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The Early Development of the Anthomedusa, *Polyorchis karafutoensis* Kishinouye[1)2)]

With 23 Text-figures

Zen NAGAO

Akkeshi Marine Biological Station, Akkeshi, Hokkaido
(*Communicated by* T. UCHIDA)

Polyorchis and *Spirocodon* are known as the highest forms in the Anthomedusae (Uchida, 1927) but their life-histories remain mostly unknown. There have been published several works on the early development of *Spirocodon saltatrix* by Uchida (1927), Dan and Dan (1947) and Dan (1950); no report has been published on the development of *Polyorchis*.

In Akkeshi Bay the young medusae of *Polyorchis karafutoensis* begin to appear from the middle of April. During the succeeding three months the medusae are very common there; they spawn from the middle of June to the end of July. In the breeding season the water temperature in their habitat ranges from 10°C to 19°C. The present paper deals with the development from eggs to planulae of *Polyorchis karafutoensis* near the Akkeshi Marine Biological Station.

The medusae were caught one day before they were used and kept in glass bowls filled with filtered sea water after sexual isolation. The eggs were obtained by induction of spawning by the light control described below, and they were inseminated shortly after the spawning. Phase-contrast microscopy was employed in the early stages.

OBSERVATIONS

It is well known that some hydromedusae are induced to spawn by light control (Perkins, 1903; Uchida, 1927; Rugh, 1929; Yoshida, 1952; Roosen Runge, 1962). In the present species, after the light adapted matured medusae had been kept in darkness for two to three hours they were brought again to light,

1) Contributions from the Akkeshi Marine Biological Station, No. 119.

2) This paper is dedicated to Professor Atsuhiko Ichikawa, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, May 20, 1964.

then they began to spawn synchronously one to one-and-a-half hours later. When the animals were kept only in the light or in darkness their spawning was inhibited. So it seems that the light-dark-light changes are neccessary for the induction of spawning. In nature the medusae probably spawn soon after sunrise.

The gonads are of sausage-shape and large in number; they hang down from the junction points of the stomach and the radial canals. They are hollow and are formed by the outgrowth of the basal part of the stomach wall. They consist of the inner endoderm, the mesolamella and the outer ectoderm which contains the germ cells. The ovarian endoderm is composed of high columnar cells filled with many nutritive drops and gland cells which are distributed among the columnar cells. In the testes the endoderm columnar cells containing few nutritive drops are shorter than those of the ovaries; they are greatly vacuolated at the basal part. In the ectoderm of ovaries grown oocytes occupy a large area and younger germ cells are located at rather the periphery. On the other hand, germ cells are regularly arranged in the testes, with older cells nearer the periphery. The ectoderm covering the gonad surface is greatly modified by the growth of the germ cells. Eggs are extruded from the outer wall of the gonad; they fall out directly into the sea water. At a spawning time numerous eggs are laid.

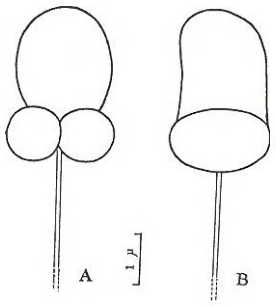


Fig. 1. Spermatozoon of *Polyorchis karafutoensis*
A: View from above.
B: Side view

The spermatozoon (Fig. 1) is about 60 μ in total length, the tail being about 57 μ long. The head is elliptic in form, about 2.0 μ in length. The middle piece is of ellipsoid shape divided into two portions, each about 1.0 μ in length.

The time for the process of the development from insemination was as follows. The water temperature ranged from 14°C to 15°C.

