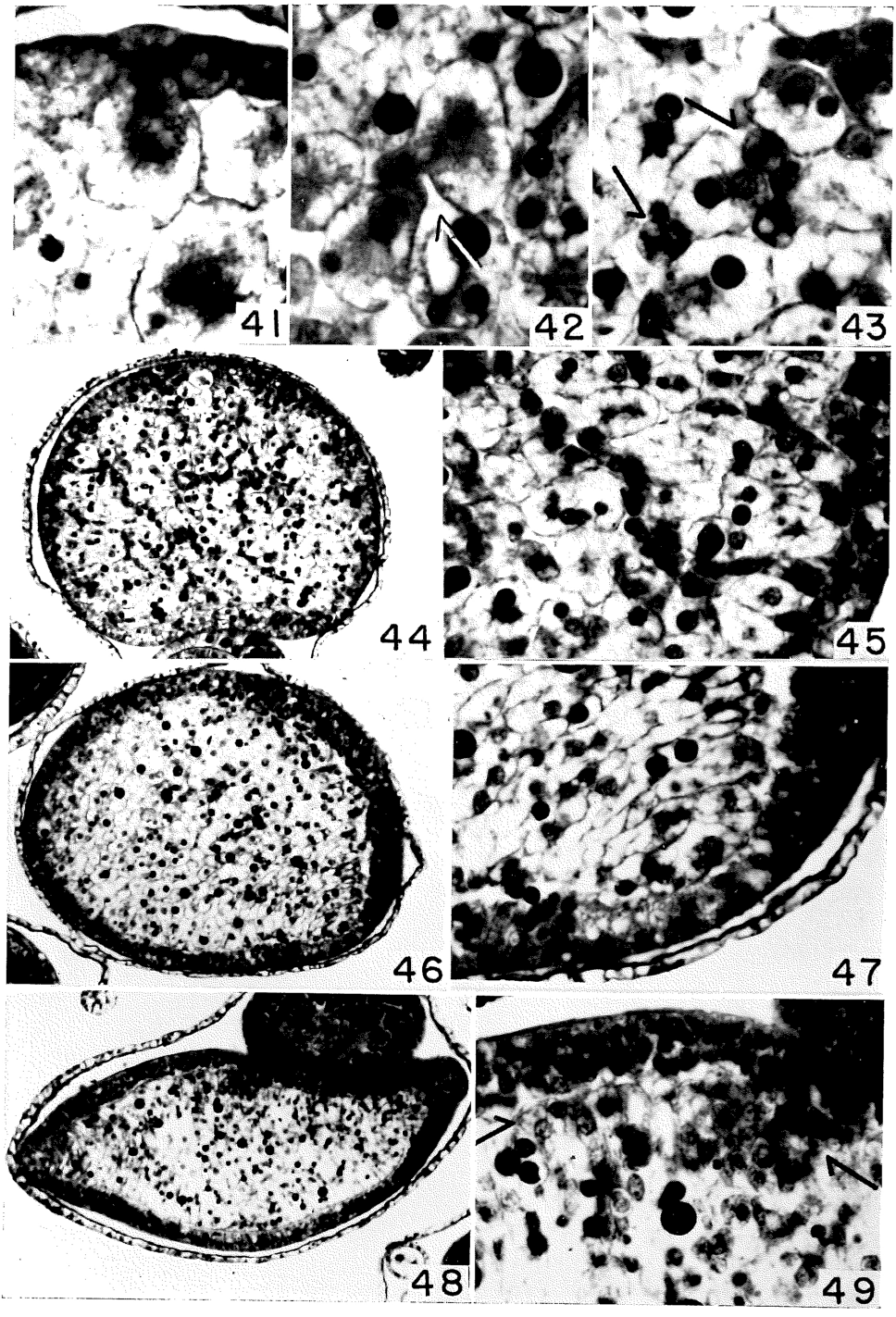
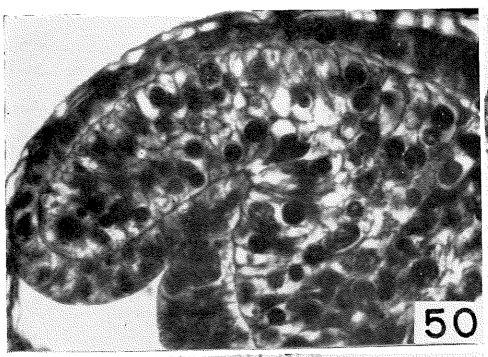
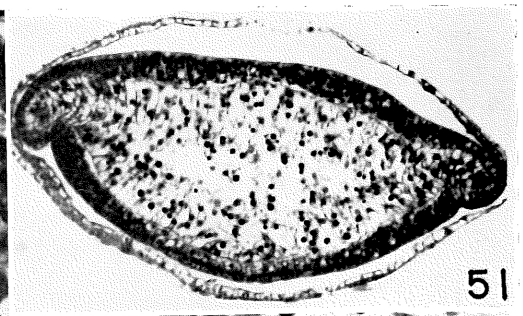

