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THE TAXONOMY, DEVELOPMENT AND BROODING BEHAVIOR OF THE ANEMONE, *CRIBRINOPSIS FERNALDI* SP. NOV.

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The Actiniidae is the largest and most diverse of the families of sea anemones (Anthozoa: Actiniaria). In addition to the morphological diversity within this family, there is considerable diversity in larval development. The Actiniidae are generally considered to be highly advanced anemones, although Hand (1966) has argued that this family should be considered primitive.

In this paper we present information on the taxonomy and life history of the anemone *Cribrinopsis fernaldi* sp. nov. The brooding behavior exhibited by this anemone is intermediate between that of the nonbrooding anemones such as *Metridium dianthus* and *Adamsia palliata* (Gemmill, 1920) and the specialized brooding anemones such as *Actinia equina* (Chia and Rostron, 1970).

Materials and Methods

Adult anemones were collected at various subtidal areas near San Juan Island, Washington using S.C.U.B.A. The anemones were maintained in sea water tanks at the Friday Harbor Laboratories.

The following taxonomic description is based on the study of thirty living or preserved specimens and follows the pattern of Hand (1954, 1955). The cnidom was determined according to the method of Hand (1954) and the descriptions of the nematocysts follow the system of Weill (1934), as modified by Carlgren (1940a). A minimum of 50 nematocysts of each type was measured. Several specimens were dissected to determine the arrangement of mesenteries and distribution of gonads and serial longitudinal and cross sections were prepared from four specimens. After relaxation in 7.5% magnesium chloride, the anemones were fixed in Bouin's fluid or 4% formalin-sea water, embedded in paraffin, sectioned at 15 μm, and subsequently stained with basic fuchsin followed by picro-indigocarmine.

Four anemones collected during the last week of March, 1971 provided the material for the developmental study. Two female and one male anemone (sex determined by postspawning analysis) were placed together in a seawater tank. A single female was placed in an adjacent tank. The male was observed releasing sperm on March 30. At the same time eggs could be seen in the gastrovascular cavity of the females. The eggs, embryos and larvae were removed from the gastrovascular cavity of the females by clipping the end from a tentacle and applying gentle suction with a basting syringe. Embryos obtained in this manner were either prepared immediately for histological observations or cultured. Cultures were started on days 1, 7, 13, 34, and 40 of development.

Embryos and larvae were cultured in jars filled with 500 ml of sea water. The cultures were maintained at approximately 12° C and the water changed every second day. After the larvae settled they were fed *Artemia* nauplii.

Histological preparations were made simultaneously of individuals removed from the adult females and those removed from the cultures. They were fixed in Bouin's fluid and embedded in paraffin. Serial sections were cut at 5–8 μm thickness and stained with Heidenhain's hematoxylin and Orange G.

Taxonomy

Family Actinidae, Gosse, 1858. The definition of Carlgren (1949) remains unchanged.

Genus *Cribrinopsis*, Carlgren, 1921. The description of Carlgren (1949) should be amended to read as follows (Italics indicate amendments): Actiniidae with usually feebly developed verrucae on the column *or verrucae absent*. Pedal disc is well developed; pseudospherules are present or absent; fosse distinct. The sphincter is strong, palmate or pinnate circumscribed. Tentacles are simple, sometimes papillose, short, thick, or *long, tapering, and more or less thickened*. Longitudinal muscles of the tentacles are principally mesogleal, radial muscles or oral disc are meso-ectodermal to ecto-mesogleal. Numerous perfect mesenteries are decamerously, hexamerously, or irregularly arranged; well developed mesenterial muscles. Gonads are on mesenteries of the first cycle and on the other stronger mesenteries, often absent on the directives. Mesenteries are more numerous proximally than distally. The basitrichs of tentacles and actinopharynx are about the same length. Cnidom: spirocysts, basitrichs, microbasic *p*-mastigophores, *atrichs*.

Cribrinopsis fernaldi sp. nov., Siebert and Spaulding.

Description

The base is circular, often irregular in outline, adherent and often transparent. The mesenterial insertions may be visible or not. The basilar muscles are strong.

The column (Figs. 3b, c) is smooth, without adherent material and the verrucae are very weak or absent. Small white tubercles arranged in numerous longitudinal rows are present and the column is usually as high as wide, or higher. The collar and fosse are well developed and numerous marginal pseudospherules are present. The color of the column is variable, generally white, pink, or yellow and as the tissue of the column is very thin, it sometimes appears translucent or transparent with the mesenteries visible along the inner face of the column. The verrucae and tubercles generally are restricted to the upper one third of the column, but sometimes extend almost to the limbus. Cinclides and pores are absent.