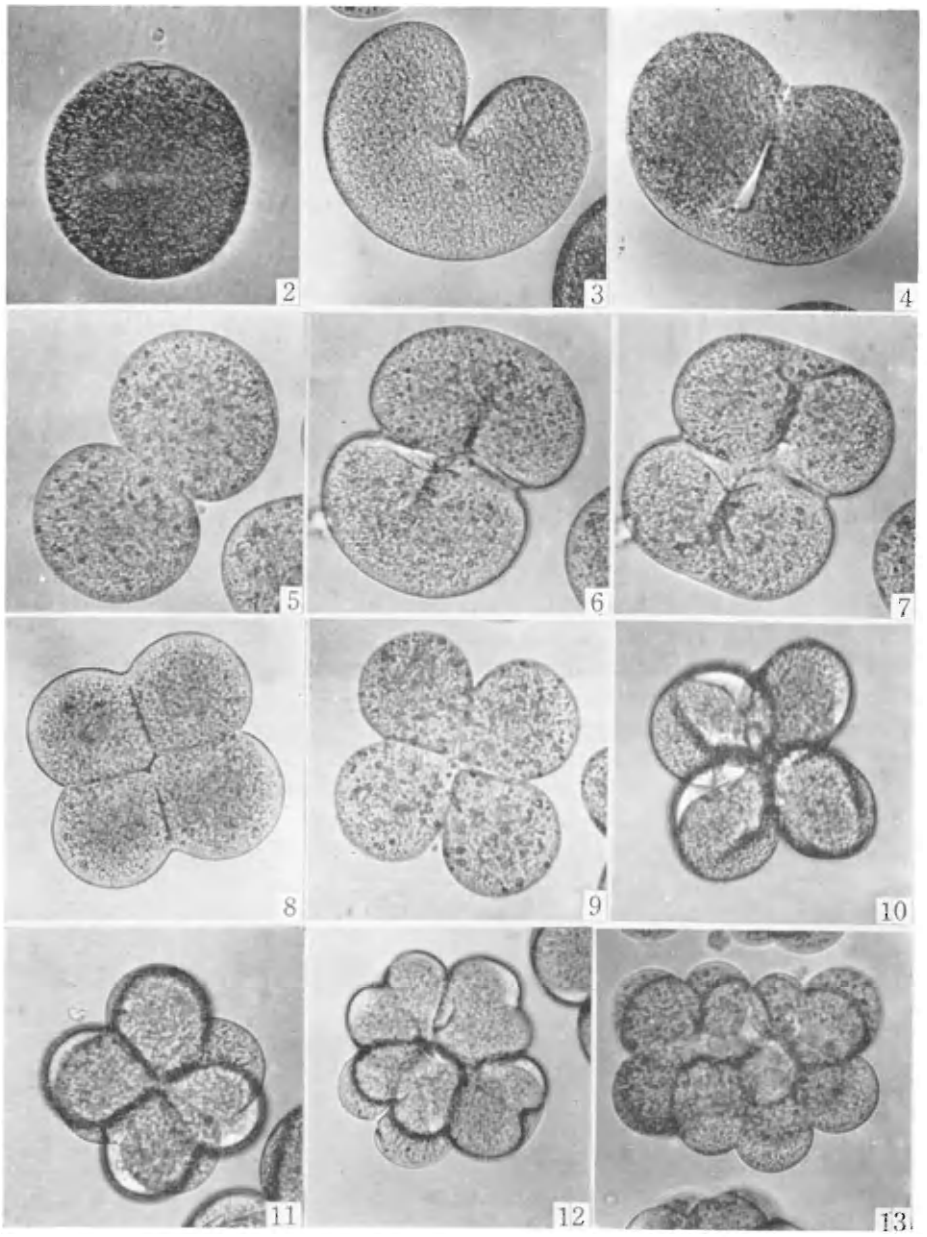
Stage	Average time
Insemination	
2-cell stage	1 hour
4-cell stage	2 hours
8-cell stage	2.5 hours
16-cell stage	3 hours
Aprox. 32-cell stage	3.5-4 hours
Blastula stage	4-5 hours
Body elongation, cilium appearance	8-9 hours
Beginning of gastrulation	9-10 hours
Sterro-planula	20 hours

Unfertilized mature eggs immediately after spawning (Fig. 2) are slightly oval in shape; they become spherical in ten to twenty minutes, 140-160 μ in diameter. They are closely covered with an extremely delicate membrane. At their animal pole, there is a small depression which disappears soon after insemination. Polar bodies are extruded immediately after spawning, shortly thereafter they fall off from the egg surface as observed by Dan (1950) in *Spirocodon saltatrix*.

Behind the second polar body, there is always found a very small spherical body. No fertilization membrane or fertilization tube is observed in the present species.