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PLATE V

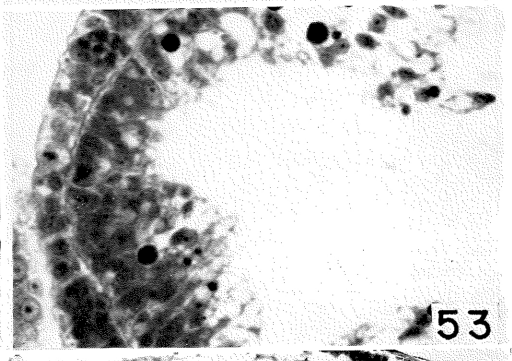
Tubularia radiata

Fig. 50. Sagittal section of young aboral tentacle. × 350. Fig. 51. Sagittal section of later stage of formation of aboral tentacles. × 110. Fig. 52. Sagittal section of stage of formation of actinula. × 110. Fig. 53. Oral part of the same stage. × 350. Fig. 54. Aboral end of the same stage. × 350. Fig. 55. Longitudinal section of preactinula stage. × 110. Fig. 56. Sagittal section of rudiment of oral tentacle. × 480. Fig. 57. Longitudinal section of young oral tentacle. × 480. Fig. 58. Longitudinal section of a part of aboral tentacle base showing the development of endoderm cushion. × 230.


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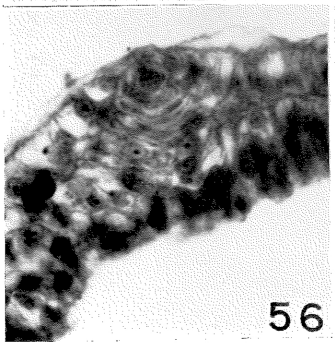

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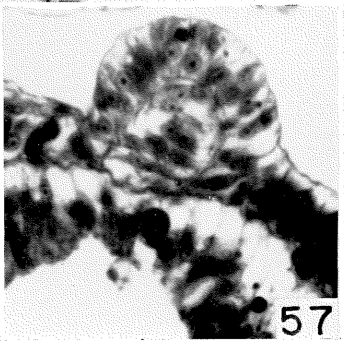

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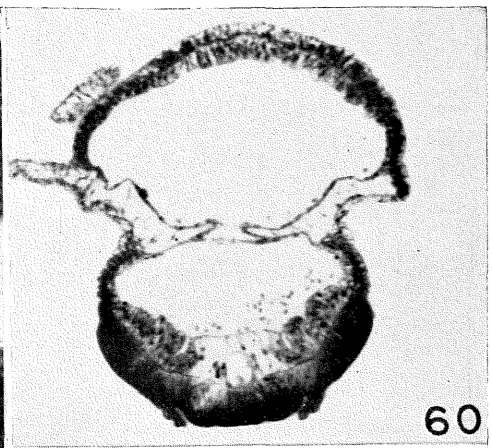