The tentacles (Figs. 3b, d) are long, tapering from base to tip with cinclides at the tip and are usually white in color with thin irregular pink or yellow stripes encircling them. The ectoderm is smooth, thin and never papillose. The arrangement is hexamerous, in up to five cycles (96 tentacles). The number may vary somewhat and individuals with 92 to 104 tentacles are not uncommon. The longitudinal muscles of tentacles are well developed, ectomesogleal.

The disc is the same color as the tentacles and the column, often semi-transparent and with mesenterial insertions visible. There may be thin red or yellow lines around the bases of the tentacles, sometimes extending from the margin to the lips.

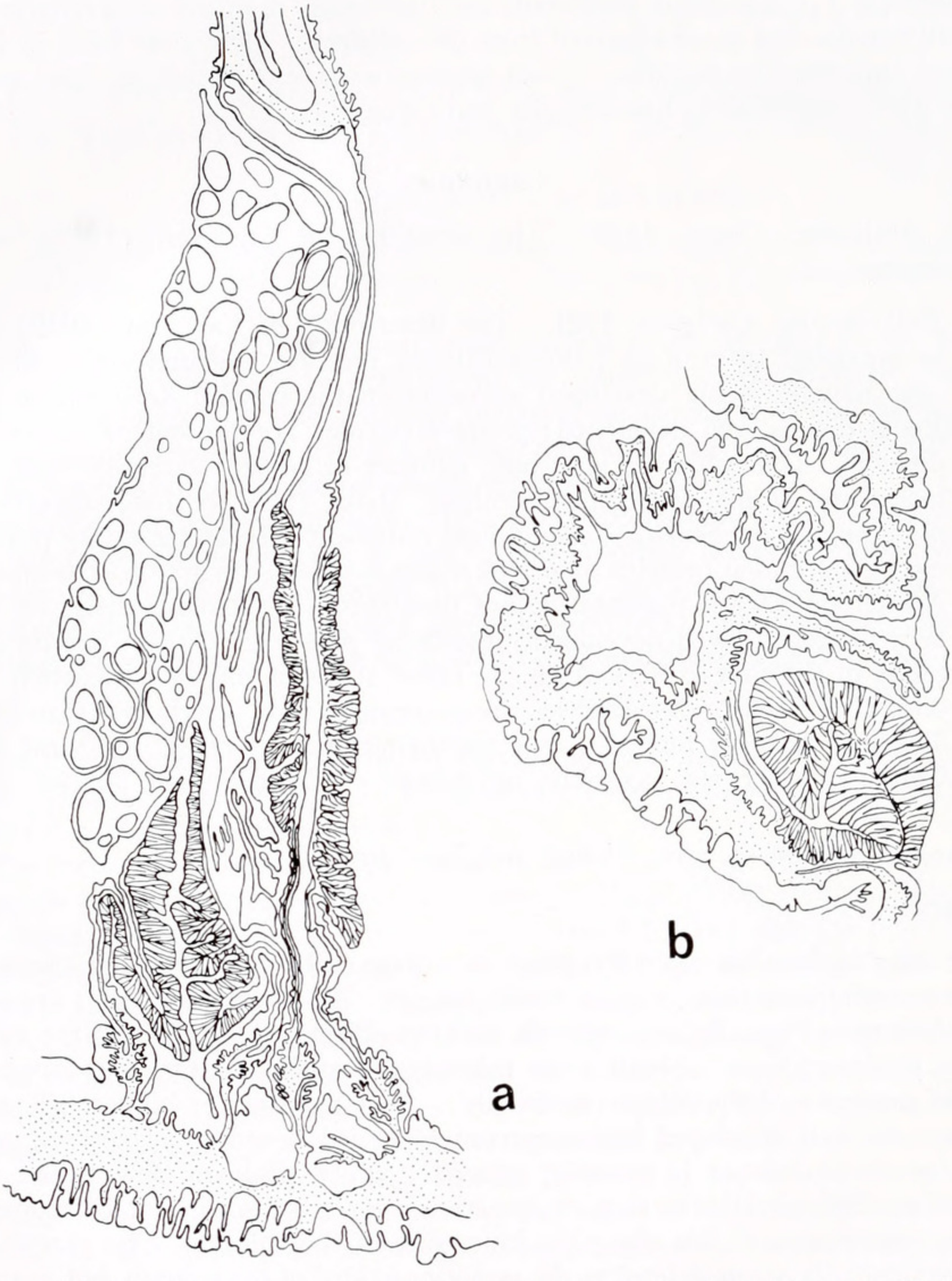

FIGURE 1. a) Cross section of directive and fifth-order mesenteries, 11×; b) longitudinal section of column and oral sphincter, 12×.