About 50 minutes after the insemination, prior to the beginning of the first cleavage the eggs are slightly elongated towards the equatorial direction, next the division is caused to take place by the formation of a meridional furrow which gradually deepens (Figs. 3, 4). During these processes the spindle assumes V shape appearance bending to the vegetal side as does that of *Spirocodon* observed by Dan and Dan (1947). At the end of the division two equal blastomeres are barely connected by a narrow plasma bridge at the vegetal pole, then the egg axis running parallel to the substratum rotates to become vertical as shown in Figure 5. Subsequent to this the second furrows which are also meridional and perpendicular to the first start oppositely to each other at the center of the first furrow and move toward the periphery, then they gradually deepen (Figs. 6, 7). Finally the two blastomeres are separated into four equal ones tending to exhibit radial arrangement (Figs. 8, 9). The third division is equatorial and appears as a transverse furrow in each blastomere (Fig. 10), thus the four blastomeres are divided into eight which are all equal in size and arranged regularly in two tiers (Fig. 11). Although slight rotations of the upper quartette to the right or the left side are observed in most cases, they show no regularity. Moreover parallel slippings of the two tiers are seen in some embryos. The fourth division is again meridional; it proceeds centripetally from the periphery of each blastomere (Fig. 12). Then the embryo arrives at the sixteen-cell stage, the cells being regularly arranged in two layers (Fig. 13). During these processes there is observed a slight dislocation of blastomeres in some embryos. On the other hand, the eight blastomeres of each layer are occasionally arranged regularly in two rows, so that the embryos present rather rectangular appearance. The fifth cleavage-furrows are equatorial, but they do not take place synchronously in each blastomere. Moreover gradual dislocation of the blastomeres begins, so the furrows often deepen obliquely in polar view (Fig. 14). Passing through the intermediate stages from sixteen to thirty-two cells, the sixteen blastomeres are gradually divided into thirty-two (Fig. 15). During these processes the blastomeres are arranged by degrees in four tiers with a central cleavage cavity.

Following the successive divisions the blastomers increase in number, become smaller in size, and take arrangement surrounding the blastocoel in a single layer (Fig. 16). The blastulae are typical coeloblastulae being nearly spherical in shape, 160-200 μ in diameter (Figs. 17, 18), but there are frequently observed somewhat rectangular ones whose shape and size show some variations. About eight to nine hours after the insemination the blastulae begin to elongate, to become about 300 μ in length and wider at the posterior portion (Fig. 19). Now numerous cilia appear on the surface of the blastulae, then the elongated blastulae begin to swim in rotating progression through the water. Although the endoderm is not yet formed, there are found several figures of cell division in the direction of the blastocoel in some blastomeres. Nine to ten hours after the insemination the elongated blastulae consist of a layer of columnar cells regularly arranged side by side, with their nuclei located rather on the periphery. Now the pro-



Figs. 2–13. All figures magnified about ×190. Fig. 2. Unfertilized mature egg immediately after spawning. Figs. 3 and 4. First cleavages. Fig. 5. Two-cell stage. Figs. 6 and 7. Second cleavages. Figs. 8 and 9. Four-cell stages. Fig. 10. Third cleavage. Fig. 11. Eight-cell stage. Fig. 12. Fourth cleavage. Fig. 13. Sixteen-cell stage.