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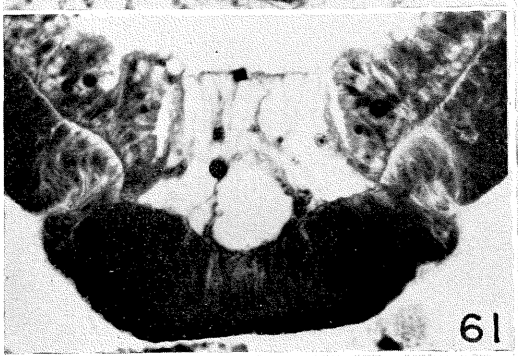

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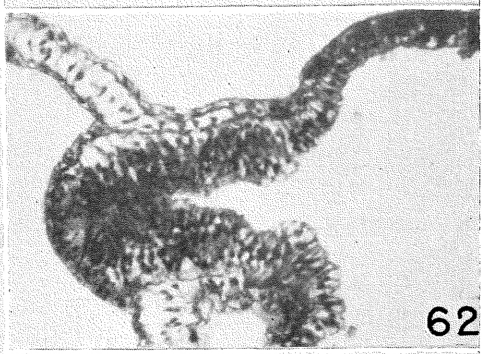
PLATE VI

Figs. 59–64. *Tubularia radiata.* Figs. 65 and 66. *Tubularia venusta.*
Fig. 59. Longitudinal section of aboral tube in preactinula stage. × 230. Fig. 60. Longitudinal section of actinula. × 110. Fig. 61. Longitudinal section of aboral end of actinula. × 230. Figs. 62–64. Longitudinal sections of young polyp, 6 days after liberation. Fig. 62. Oral part. × 300. Fig. 63. Aboral tentacle circlet and upper part of hydrocaulus. × 300. Fig. 64. Lower part of hydrocaulus. × 230. Fig. 65. A section of a mature egg. × 130. Fig. 66. Vertical section of early stage of irregular cleavage. × 120.


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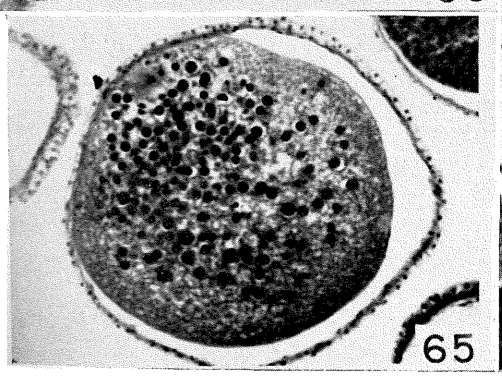

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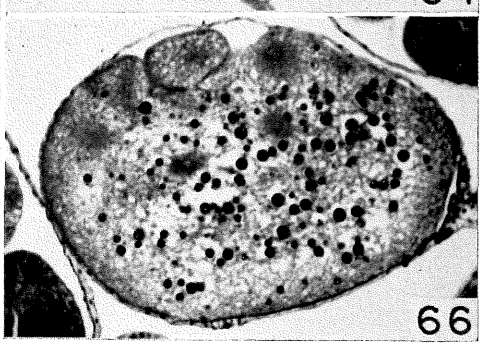

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PLATE VII

Tubularia venusta

Fig. 67. Vertical section of embryo showing irregular cleavage. × 120. Fig. 68. Vertical section of blastula with small blastocoels. × 120. Fig. 69. Early gastrula with large blastocoels. × 120. Fig. 70. Blastula derived from second irregular type. × 120. Fig. 71. Early gastrula. × 120. Fig. 72. Gastrula forming a solid mass of cells. × 120. Fig. 73. Second stage of gastrula. × 120. Fig. 74. A part of the same stage showing unequal divisions of primary endoderm cells (arrows) and divisions of the interstitial cells (arrows). × 350.

