

A Description of the Sea Anemone *Stomphia didemon* sp. nov. and Its Development¹

ARTHUR E. SIEBERT, JR.²

ABSTRACT: *Stomphia didemon* is described as a new species of sea anemone from the San Juan Archipelago, Washington. It possesses about 192 tentacles and 96 pairs of mesenteries. The cnidom is spirocysts, basitrichs, microbasic b-mastigophores, and microbasic p-mastigophores. *S. didemon* spawned in April and May. The eggs are 750 to 800 μm in diameter, orange, and centrolecithal; cleavage is superficial, equal, and incomplete. Endoderm formation is by unipolar ingression from a fold in the surface of the blastula. Settlement of the planulae is favored by the presence of sand or gravel. Attempts were made to fertilize eggs of *S. coccinea* with sperm from *S. didemon*, but the cross-fertilized eggs failed to develop.

MANY ASPECTS of the biology of the Actiniaria of the waters of the San Juan Archipelago have been incompletely studied. Recently, though, more attention has been given this group. Chia and Spaulding (1972) observed the development of *Tealia crassicornis*; Spaulding (1971), the development and brooding behavior of *Cribrinopsis williamsi*; and Spaulding (in press), the life history of *Peachia quinquecapitata*. Ross and Sutton (1964a, b; 1967a, b) have investigated the response of *Stomphia coccinea* to stimulation by *Dermasterias*, *Aeolidia papillosa*, and electrical stimuli.

There is some question as to the species of anemone utilized in the experiments of Ross and Sutton. Although one anemone was *S. coccinea*, the possibility exists that some of the anemones studied were not of this species. Indeed, they often stated (Ross and Sutton, 1967a, b) that they used *Actinostola*, as yet undescribed, in their work. While studying the early development of *Stomphia*, it became evident to me that there are two distinct species of anemones in the San Juan region which had variously been termed *Stomphia* and *Actinostola*. Both are members of the genus *Stomphia*, and *Stomphia didemon* is here described as a new species.

Although the development of *Stomphia* is somewhat similar to that of other anemones, there are differences. A discussion of the embryology and early development is presented in this work.

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MATERIALS AND METHODS

Stomphia coccinea and *Stomphia didemon* were collected in the San Juan Island area and kept in seawater tanks in the Friday Harbor Laboratories during the spring and summer of 1972. Spawning occurred in April and May at various times of the day and gametes of both sexes were collected on several occasions as they were released. When a female spawned but a male did not, sperm were obtained by dissecting a small piece of testis from a male and washing it in seawater. The sperm were shed and then used to inseminate the eggs which had been collected previously. This technique was generally successful, as most males were ripe at the time the females spawned. Eggs from spawning females were placed in culture dishes, washed in seawater, and inseminated. A few minutes

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² University of Washington, Friday Harbor Laboratories, Friday Harbor, Washington 98250. Present address: Department of Zoology, University of California at Berkeley, Berkeley, California 94720.

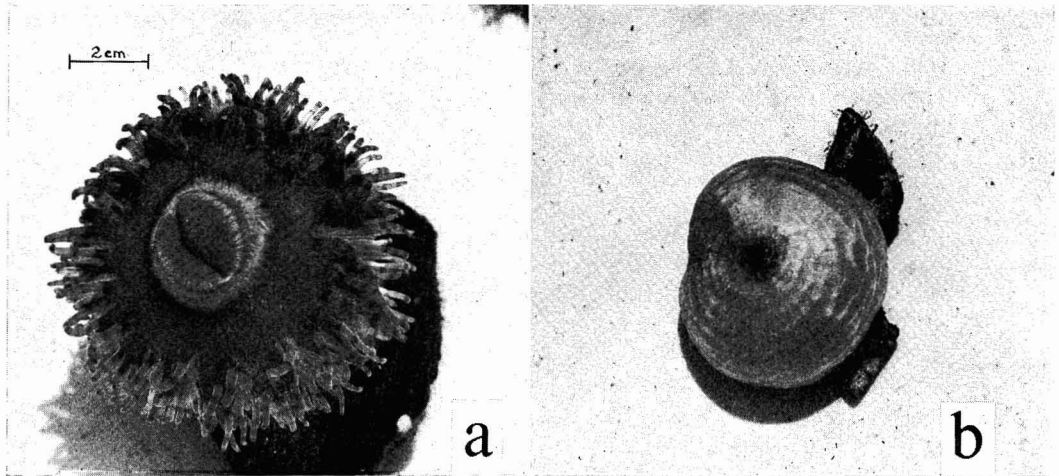


FIG. 1. *a*, *Stomphia didemon* sp. nov., live specimen showing oral disc, lips raised due to recent feeding; *b*, *S. didemon* fully contracted with the strong sphincter completely covering the retracted tentacles.

later, excess sperm was washed from the culture and discarded; the water was subsequently changed daily. Development continued in these dishes for 3 months at temperatures ranging from 8° to 12° C. After settling behavior had begun, various substrates were introduced into the dishes. Once settling had occurred, the dishes were submerged in a seawater table and the water was allowed to flow freely over them. The young anemones were never fed but they were allowed to capture small crustaceans from the water in the sea table.

Histological preparations of the embryos were made at various stages of development. Embryos were fixed in Bouin's fluid, embedded in paraffin, and sectioned at 8 μ m. The sections were then stained with Harris' haematoxylin and counterstained with eosin.

Careful examination of the morphology of many specimens of both species was undertaken. Live animals were examined for size, color, shape, and cnidom determination. Preserved individuals, relaxed in magnesium chloride, were dissected and sectioned. Serial sections were cut at 20 μ m and stained with basic fuchsin and picroindigo carmine. More than 25 animals of each species were examined.