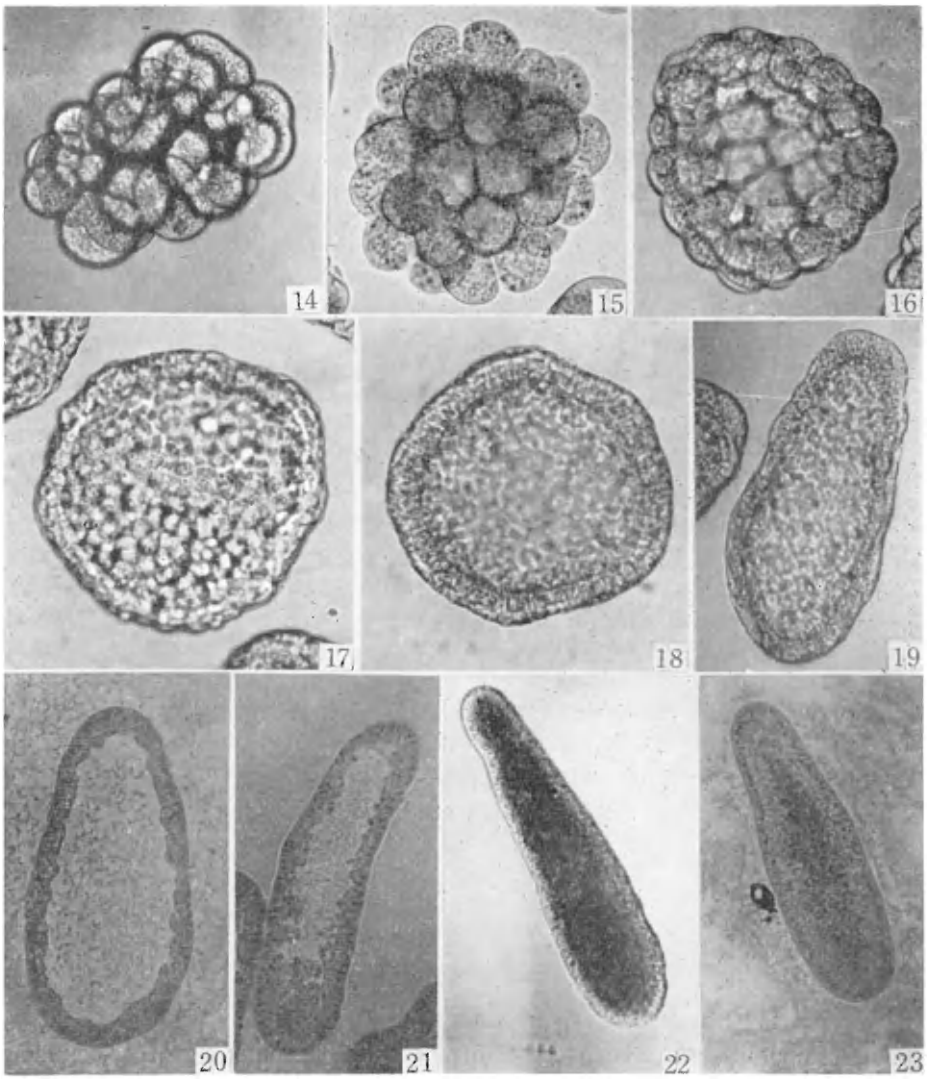


Fig. 14. Beginning of the fifth cleavage. ×190. Fig. 15. About thirty-two-cell stage. ×190. Figs. 16-18. Blastulae. ×190. Fig. 19. Elongated blastula with fine cilia, 8 hours after insemination. ×150. Fig. 20. Longitudinal section of the elongated blastula, 10 hours after insemination. ×210. Fig. 21. Longitudinal section of the early gastrula, 11 hours after insemination. ×160. Fig. 22. Planula, 24 hours after insemination. ×150. Fig. 23. Longitudinal section of the same. ×130.

liferations of blastomeres toward the blastocoel begin here and there (Fig. 20); subsequently multipolar proliferation on the whole surface of the germ layer takes place. Thus the many small endoderm cells are gradually liberated into the blastocoel and brought to the posterior end of it along the inner wall (Fig.

21). Consequently the blastocoel is gradually filled with a rather compact mass of small endoderm cells from the posterior portion. At the time the columnar ectoderm cells at the anterior and the posterior ends are relatively taller than those of the side wall. About twenty hours after the insemination the blastocoel is almost completely packed with a mass of endoderm cells, but they ramain comparatively few at the anterior portion where the blastocoel is greatly reduced by remarkable elongation of the ectoderm cells. The endoderm cells usually deeply stained by hematoxylin are small in size and slightly irregular in form.

The planulae, twenty-four hours after the insemination, are of club-shape tapering toward the anterior end, 350-450 μ in length, and are actively swimming by means of a rotating movement (Fig. 22). Now the blastocoel is completely packed with the endoderm cells. The ectoderm cells of the anterior portion again become as short as those of other portions. In more developed larvae the large endoderm cells begin to take arrangement as a layer along the inner surface of the ectoderm (Fig. 23). One to two days after that the planulae become more slender in form and 400-500 μ in length. After four to seven day's swimming they sink to the bottom. Unfortunately, further rearing was unsuccessful.