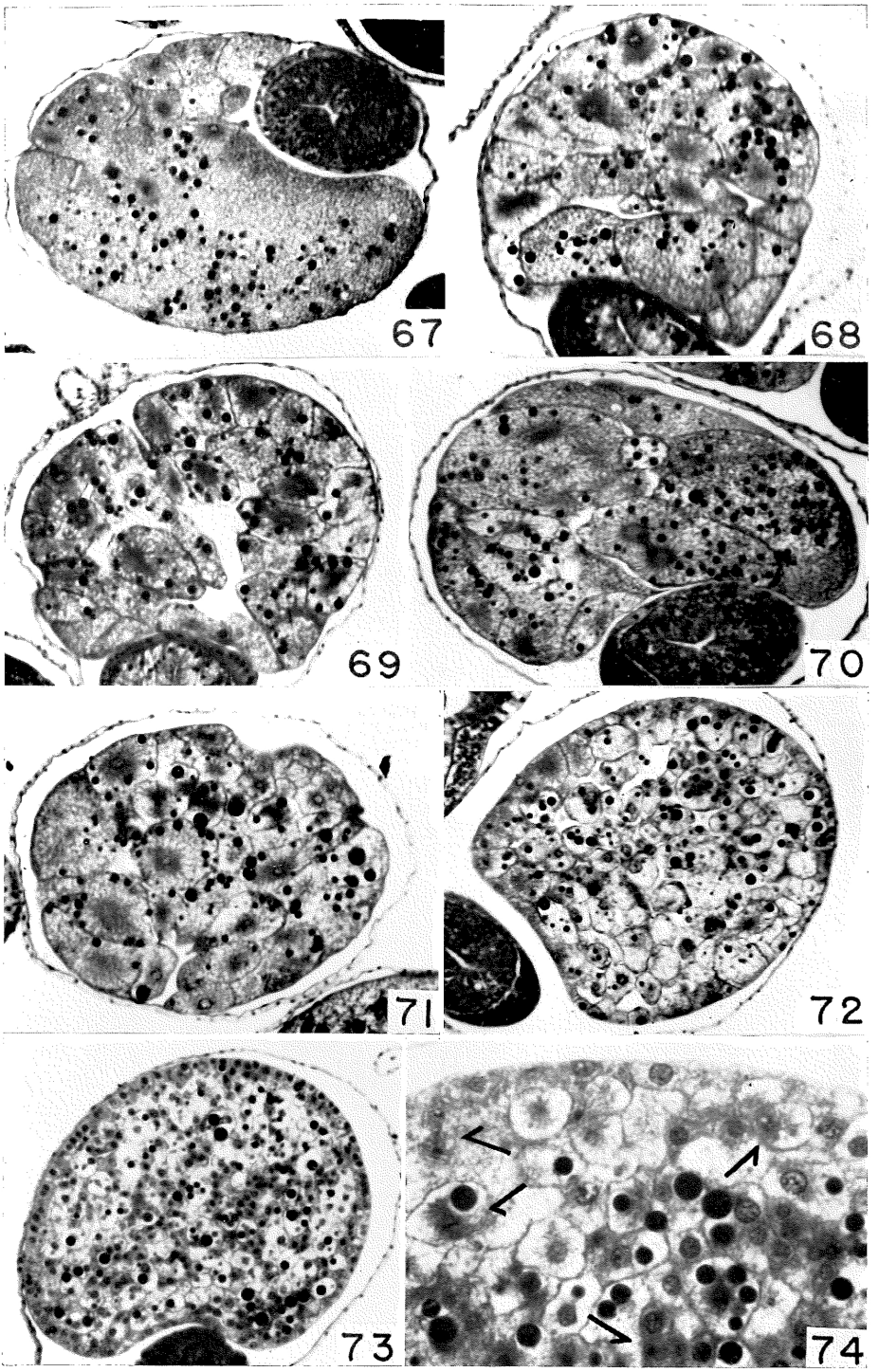

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PLATE VIII

Tubularia venusta

Fig. 75. End of first stage of gastrula derived from the second irregular type. × 120. Fig. 76. Second stage of gastrula. × 120. Fig. 77. Second stage of gastrula derived from the second irregular type. × 120. Fig. 78. Third stage of gastrula. × 130. Fig. 79. Sagittal section of later stage of formation of aboral tentacles. × 130. Fig. 80. Sagittal section of oral part at the same stage, showing remigration of interstitial cells (arrows). × 350. Fig. 81. Sagittal section of stage of formation of actinula. × 130. Fig. 82. Longitudinal section of early preactinula stage. × 230.