The cnidom was determined by studying tissue squashes taken from various places in the anemones. All measurements are of nematocysts from fresh squashes and were taken

according to the method of Hand (1954) in which only the largest and smallest nematocysts seen of each type are recorded. This establishes a range for unexploded nematocysts. Fifty or more of each type from various regions of the body were measured and used to determine the range.

The descriptions of the two species are patterned after those of Stephenson (1935) and Hand (1954; 1955*a*, *b*). The terminology is that of Stephenson (1928, 1935) or Carlgren (1949). The terminology and definitions of the nematocysts follow the system of Weill (1934) as modified by Carlgren (1940).

Stomphia didemon Siebert sp. nov.

Tribe Theneria Carlgren, 1899

Subtribe Mesomyaria Stephenson, 1921

Family Actinostolidae Carlgren, 1932

Base

Base is round, not strongly adherent, slightly wider than the column. Well-developed basilar muscles are present.

Column

Column is cylindrical, and is not divided into regions; it is almost as wide as the base and disc, though sometimes it is distinctly smaller.

Column is never as wide as long, and its diameter is uniform throughout its length. Ectoderm is thin, but generally not transparent; always smooth. Mesoglea is thicker than the endoderm or ectoderm. When contracted, the column is a dome and appears wrinkled. Insertions of mesenteries are not visible. Color is usually orange with irregular darker orange patches. Sphincter is mesogleal, diffuse, wide, and strong; it extends from immediately below the margin to some distance down the column.

Tentacles

Tentacles are up to 2.0 cm long, conical, and never branched. They are fully retractile and fully covered when retracted. The definitive number is 192, arranged in six cycles (6 + 10 + 16 + 32 + 64 + 64). Individuals with fewer tentacles are often found. Inner tentacles are slightly longer than the outer ones and all bend toward the margin; seldom are any held upright. They are white with orange bands. The longitudinal muscles are ectomesogleal.

Disc

The disc is circular, with the central area free of tentacles. Insertions of mesenteries may or may not be visible. Surface is fairly smooth with shallow folds caused by the mesenterial insertions. Color varies from orange to almost colorless.

Actinopharynx

The actinopharynx extends one-half the length of the column, and is not strongly ribbed. Lips are the same color as disc, or colorless, are sometimes surrounded by a thin orange ring, and are seldom raised above the disc, though this may occur after feeding (Fig. 1a). Two well-developed siphonoglyphs are present.

Mesenteries and Internal Anatomy

Usually 16 to 18 pairs of perfect mesenteries are present in two cycles, with three cycles of imperfect ones. All but the first two cycles follow the *Actinostola*-rule (Carlgren 1949), so that the mesenteries of the younger cycles are

unequally developed in such a way that the mesentery which turns its longitudinal muscle toward the nearest mesentery of the preceding cycle is larger than its partner. In the third cycle, one member of a pair may be perfect and the other imperfect; all of the perfect mesenteries are infertile, whereas all but the youngest of the imperfect ones are fertile and all but the youngest bear filaments. Generally, more mesenteries are present at the base than at the margin, but the number varies. All but the last cycle extend from base to margin, so there is nearly always a 1:1 ratio of tentacles to mesenteries. The mesenteries are arranged in five cycles (6 + 10 or 12 + 16 + 32 + 32 mesentery pairs), for a total of 96 or 98 pairs of mesenteries. The retractors are mesogleal, well developed, and diffuse. The muscle processes are longest and most prominent toward the middle of the mesentery and gradually shorten closer to the column (Fig. 2a). Often the processes are almost the same length throughout the length of the mesentery. The parieto-basilar muscles are not exceedingly well developed and extend approximately to the level of the actinopharynx. The basilar muscles are well developed. *Stomphia didemon* is dioecious.

Cnidom

Spirocysts, basitrichs, microbasic b-mastigophores, and microbasic p-mastigophores are present.

Size and Distribution of Nematocysts

Fig. 2c

Tentacles

Spirocysts	15.0–55.0 μm \times 2.0–5.0 μm
Basitrichs	12.0–28.0 μm \times 1.5–4.0 μm
Microbasic b-mastigophores	31.0–49.0 μm \times 4.0–8.0 μm

Filaments

Basitrichs	11.0–25.0 μm \times 2.5–5.5 μm
Microbasic p-mastigophores	16.0–29.0 μm \times 3.0–7.0 μm

Actinopharynx

Basitrichs	11.0–30.0 μm \times 2.5–5.5 μm
Microbasic p-mastigophores	22.0–30.0 μm \times 3.5–6.0 μm