The disc is broad and flat, with the lips usually raised and folded and they are light pink in color. The actinopharynx is thick, glandular, and heavily ribbed and two thick-walled siphonoglyphs are present. The radial muscles of the disc are well developed and mesoectodermal.

In fully developed specimens 96 pairs of mesenteries are present, hexamerously arranged, through variations in this number my occur in some specimens. Gonads are present on all mesenteries except the two directive pairs. The retractors are large, strong and diffuse to somewhat restricted (Fig. 1a) and the laminae of the

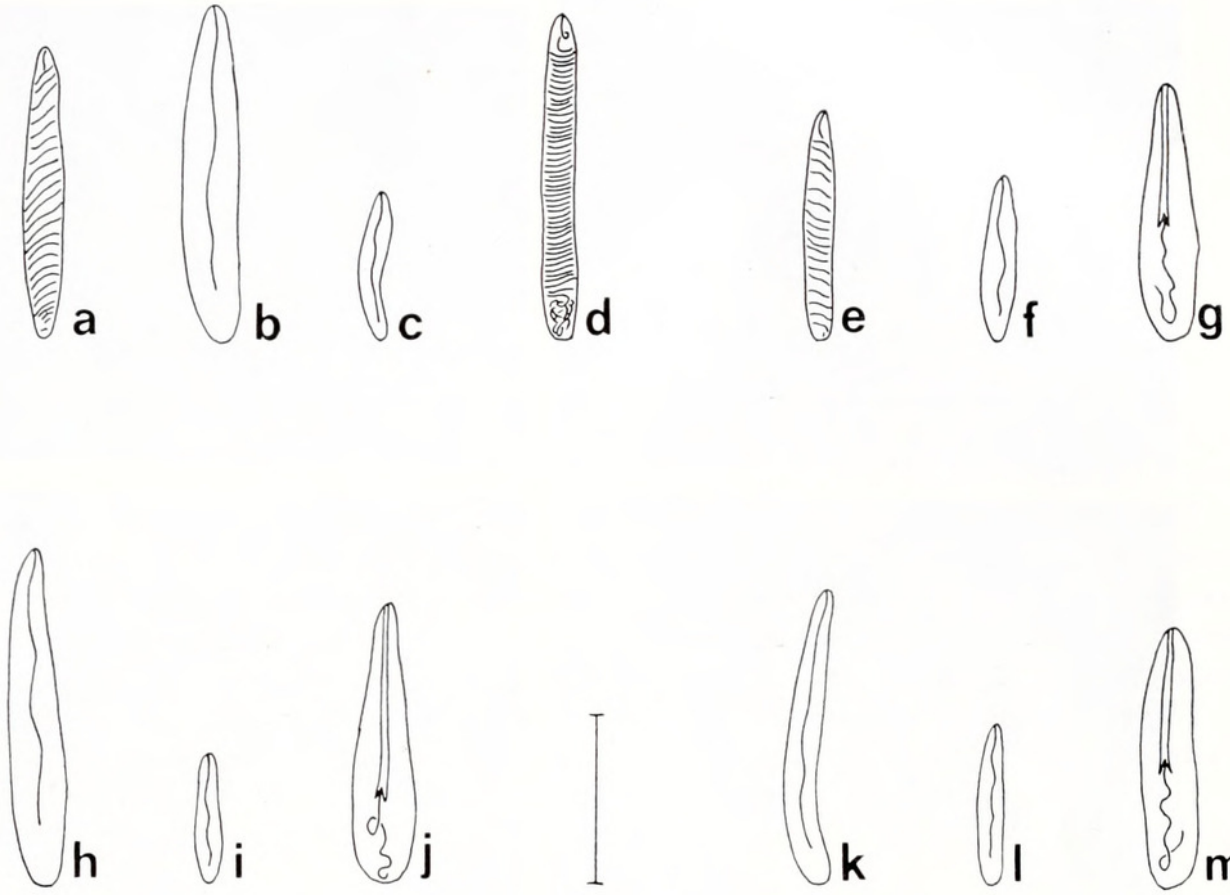

FIGURE 2. Nematocysts of *Cribrinopsis fernaldi* (all nematocysts are drawn to same scale, bar equals 20 μm). a-d) Tentacles; e-g) column; h-j) actinopharynx; k-m) filaments; (a and e) spirocysts, (b, c, f, h, i, k, and l) basitrichs, (d) atrich, (g, j, and m) microbasic *p*-mastigophores.

muscles are thin and finely branched. The parietal and parietobasilar muscles are well developed.