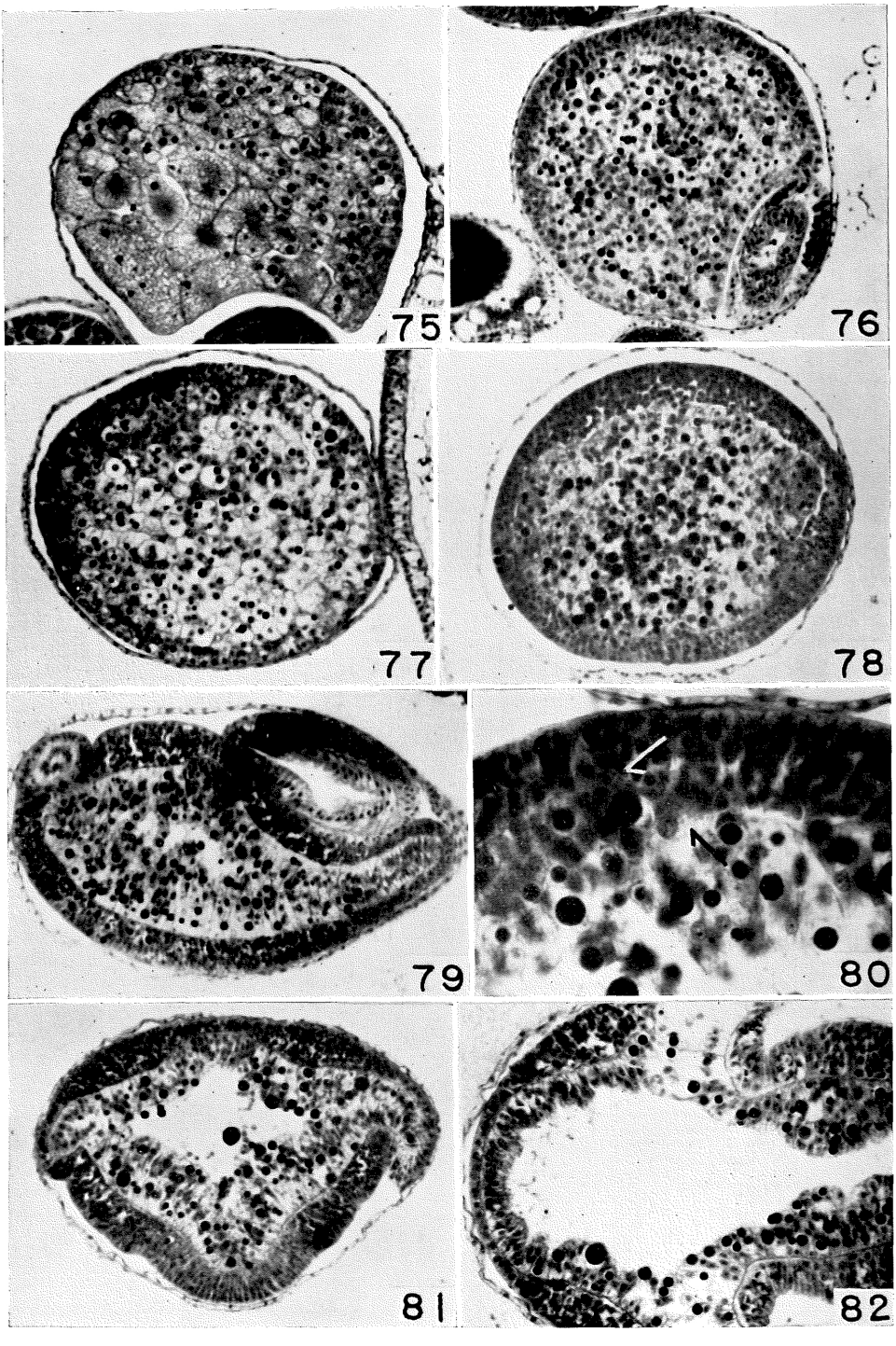
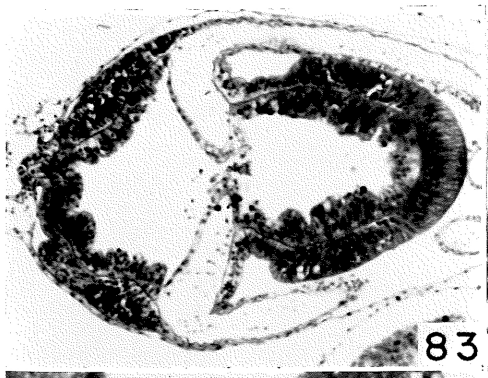