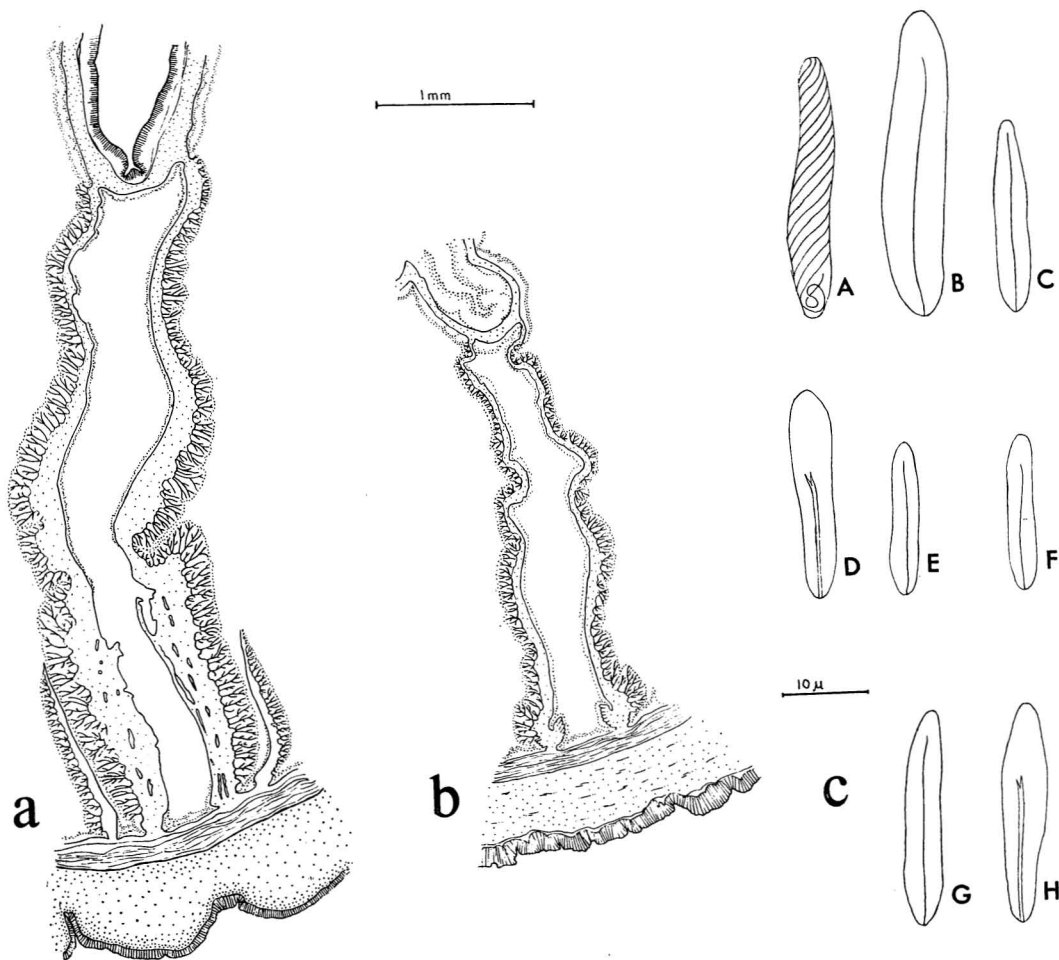


FIG. 2. *a*, Cross section of a small specimen of *Stomphia didemon* at the level of the actinopharynx showing a directive pair of mesenteries and siphonoglyph; *b*, cross section of *S. coccinea* at the level of the actinopharynx showing a directive pair of mesenteries and siphonoglyph; *c*, nematocysts of *S. didemon*: A–C, tentacles; D–E, actinopharynx; F, column; G–H, filaments. A, spirocyst; B, microbasic b-mastigophore; D and H, microbasic p-mastigophores; C, E, F, and G, basitrichs. Note: all nematocysts drawn to scale.

Column

Basitrichs 17.0–22.0 $\mu\text{m} \times 1.5\text{--}3.5 \mu\text{m}$

Size

A larger-than-average specimen of *S. didemon* will be up to 21 cm tall with the column about 5 cm in diameter. The disc in a specimen this size would be approximately 6.0 to 6.5 cm in diameter and the base about the same dimensions. When retracted, the column is less than 12.0 cm in height.

Habitat

This anemone will attach to any hard substrate, and specimens have been collected from rocks, bottles, and old shells. They have been dredged from depths of 30 to 60 fathoms.

Type Locality

Type locality is the San Juan Channel, east of San Juan Island, Washington.

Type Specimens

Though holotype and paratype specimens of the anemone *Stomphia didemon* described in this paper were designated and mailed to the United States National Museum, they were never received. Inquiry into the matter revealed that the United States Postal Service apparently lost the type specimens and was unable to locate them. Subsequently, two additional specimens were sent to the United States National Museum but were not designated as type specimens. These are USNM nos. 53856 and 53857 and were collected from the type locality for the species.

Remarks

This anemone has been much utilized in the past few years for the study of swimming and escape behavior in anthozoans. However, due to the uncertain taxonomic position of this species, it has variously been referred to as "large" *Stomphia* (Ross and Sutton 1964a, b), *Actinostola* n.sp. (Robson 1966, Ross and Sutton 1967a, b); and it probably has been included in other experiments which also involved *Stomphia coccinea* but in which a clear distinction was not made between the two species.

Physiological and behavioral differences gave the first indications that the two forms of *Stomphia* found in the San Juan Archipelago were different. Ross and Sutton (1964a) found that the large and small *Stomphia* respond to different intensities and frequencies of electrical shock which may cause the initiation of swimming behavior in both. Robson (1966) presented an extensive discussion of the differences in swimming behavior between *S. coccinea* and *S. didemon*, both of which she terms *Actinostola* n.sp. Also, *S. didemon* ("*Actinostola*") exhibits the swimming response when touched with *S. coccinea*, but the reverse is never true (Ross and Sutton 1967b). Ross and Sutton (1967a) showed that *S. coccinea* and *S. didemon* ("*Actinostola*") exhibit definite experimental preferences for the shells of *Modiolus modiolus*, though I have never collected any *Stomphia didemon* attached to these shells. *Stomphia coccinea* is inevitably collected attached to *Modiolus modiolus* shells but Dr. Ross (personal communication) has also indicated that, despite extensive col-

lecting, he has not found *Stomphia didemon* ("*Actinostola*") on them. This may indicate a possible mechanism for habitat selection in the natural populations of the two species, as they are usually found attached to different substrates.