The sphincter (Fig. 1b) is strong, pinnate circumscribed and attached to the column just below the fosse. The laminae are of unequal length throughout the sphincter and branched.

The cnidom (Fig. 2) consists of spirocysts, basitriches, microbasic *p*-mastigophores and atrichs.

The distribution and size of the nematocysts (all measurements in microns) is as follows:

Tentacles

Spirocysts	16–55 × 2.0–4.5
Basitrichs	28–67 × 2.5–5.0
Basitrichs	12–23 × 2.0–3.0
Atrichs (Rare)	27–51 × 3.0–4.0

Column

Spirocysts	15–39 × 1.5–3.5
Basitrichs	15–26 × 2.0–3.0
Microbasic *p*-mastigophores	23–37 × 4.0–7.0

Pharynx

Basitrichs	26–59 × 3.0–6.0
Basitrichs	12–18 × 2.0–3.0
Microbasic *p*-mastigophores	29–38 × 4.5–7.0

Filaments

Basitrichs	29–42 × 5.0–6.0
Basitrichs	12–30 × 2.0–3.5
Microbasic *p*-mastigophores	26–40 × 4.5–7.0

The size of an average individual may be 5–8 cm in diameter at the base, 6–9 cm tall, and 6–8 cm in diameter across the disc. The tentacles in a specimen of these dimensions would be 6–8 cm in length.

This anemone is found on rock walls sloping to vertical, which are heavily encrusted with coralline algae, *Allopora* (a stylasterine hydrozoan), bryozoans, and sponges at a depth of 25–35 meters. Other larger invertebrates common in the same area include *Metridium senile, Terabratalia transversa, Cucumaria miniata, Psolus chitinoides, Evasterias troschellii,* and *Chlamys* spp.

The type locality is about ten meters northeast of Cantilever pier, University of Washington, Friday Harbor Laboratories, San Juan Island, Washington.

The type specimens were deposited in the U. S. National Museum. The holotype is USNM No. 54132; and the paratype is USNM No. 54133.

Diagnosis and remarks

This anemone may be distinguished by the long, tapering tentacles which are usually white with irregular red or yellow stripes, the presence of marginal pseudospherules, and the rows of white spots along the column.

The genus *Cribrinopsis* was established by Carlgren (1921) and at that time he described *C. similis* from the North Atlantic as the type for the genus. Later, he (Carlgren, 1940b) described *C. williamsi* from the Northeast Pacific. Parulikar (1966) records an undescribed species of *Cribrinopsis* from the Indian coast, but gives scant information concerning it, and Schmidt (1972) added *C. crassa* (Andres, 1884). *Cribrinopsis fernaldi* differs significantly from each of the previously described species of the genus in several respects.

The three previously described species of *Cribrinopsis* all have thick tentacles which may be papillose while those of *C. fernaldi* are thin and tapering. There are 60–90 tentacles in *C. similis,* approximately 48 in *C. williamsi,* and 96 in *C. crassa. Cribrinopsis fernaldi* generally possesses 96. All species have verrucae on the column, though in *C. fernaldi* they may be so weak as to be absent. *Cribrinopsis similis* lacks marginal pseudospherules while the other three described species possess them. In *C. similis* the mesenteries are decamerously arranged, those in *C. williamsi* may be decamerous or hexamerous, and in *C. crassa* hexamerous. In *C. fernaldi* the mesenteries are hexamerous.