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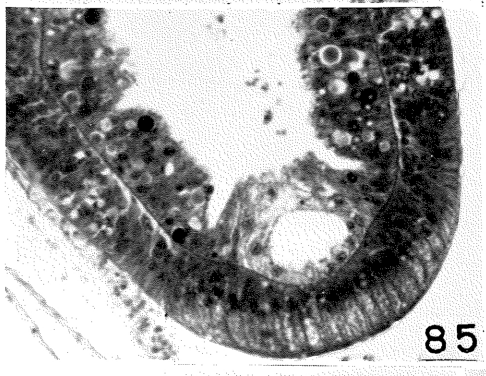
PLATE IX

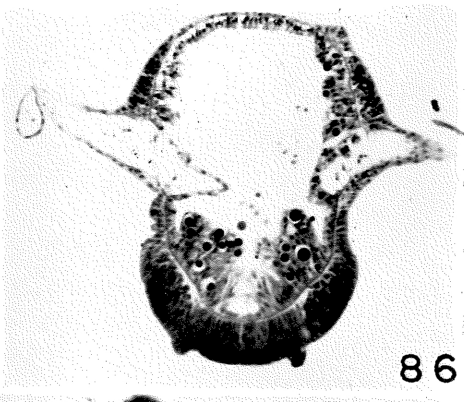
Tubularia venusta

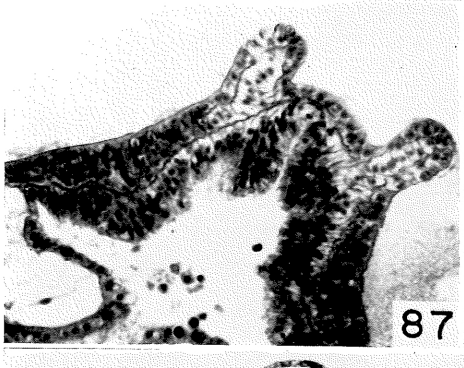
Fig. 83. Longitudinal section of late preactinula stage. × 140. Fig. 84. Longitudinal section of base of aboral tentacle showing formation of endoderm cushion. × 350. Fig. 85. Longitudinal section of aboral tube at preactinula stage. × 280. Fig. 86. Longitudinal section of actinula. × 120. Fig. 87. Longitudinal section of oral part of actinula. × 230. Fig. 88. Longitudinal section of metamorphosing actinula. × 130. Figs. 89 and 90. Longitudinal section of young polyp, 4 days after liberation. Fig. 89. Hydranth and upper part of hydrocaulus. × 120. Fig. 90. Middle part of hydrocaulus. × 300.


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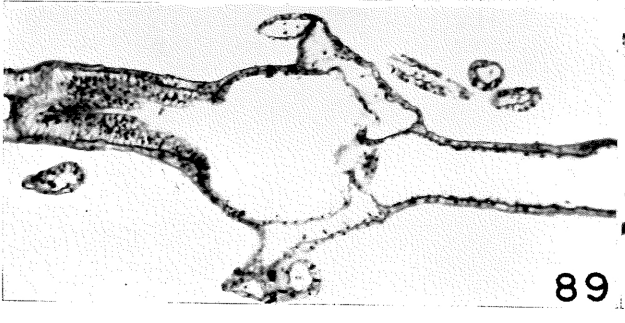

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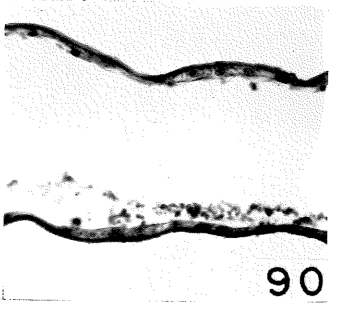

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