Why *S. didemon* was previously assigned to the genus *Actinostola* is unknown. Carlgren (1949) has differentiated the two genera on the basis of several traits. First, the sphincter in *Actinostola* is weak and does not fully cover the tentacles when the anemone is completely retracted. In *Stomphia* it does do so. Second, *Actinostola* has a characteristic cup or goblet shape when contracted, due to the weak sphincter which allows the disc to flair when the column is more fully contracted. In *Stomphia*, a tightly contracted individual will form a low mound (Fig. 1b). Third, the several cycles of mesenteries extend from the base to disc in *Actinostola*, whereas the younger mesenteries do not extend from base to disc in *Stomphia*. In *S. didemon*, the youngest cycle generally does not extend from base to disc. Finally, the longitudinal muscles of the tentacles of *Stomphia* are ectomesogleal, whereas those of *Actinostola* are not of this form. All of the traits listed above which are characteristic of the genus *Stomphia* are also found in *S. didemon*. Thus, there can be little question that the anemone under discussion belongs in the genus *Stomphia*, not *Actinostola*.

My initial indication that the two forms were indeed different species came during attempts to obtain viable zygotes so that the early development of *S. coccinea* could be studied. Due to chance, the species were separated in holding tanks to spawn. Thus, one animal from each tank could be used in each fertilization and various combinations of gametes could be tried. These fertilizations were unsuccessful and will be discussed later in this paper. This result suggested that these two forms were different species and so I instituted a detailed morphological study of both.

The following description of *S. coccinea* is presented to distinguish clearly the two species. Also, *S. coccinea* has not previously been described from this region, though it has long been known from the Pacific coast of North America.

Stomphia coccinea (Mueller 1776)

For synonymy see Stephenson 1935.

Base

The base is slightly irregular, adherent, and much wider than the column. Well-developed basilar muscles are present.

Column

This is cylindrical and not divided into regions. It is never as wide as the base or disc. The ectoderm is generally thin and often transparent, always smooth. Mesoglea thicker than endoderm or ectoderm. In contraction, the column is a low, firm mound. Color ranges from solid orange to pale white. Many individuals are pale orange with irregular orange or red areas scattered on the column. Sphincter mesogleal, wide, and strong.

Tentacles

Tentacles may be up to 1.5 cm long, conical, and fully covered when retracted; there are usually about 72, but individuals with as many as 86 can be found. They are generally arranged in four or five cycles with those of the inner cycles being slightly longer than those of the outer ones. The six tentacles of the first cycle are usually held upright, whereas those of the outer two or three cycles bend down over the margin. The color of the tentacles is white or transparent with two orange rings encircling them and a small white spot at the base of each. The longitudinal muscles are ectomesogleal.

Disc

The disc is circular, with the large central area being free of tentacles. Insertions of the mesenteries are visible from the exterior. The surface of the disc is often irregular and not smooth, but with furrows formed by the mesenterial insertions. The disc is transparent with orange patches scattered on it.

Actinopharynx

This extends one-half to three-fourths the length of the column and is well ribbed. Lips are deep orange and raised well above the surface of the disc. Two well-developed siphonoglyphs are present.

Mesenteries and Internal Anatomy

Usually 16 to 18 pairs of perfect mesenteries, with two or three cycles of imperfect ones, are present. The imperfect mesenteries follow the *Actinostola*-rule. All imperfect mesenteries, with the exception of the youngest, are fertile and all but these bear filaments. Often one partner of a pair may be perfect while the other is not. If this is so, the imperfect one will be fertile while the perfect mesentery will be infertile. Generally twice as many mesenteries are at the base than are at the margin. The retractor muscles are mesogleal, well developed, and diffuse, with the processes longest near the column, so as to form an almost circumscribed mass at that position (Fig. 2*b*). *Stomphia coccinea* is dioecious.

Cnidom

The cnidom bears spirocysts, basitrichs, microbasic b-mastigophores, and microbasic p-mastigophores.

*Size and Distribution of Nematocysts**Tentacles*

Spirocysts	11.0–46.0 μm \times 1.5–4.0 μm
Basitrichs	12.0–25.0 μm \times 1.5–4.0 μm
Microbasic b-mastigophores	35.0–50.0 μm \times 5.0–7.0 μm

Filaments

Basitrichs	10.0–17.0 μm \times 1.5–3.5 μm
Microbasic p-mastigophores	16.0–27.0 μm \times 1.5–4.0 μm

Actinopharynx

Basitrichs	15.0–27.0 μm \times 1.5–4.0 μm
Microbasic p-mastigophores	20.0–30.0 μm \times 3.0–7.0 μm

Column

Basitrichs	11.0–17.0 μm \times 1.5–3.5 μm
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Size

S. coccinea is up to 3 or 4 cm tall with the column being usually less than 3 cm in diameter. The disc of a specimen this size would be about 3 cm wide and the base about 5 cm.

Habitat

S. coccinea is generally found attached to shells of *Modiolus modiolus* at depths of 25 fathoms or more.

Remarks

Stomphia coccinea is commonly found in the deeper waters of both Puget Sound and the San Juan Archipelago. Stephenson (1935) gave a more detailed account of the anatomy of *S. coccinea*; the present account agrees well both with it and with that of Carlgren (1921).

Stomphia didemon closely resembles *S. coccinea*. The color and external appearance of both species is very similar and a superficial observation might give the impression that the two were size morphs of the same species. However, upon a closer examination of the two species, several differences become apparent. *Stomphia didemon* possesses more tentacles than does *S. coccinea* (192 vs. 72) and more mesenteries. An examination of the mesenteries of the two species also reveals differences. The retractor muscles of *S. coccinea* are slightly restricted near the site of the mesentery to the column and the muscle processes are longest in this region. The retractors of *S. didemon* are not of this form, as the muscle processes are about the same length throughout the mesentery and are not restricted near the column. The nematocysts of both are very similar in size and distribution, though some minor differences can be noted.