From the above comparison, it can be determined that *C. fernaldi* is similar to *C. crassa* in the number and arrangement of the tentacles and mesenteries and distinct from *C. similis* and *C. williamsi.* However, *C. fernaldi* and *C. crassa* differ greatly in the shape and patterns of the bands of color on the tentacles and in the

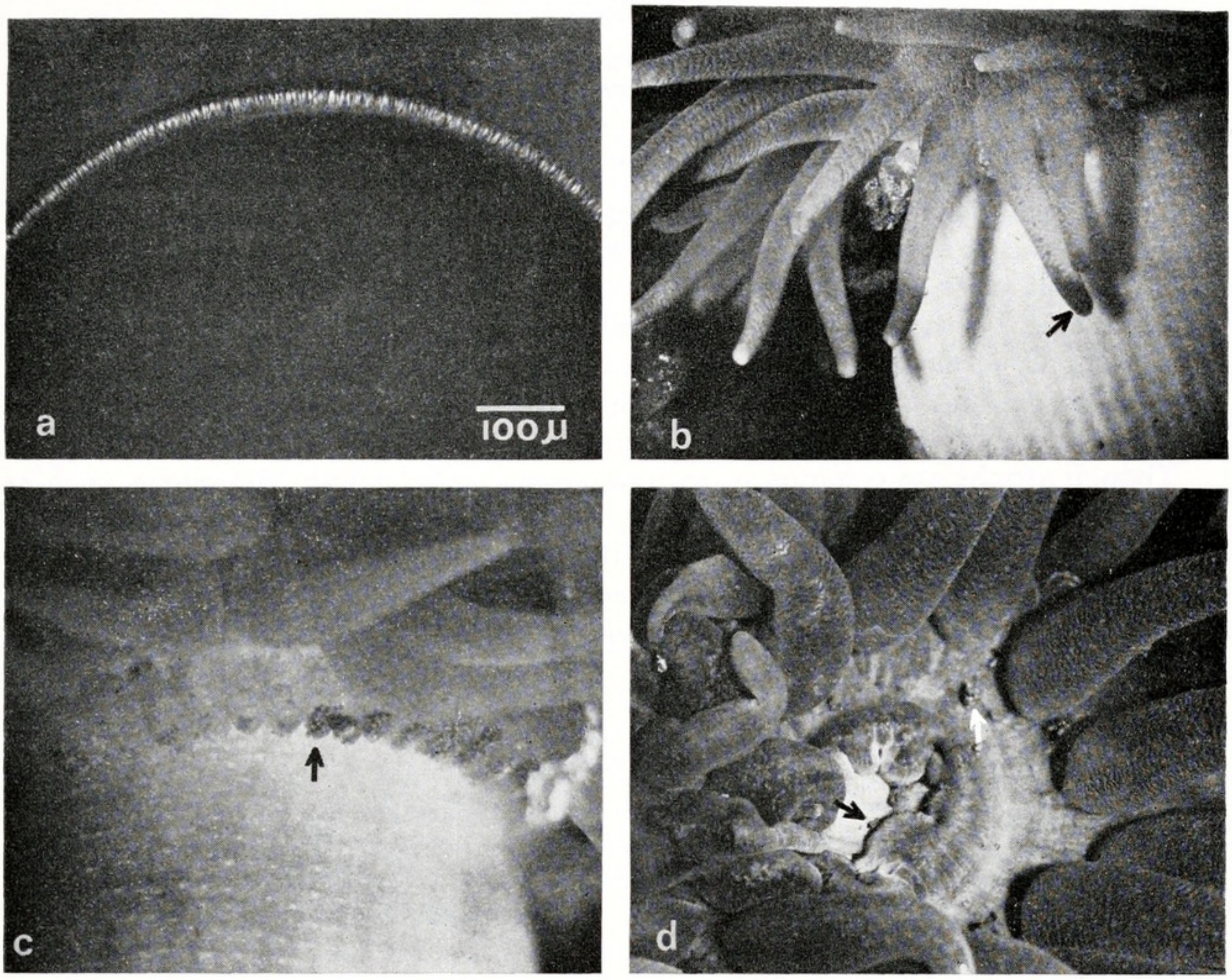

FIGURE 3. a) Recently spawned egg. Spiny surface is emphasized by phase contrast; b), an adult female with fertilized eggs in the tentacles and pseudospherules (arrows); c), a closer view of the eggs in pseudospherules (arrow); d), the same female releasing planulae (arrows).

size and shape of the verrucae. In *C. crassa* these are quite large and distinct while in *C. fernaldi* the verrucae are small and inconspicuous, if not absent. The ectoderm and mesoglea of the column, disc, and base is much thicker and better developed in *C. crassa* than in *C. fernaldi*. In addition, the cnidom of each species is distinctive.

Available data concerning the cnidom of *C. crassa* is not complete, though there is enough information available so that a meaningful comparison of the cnidoms of *C. crassa* and *C. fernaldi* is possible. The data of Schmidt (1972) are summarized below.