Remarks. The unfertilized mature eggs of the present species are, in the shape and in the small depression at the animal pole, closely alike to those of *Spirocodon saltatrix* observed by Dan (1950), though the latter lacks any membranous envelop. In *Polyorchis karafutoensis* there was observed neither the fertilization tube as reported in *Spirocodon* by Dan (1950) nor the fertilization membrane as observed in *Merga tergestina* by Vannucci (1960).

Although the general mode of cleavage till the eight-cell stage of the present species nearly resembles that of *Spirocodon* observed by Uchida (1927), there are some differences between them; moreover, in the former species the regularity of the cleavage is maintained almost until the fifth division, but it is lost after the third division in the latter species. It was often observed by several authors in some hydromedusae that the second cleavage furrows start from the center of the first furrow toward the periphery, for example, in *Koellikerina fasciculata* and *Phialidium hemisphaericum* by Metschnikoff (1886), *Turritopsis nutricula* by Brooks and Rittenhouse (1907), *Stomotoca apicata* = *Amphinema dinema* (?) by Rittenhouse (1910), and *Spirocodon saltatrix* by Uchida (1927). It seems to be rather a common character in hydromedusan eggs.

The temporary upward movement of a single blastomere at four-cell stage was pointed out by Uchida (1927) in *Spirocodon*. In the present species such a movement was not observed. Moreover the rotation of the upper quartette through 45° was frequently reported in some hydromedusae such as in *Koellikerina fasciculata* and *Neoturris pileata* by Metschnikoff (1886), *Gonionemus vertens* by Perkins (1903) and *Spirocodon saltatrix* by Uchida (1927). In the present medusae such a rotation was also observed, though there was no regularity in its direction of the rotation. It seems to be a temporary slipping caused by a loose connection of the two quartettes.

In the present medusa the elongated coeloblastula with fine cilia is formed as in *Spirocodon*. On the other hand, a typical flagellated coeloblastula is characteristic of two species of Pandeidae, *Neoturris pileata* reported by Metschni-

koff (1886) and *Merga tergestina* by Vannucci (1960). According to Uchida (1927), the endoderm formation of *Spirocodon* seemed to be due to the unipolar ingression from the posterior portion, while that of the present species is carried out by multipolar proliferation from the whole area of the ectoderm. Judging from the early developmental processes, *Polyorchis* and *Spirocodon*, though agreeing in several ways, have also several different points.

Summary

The development of *Polyorchis karafutoensis* from eggs to planulae is described. Spawning is easily induced by light control. The cleavage is total, equal and radial in type. The first and second divisions are meridional, the third one is equatorial, the fourth one is again meridional, and the fifth is equatorial but becomes rather irregular; then an elongated coeloblastula with fine cilia is produced passing through the spherical blastula stage. The endodorm is formed by multipolar proliferation. Subsequently the embryo becomes a swimming planula of club-shape tapering anteriorly.

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References

Brooks, W. K. and S. Rittenhouse 1907 Proc. Boston Soc. Nat. Hist., **33**, 429.
Dan, K., and J. C. Dan 1947 Biol. Bull., **93**, 163.
Dan, J. C. 1950 ibid., **99**, 412.
Metschnikoff, E. 1886 Embryologische Studien an Medusen. Wien.
Perkins, H. F. 1903 Proc. Acad. Nat. Sci. Philadel., **54**, 750.
Rittenhouse, S. 1910 J. Exp. Zool., **9**, 333.
Roosen-Runge, E. C. 1962 Pacific Sci., **16**, 15.
Rugh, R. 1929 Biol. Bull., **57**, 261.
Uchida, T. 1927 J. Fac. Sci. Imper. Univ. Tokyo, Sect. IV, Zool., **1**, 145.
Vannucci, M. 1960 Publ. Staz. Zool. Napoli, **31**, 393.
Yoshida, M. 1952 Zool. Mag., **61**, 358.