In total, the physiological, behavioral, and morphological differences exhibited by these two anemones are more than sufficient to warrant their recognition as distinct species. The name *didemon* is derived from the Greek word meaning cowardly or fearful, and it describes the behavior of *S. didemon* when touched by the smaller *S. coccinea*.

DEVELOPMENT OF *Stomphia didemon*

Spawning

S. didemon spawned in the laboratory on three occasions during the spring of 1972. All were females, so sperm were obtained by removing a small piece of ripe gonad from a male and treating it as previously described. The animals spawned during both day and night, with no particular time favored over any other. The spawned eggs were collected and held until artificially fertilized. In addition, one group of embryos was obtained from a seawater tank in which simultaneous spawning of a male and female had occurred.

When expelling gametes, the animals assumed a "spawning posture" and became virtually insensible to external stimulation. In this posture, the tentacles were held erect and over the edge of the disc and the mouth was relaxed and open. Both the tentacles and column were extended. Eggs were emitted from the mouth in a thick mass of mucus which persisted for several minutes. The eggs within the mucus floated to the surface of the water and, even after the mucus had dissolved, the eggs continued to float and settled only as planulae. Throughout spawning, the animals retained their characteristic posture despite prodding or other mechanical stimuli which, under ordinary circumstances, would cause contraction of the disc and column.

On one occasion, the spawning of a male was observed. Sperm were released in the same manner as eggs, but no mucous sheath was present. The sperm formed a dense, milky cloud in the water of the holding tank, but females placed in their presence did not spawn in response to the sperm.

Gametes

The eggs were orange colored, spherical in shape, and measured about 750 to 800 μm in diameter. The surface was much folded and creased initially (Fig. 3a), but soon became smoother and rounder. When placed on a slide the eggs tended to flatten. They were centrolecithal; very yolky; and, if handled roughly, ruptured and released a stream of yolk from

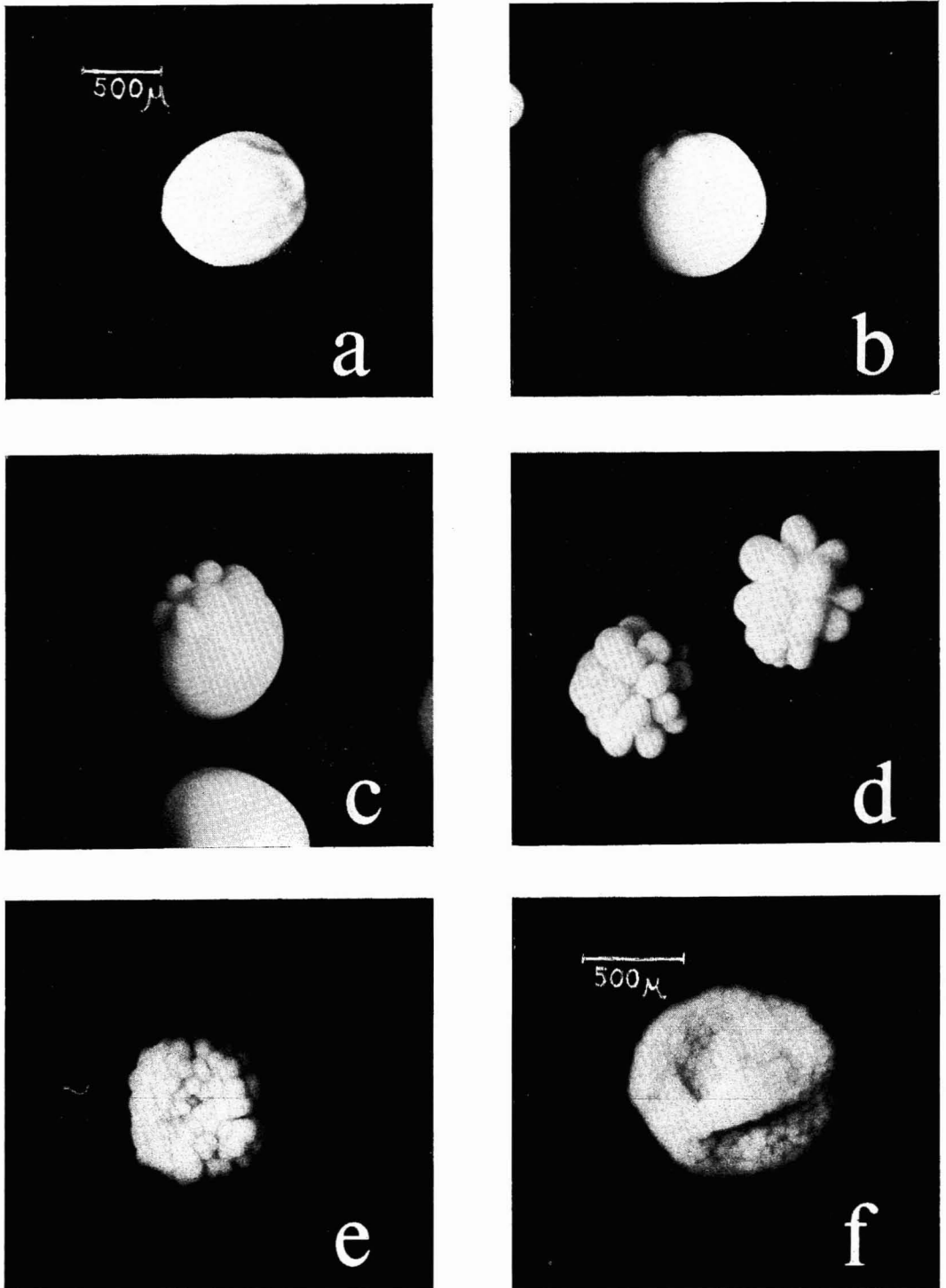


FIG. 3. Development of *Stomphia didemon*: *a* through *e* to scale on *a*. *a*, Unfertilized egg; *b*, first cleavage, blastomeres at top of embryo visible; *c*, third cleavage, seven blastomeres visible; *d*, 32-cell stage, cleavage is superficial and incomplete; *e*, early blastula, central mass of yolk remains uncleaved; *f*, blastula showing two folds in the surface.