Distribution and size of nematocysts of *C. crassa* (all measurements in microns).

Tentacles

Spirocysts	21–39 × 2.5–4.0
Basitrichs	21–32 × 2.0–3.0

Pharynx

Basitrichs	26–38 × 2.5–4.5

Filaments

Basitrichs	30–46 × 3.0–5.5
Basitrichs	13–20 × 1.5–2.5
Microbasic *p*-mastigophores	21–28 × 3.5–5.5

The distribution of nematocysts appears to be somewhat different in the two species and the sizes of the nematocysts are also very dissimilar. This is particularly noticable when comparing the large basitrichs of the tentacles and pharynx of both species. The largest basitrich recorded from the tentacles of *C. crassa* is 39 μm and for *C. fernaldi* 55 μm, while the largest basitrich from the pharynx of *C. crassa* is 38 μm and for *C. fernaldi* 59 μm. A further comparison of the cnidoms of these species will reveal additional differences.

Though atrichs are common in various genera in the family Actiniidae, such as *Anthopleura* and *Actinia,* they were previously unrecorded from the genus *Cribrinopsis.* As these were located only on the tentacles and are rather rare, it is not surprising that they have been overlooked until now. No great significance can be attached to their presence in *C. fernaldi* until further studies are made of the cnidoms of the other species in the genus.

Development

Spawning

C. fernaldi is dioecious. The male was observed releasing sperm from its mouth late in the morning of March 30. These sperm were being taken into the gastrovascular cavities of the females in currents of water. Eggs could be seen free of the gonads inside the gastrovascular cavities of all three females. These eggs were primarily in the tentacles and pseudospherules (Figs. 3b, c).

The eggs are 700–750 μm in diameter and cream to pale pink in color. Observations of the living eggs using phase contrast microscopy revealed that the oölemma is formed into conical spines about 15 μm in length (Fig. 3a). Histological examination of sectioned material indicated that each spine visible in a living egg was composed of a group of cytospines (Dewel & Clark 1974).

Development

Comparison of developing individuals removed from the females at various times and cultured prior to fixation and those fixed immediately upon removal showed no difference in morphology or rate of development. The following description applies to both the brooded and cultured organisms. A summary of the development and experimental observations is given in Table I.

The eggs in the gastrovascular cavity of the two females in the tank with the male began to show cleavage by late afternoon. Those in the isolated female did not develop, but remained in the gastrovascular cavity. Cleavage is preceded by a few rounds of nuclear division. Initial cleavage is superficial. The cells do not become separated from the yolky interior of the blastula until the second day of development. At this time the outer cell membranes of the blastula are still covered with 15 μm spines.

TABLE I

Development, settling and metamorphosis.

Day	Event	Cultures 1	2	3	4	5	6
1	Fertilization and early cleavage	start*					
5	Gastrulation and cilia formation		start*				
10	Planulae begin swimming			start*			
15	Septa forming, planulae able to change shape, adults begin releasing planulae						
20	*Phyllochaetopterus* added		yes	yes			
25			+	+			
30			+	+	start[x]		
35			++	++		start[x] (dense)	
40	8 tentacles on settled anemones able to ingest *Artemia nauplii*		yes	yes			start[o]
45	*Phyllochaetopterus* added	yes				+	yes
50	12 tentacles on settled anemones	++	++	++		+	++
75		+++	+++	+++		++	+++
125		+++	+++	+++	++	+++	+++

* Removed from the gastrovascular cavity of a female.
[x] Put in culture after being released by a female.
[o] Subcultured from number 5.
Settling and metamorphosis: + = a few; ++ = many; +++ = most.

Gastrulation begins on day 3. The endodermal cells arise by multipolar ingression. Some cells may move into the endoderm by epiboly as in *Tealia crassicornis* (Chia and Spaulding, 1972), although the yolk plug structure reported for *Tealia* is not as prominent. As in the case of *T. crassicornis* the endoderm is difficult to define in histological preparations because of the presence of the yolk mass. At this time cilia begin to appear between the bases of the spines. At first they are sparse, but become more numerous within a few days. As the cilia develop the spines begin to shorten. By day 7 the spines are completely gone and the cilia completely cover the embryo. The beating of the cilia is uncoordinated at this time.

Gastrulation is complete by day 10 and the ciliary beating becomes coordinated. These planula larvae begin to move in the gastrovascular cavity of the female. Their movements become progressively more vigorous. They become more generally dispersed throughout the gastrovascular cavity, but the greatest concentrations are still in the tentacles and pseudospherules.