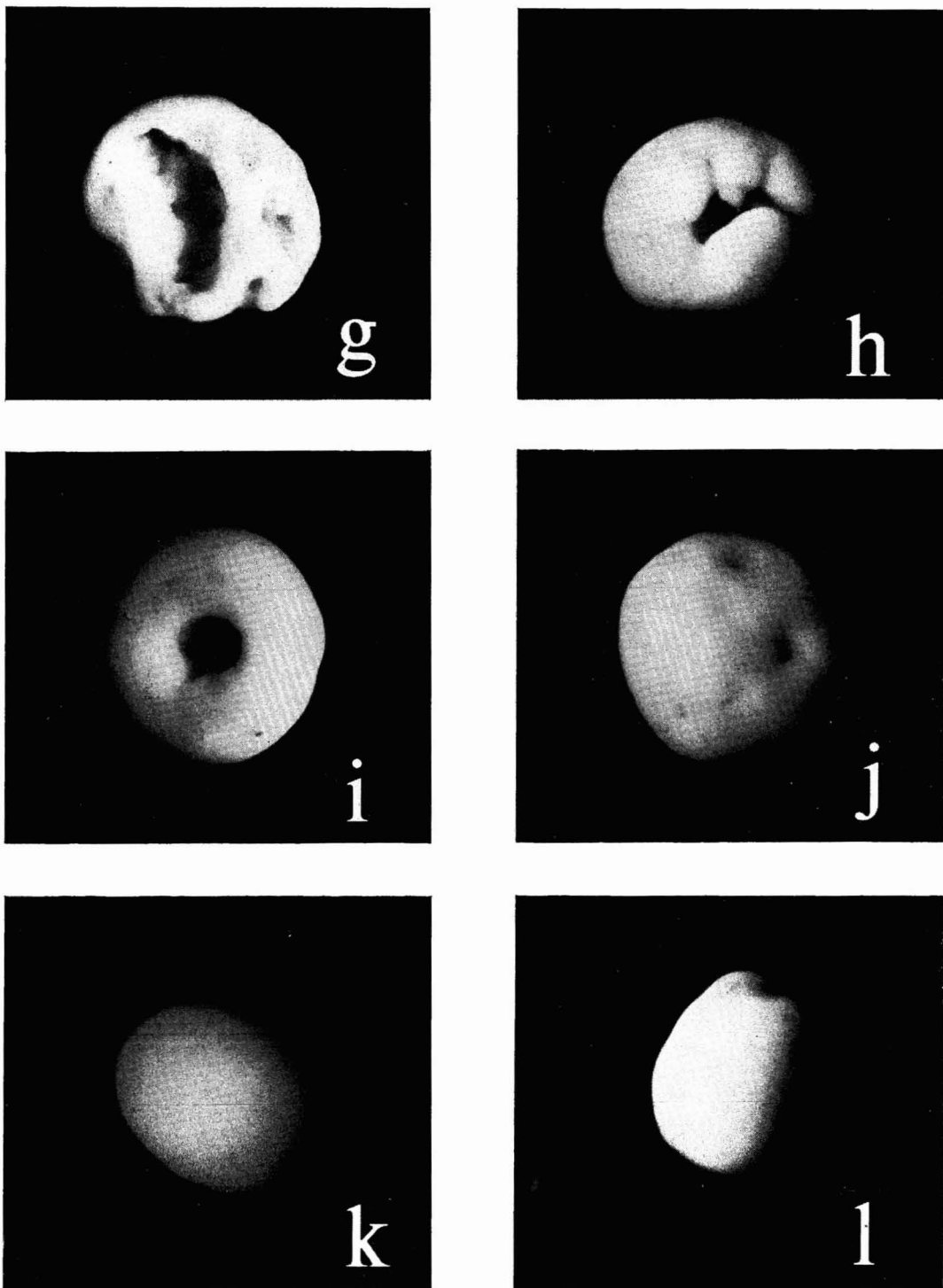


FIG. 4. Development of *Stomphia didemon*: *g* through *l* to scale on Fig. 3*f*. *g*, Blastula with deep wrinkle in the surface which will become the blastopore; *h*, formation of blastopore, fold has deepened and become more circular; *i*, blastopore fully formed, blastula flattened and abnormally wide from resting on slide; *j*, opposite surface of blastula in *i*, other folds in the surface are gradually disappearing; *k*, planula, anterior to left, tentacle buds just beginning and not readily discernible, mouth not yet present; *l*, settled planula, oral surface to upper right, tentacle buds present, mouth forming.

the interior. Numerous fine spines about 15 to 20 μm long covered the surface of the eggs. No polar bodies were observed at any time.

In cross section, the unfertilized egg appeared to have three distinct components. The outermost layer was thin and composed of dense material which may constitute a cortical region. Beneath this was a thicker layer of cytoplasm and yolk; and in the center of the egg was a mass of yolk platelets. The yolk granules were round and bright orange in the unstained egg.

The sperm were conical and mature when released. The midpiece was visible and the tail was quite long; the head was about 2 μm long and was pointed at the acrosome.

During insemination, many sperm were seen to be trapped in the spines on the surface of the eggs; but penetration failed to occur. Polyspermy normally was rare, but it did occur in large numbers in one instance. The zygotes produced underwent a short period of aberrant cleavage; then development was arrested.

Cleavage

After being fertilized, the zygotes floated at the surface of the water with the heavier animal pole downward. Cleavage began within 2 hours after fertilization; but this process often was difficult to observe, as the initial furrow started at the animal pole and was not visible to an observer viewing the zygotes from above. Cleavage was superficial, equal, and incomplete (Fig. 3*b*). The blastomeres appeared as mounds on the surface of the zygote (Fig. 3*c*). The cell membranes, as determined by subsequent histological examination, were indistinct or absent. The central mass of yolk remained uncleaved and gradually became surrounded by a single layer of cells. At the 32-cell stage, the embryo resembled a morula (Fig. 3*d*). However, it was not a true morula but a blastula, as the cell mass was not solid but only covered the yolk (Fig. 3*e*). No blastocoel was ever present. Not until late cleavage did the cell boundaries become fully apparent in histological preparations.

Endoderm Formation

As cell division continued, the size of the cells decreased while the number increased.

The overall size of the embryos changed very little from that of the unfertilized eggs, though the surface area increased. This caused a marked folding or wrinkling of the surface of the blastulae (Fig. 3*f*). Gemmill (1920) termed this a "wrinkled blastula" in *Adamsia palliata*; and the appearance in *S. didemon* is similar. These wrinkles might have been construed to be sites of ingression and endoderm formation, and this would be in agreement with the descriptions of the origin of the endoderm in *Halcampa duodecimcirrata* (Nyholm 1949) and *Tealia crassicornis* (Chia and Spaulding 1972). But in *S. didemon* this was not the case. Paraffin sections indicated that the endoderm arose by unipolar, not multipolar, ingression. As the blastulae developed, the numerous wrinkles smoothed out and disappeared, though one such wrinkle persisted and deepened. This eventually formed a blastopore and the endoderm proliferated from this region. Thus, the endodermal cells arose as a sheet and not as a scattered layer under the ectoderm as described by Chia and Spaulding for *Tealia*.

As the wrinkle that was to become the blastopore deepened, it also became more regular in outline. At first it was only a long irregular trench in the surface of the blastula (Fig. 4*a*). Then, the sides began to round off and to form an irregular oval (Fig. 4*b*) which soon became circular and began to deepen (Fig. 4*c*) while the other furrows gradually disappeared (Fig. 4*d*). At this time, the blastulae developed short cilia and were, in fact, early planulae. The time of the disappearance of the surface spines was not noted. Chia and Spaulding (1972) also described the formation of a structure similar to the yolk plug of amphibian gastrulae. No such "yolk plug" was formed during gastrulation of *S. didemon*. The blastopore remained open until formation of such a structure in the late planula. The embryos began to elongate slightly in the oral-aboral axis and attained the shape of the planula. The central mass of yolk present in the embryos remained intact even after the planulae had settled.

Planulae

Th planeulae were cylindrical, rounded at

TABLE 1

CHRONOLOGY OF LARVAL DEVELOPMENT

TIME	DEVELOPMENT EVENTS
2 hrs.	Fertilization; superficial cleavage to eight-cell stage
1 day	Superficial cleavage to 16-cell stage
2 days	"Morula"; wrinkling of surface begins; cells numerous
3 days	Blastula; blastopore forming; early gastrula; cilia evident
4 days	Early planula; motile with functional cilia
5-7 days	Planula; swimming at surface of water; mesenteries developing
8 days	Substrate selection begins; some settlement begins on gravel and coarse sand, but not on <i>Chlamys</i> shell
10 days	Base forming on settled planulae; attachment complete
12 days	First muscular contractions noted
13-14 days	Tentacle buds forming; additional septa forming
19 days	Young anemones; 12 tentacles
90 days	Young anemones about 1.0 mm across oral disc

the apical end, and with the blastopore located at the posterior; they were about 800 μm in length and swam at the surface of the water (Fig. 4e). They retained the orange color of the eggs. No apical organ or tuft was found in the serial sections of the larvae, and the ectoderm consisted of columnar epithelium with nematocysts scattered throughout the surface. These were mostly spirocysts, though other types were present. Although these unfortunately were not closely studied, they are believed to have been basitrichs. The endoderm formed a thin layer of cells lining the ectoderm and contained a large number of yolk granules. The primary cycle of mesenteries was forming; no contractile elements were observed until the planulae were ready to settle. The central mass of yolk was slowly utilized and absorbed, though a large amount remained, some of which formed the yolk plug just before the planulae settled; the blastopore developed into the mouth and the actinopharynx formed. There was no sign of tentacle buds until after settlement had occurred.

The larvae continued to swim at the surface until ready to settle. At that time, they proceeded to the bottom of the culture dishes where they swam with the apical end down. This indicated that some sort of apical sense organ is used in substrate selection during larval settling, though no such organ could be found in histological sections. This is not unusual, as the apical organ is difficult to fix and does not show well in paraffin sections. When the larvae were ready to attach to the substrate, they ceased swimming and adhered to the bottom with the apical end. For some time the attachments were rather weak, but eventually they became secure. This occurred when the larvae were about 8 days old. The chronological development is summarized in Table 1.