By day 15 longitudinal depressions are visible on the surface of the planulae, indicating the formation of the septa. The planulae begin to change shape. At this time they average about 1000 μm long by 600 μm in diameter. They may, however, contract into spheres or assume numerous other shapes.

On day 15 the female holding the nonfertilized eggs released most of them. On day 18 the two females brooding planulae began releasing them. The planulae were released from the mouth in small clumps of mucus which soon dispersed and the planulae swam away (Fig. 3d). By day 30 these females were releasing large numbers of planulae. Most of the planulae were released by 75 days.

Larval Settlement

Initially all culture jars contained a few stones; the sides of the jars gradually became covered with algae during the course of the observations. On day 20, tubes containing the polychaete *Phyllochaetopterus* sp. were added to culture jars 2 and 3 (Table I). The planulae began to settle and undergo metamorphosis in these two jars by day 25. In the other culture jars the planulae did not begin to settle until day 35 and then in fewer numbers than in jars 2 and 3. On day 45 *Phyllochaetopterus* tubes were added to culture jar 1 in which no planulae had settled. Soon after this the planulae began to settle in this jar in large numbers. The best settlement of planulae in jars without the *Phyllochaetopterus* tubes occurred in jar 5 in which the density of the planulae was approximately double that of the other jars.

Between days 35 and 40, the planulae that settled formed the eight primary tentacles and were able to capture and ingest *Artemia* nauplii. By day 50 these young anemones had developed 12 tentacles.

Discussion

The eggs of *C. fernaldi* are similar to those described for *Tealia crassicornis* (Chia and Spaulding, 1972) and other anemones (Spaulding, 1974; Siebert, 1973; Dewel & Clark, 1974). The sperm were not observed in any detail.

We do not have any information concerning the time and site of fertilization. The eggs may be fertilized prior to their release from the gonads as is the case in *Actinia equina* (Chia and Rostron, 1970) or they may be fertilized in the gastro-vascular cavity after release from the gonads.

The embryonic development follows a course very similar to that of *Tealia crassicornis* and which has been described in greater detail (Chia and Spaulding, 1972).

Chia and Spaulding (1972) reported experiments with the effect of various substrata on the settlement of *T. crassicornis* planulae. Of the various materials tried, the tubes of *Phyllochaetopterus* were most effective. As in the case of *T. crassicornis,* a specialized substrate was not necessary for the planulae to settle, but appeared to hasten the process. High densities of planulae also appear to promote settlement in *C. fernaldi.*

Of special interest in the development of *C. fernaldi* is the brooding behavior. The eggs of this anemone are large and yolky. The developing larvae in culture were not fed until after settlement and metamorphosis. Sections of early planulae show a solid core of yolk filling the gastro-vascular cavity. Sections of planulae 20 days later, when they are capable of settlement, show much less yolky material remaining. Embryos and larvae removed from the adults at various times and put in culture developed at the same rate as those which were allowed to remain in the females. The larvae are lecithotrophic and do not appear to derive any nutritional benefits from brooding.

The brooding behavior may be protective in function, greatly reducing the loss of larvae by predation. The active part of the brooding is played by the females which retain the eggs and larvae. This is a more simple situation than that of *Actinia equina* in which the early embryos, perhaps at the morula or blastula stage, leave the female, spend a period of time in the plankton and then re-enter adults to complete their development. Larvae of *A. equina* do not seem to develop without this brooding (Chia and Rostron, 1970). Appellof (1900) reported that *T. crassicornis* brooded its larvae while Chia and Spaulding (1972) showed that what is presumed to be the same species, living in a different area, does not brood its young.

We classify the type of brooding behavior exhibited by *C. fernaldi* and possibly by *T. crassicornis* as facultative brooding. In these anemones it is conceivable that certain environmental conditions may promote brooding behavior, or it may be a matter of chance that the females retain the fertilized eggs. Another possibility is that we are in fact dealing with two species in the case of *T. crassicornis*. The answer to this question awaits further study.

We would speculate that the facultative brooding behavior exhibited by *C. fernaldi* and possibly *T. crassicornis* represents an intermediate step in the process of evolution that led to the more complex brooding behavior exhibited by *A. equina*.

Hand (1966) suggested that Actiniarians evolved from the Madreporaria by the loss of skeleton forming ability. He further suggests that the Actiniarian family Actiniidae is most closely related to the corals, citing similarities in the cnidoms and the sphincter muscles and the heavy symbiotic algal associations characteristic of these anemones. Development and larval behavior are also similar with internally brooding planulae being found in both groups (Hyman, 1940). We would like to see more work done on the early development and larval behavior of other members of the Actiniidae and the corals. Information thus obtained may help in the evaluation of the hypothesis that the Actiniidae is the anemone family most closely related to the corals. In addition, considerably more information is needed on the cnidom characteristics and taxonomy of the Anthozoa, particularly members of species in which different types of development have been reported.

Summary

Cribrinopsis fernaldi is described as a new species of sea anemone from the San Juan Archipelago, Washington. This species is distinguished from the other members of the genus by the presence of approximately 96 tentacles and 96 pairs of mesenteries arranged hexamerously. The cnidom is distinctive and consists of spirocysts, basitrichs, microbasic *p*-mastigophores, and atrichs.

During March, freshly collected specimens of *C. fernaldi* spawned in the laboratory. The female anemones retained their eggs in the gastrovascular cavity and fertilization occurred internally. The embryos developed in the cavities of the tentacles and pseudospherules.

Some embryos were removed from the tentacles of the adult on day 1 (early cleavage), and day 7, day 13 (planula), and day 34. These continued their development and metamorphosed and settled at the same time as the larvae which remained in the adult until natural release. It is concluded that the brooding behavior is protective rather than nutritive in function.

LITERATURE CITED

Appelof, A., 1900. Studienuber Actinien-Entwicklung. *Bergens Museums Aabog,* **1**: 3–99.

Carlgren, O., 1921. Actiniaria. Part I. *Danish Ingolf Expedition,* **5**: 1–241.

Carlgren, O., 1940a. A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. *Lunds Universitets Arrsk., Ny Fold Avdel., 2,* **36**: 1–62.

Carlgren, O., 1940b. Actiniaria from Alaska and Arctic waters. *J. Washington Acad. Sci.,* **30**: 21–27.

Carlgren, O., 1949. A survey of the Ptychodactaria, Corallimorphia, and Actiniaria. *Kgl. Svenska Vetens. Handl., Fjarden Serien,* **1**: 1–121.

Chia, F. S., and M. A. Rostron, 1970. Some aspects of the reproductive biology of *Actinia equina* (Cnidaria: Anthozoa). *J. Mar. Biol. Ass. U. K.,* **50**: 253–264.

Chia, F. S., and J. G. Spaulding, 1972. Development and juvenile growth of the sea anemone, *Tealia crassicornis. Biol. Bull.,* **142**: 206–218.

Dewel, W. C., and W. H. Clark, Jr., 1974. A fine structural investigation of surface specializations and the cortical reaction in eggs of the cnidarian *Bunodosoma cavernata. J. Cell. Biol.,* **60**: 78–91.

Gemmill, J. F., 1920. The development of the sea anemones, *Metridium dianthus* (Ellis) and *Adamsia palliata* (Bohad). *Phil. Trans. Roy. Soc. London, Series B.,* **209**: 352–375.

Hand, C., 1954. The sea anemones of Central California. Part I. The corallimorpharian and anthenarian anemones. *Wasmann J. Biol.,* **12**: 345–375.

Hand, C., 1955. The sea anemones of Central California. Part II. The endomyarian and mesomyarian anemones. *Wasmann J. Biol.,* **13**: 37–99.

Hand, C., 1966. On the evolution of the Actiniaria. Pages 135–146 in W. J. Rees, Ed., *The Cnidaria and their evolution.* Academic Press, New York.

Hyman, L. H., 1940. *The invertebrates. Volume 1. Protozoa through Ctenophora.* McGraw-Hill Book Co., New York, 661 pp.

Parulikar, A., 1966. Cnidae in the actinians of Maharashtra. *J. Biol. Sci.,* **9**: 36–42.

Schmidt, H., 1972. Prodromus zu einer Monographie der mediterranen Aktinien. *Zoologica,* **121**: 1–146.

Siebert, A. E., Jr., 1973. A description of the sea anemone *Stomphia didemon* sp. nov. and its development. *Pacific Sci.,* **27**: 363–376.

Spaulding, J. G., 1974. Embryonic and larval development in sea anemones (Anthozoa: Actiniaria). *Amer. Zool.,* **14**: 511–520.

Weill, R., 1934. Contribution de l'étude des Cnidaires et de leurs Nématocytes, I, II. *Trav. Stat. Zool. Wimereux,* **10–11**. Paris. 692 pp.