Effect of Substrate on Larval Settlement

Chia and Spaulding (1972) demonstrated for *Tealia crassicornis* that the settlement of the planulae is enhanced by the presence of the tubes of *Phylochaetopterus* sp. (a polychaete worm), a substrate found effective in promoting metamorphosis in *Mediaster aequilis* (Birkeland et al. 1971). Tubes of *Sabellaria cementarium* (another polychaete) also were used by Chia and Spaulding to induce settling in *Tealia*, a technique first described by Long (1964), who used the tubes to facilitate brachiopod metamorphosis. Planulae of *Tealia* did settle when not exposed to these substrates, but only after significant delay.

Planulae of *Stomphia didemon* were exposed to (1) coarse sand, (2) small gravel, (3) *Chlamys* sp. shell broken to the size of the gravel, and (4) seawater in plain glass dishes in which the planulae were reared. Substrate was added when settling behavior became evident and the planulae left the surface of the water. Those with substrates (1) and (2) settled about 8 days after fertilization, with those in dish (1) settling in larger numbers more firmly attached than were those in dish (2). The larvae in dish (3) settled about 12 days after fertilization; and those in dish (4), 14 to 16 days after fertilization.

It is interesting to note that the presence of those substrates used by Chia and Spaulding apparently was not necessary to induce settling

in *Stomphia didemon*. Even in those dishes containing substrates on which the planulae most readily settled (1 and 2), it was not necessarily the substrate on which actual settlement occurred. Often the planulae settled on the sides of the glass dish when the substrate was at the bottom. However, these attachments were usually short and not very firm. The planulae later became free-swimming and the process repeated until permanent adhesion was effected.

Once settled, the young anemones of all groups required about the same length of time to develop tentacles and septa. By the end of 3 months, almost all the anemones were about 1.0 mm in diameter across the oral disc and possessed 12 tentacles.

Cross-Fertilization Experiments

On three separate occasions, freshly spawned eggs of *S. coccinea* were collected, placed in culture dishes, and mixed with sperm from *S. didemon*. Eggs from *S. didemon* were obtained and utilized as controls; these were inseminated with sperm from the same group which had been used in attempts to fertilize the *S. coccinea* eggs.

Eggs from both *S. coccinea* and *S. didemon* were inseminated simultaneously with the sperm from *S. didemon*. The eggs of *S. coccinea* failed to show any signs of cleavage or development in three tests, whereas those of *S. didemon* proceeded through normal development. The cultures of *S. coccinea* were kept for 2 days until they began to disintegrate.

Cnidom of Juvenile Anemones

When the young *S. didemon* were approximately 2.5 months old and the diameter of the oral disc was slightly less than 1.0 mm, the nematocysts of five specimens were examined to determine whether or not they were the same as those of the adults. Squashes were made of the whole juvenile anemones and measurements of the largest and smallest examples of each type of nematocyst were recorded. The cnidom comprised spirocysts, basitrichs, microbasic b-mastigophores, and microbasic p-mastigophores. The sizes of the nematocysts were found to be as follows: spirocysts, 8.0–25.0 $\mu\text{m} \times 1.0$ –4.0 μm ; basitrichs, 9.0–16.0 μm

$\times 1.5$ –3.5 μm ; microbasic b-mastigophores, 16.0–32.0 $\mu\text{m} \times 3.0$ –6.0 μm ; microbasic p-mastigophores, 15.0–24.0 $\mu\text{m} \times 3.0$ –6.0 μm .

If these figures are compared with the measurements previously given for adults of *S. didemon*, differences become evident. In every class, the nematocysts are significantly smaller than in the adults, though their morphology is similar.

Although it was difficult to determine the distribution of the nematocysts in the juveniles, one interesting thing was noted. The microbasic b-mastigophores, which were rare in the adults and found only in batteries at the tips of the tentacles, were relatively abundant in the juveniles where they were scattered about the tentacles. Here they formed a large percentage of the nematocysts present, whereas in the adults they contributed only a small percentage.

Discussion

The eggs of *S. didemon* are similar to those of numerous other anemones. *Adamsia* (Gemmill 1920), *Tealia* (= *Utricina*) (Appellöf 1900), and *Bolocera* (Gemmill 1921) all have this type of yolky, centrolecithal egg. The spiny egg membrane, present only in actinarians, has been observed in *Peachia* (Faurot 1895), *Bolocera* (Gemmill 1921), *Actinia* (Gemmill 1921), and *Tealia* (Chia and Spaulding 1972). Although the function of this membrane remains unknown, there is the possibility that it might prevent polyspermy. This phenomenon is rare in anemones (Spaulding, personal communication) and I have seen it only once, even though I have used large numbers of sperm to fertilize eggs. In these cases, sperm became entangled in the spines and were unable to penetrate the eggs. Some eggs were so coated with sperm that the presence of numerous sperm tails gave them the appearance of being ciliated; yet they continued to develop normally. At the present time, this is only a hypothesis and remains to be proven, but initial observations suggest that the presence of the spines and absence of polyspermy in anemones might be related.

The folds and wrinkling of the surface of the early blastula stage in the development of *S. didemon* agree well with the description of a similar phenomenon in *Adamsia* (Gemmill

1920). These have also been reported in *Cribella* (Masterman 1898), *Solaster* (Gemmill 1912), *Luidia* (Mortensen 1913), and *Cucumaria* (Newth 1916); and, although Appellöf (1900) does not mention this, Gemmill (1920) claims to have seen these folds in *Tealia* (= *Utricina*). However, Chia and Spaulding (1972) do not mention them in their discussion of the development of *Tealia crassicornis*, the same species studied both by Appellöf (1900) and Gemmill (1920).

Gemmill (1920) also described the process of invagination of the blastopore and the smoothing of the surface of the blastula in *Adamsia*, the blastula in this species being essentially the same as that of *S. didemon*. Formation of the endoderm by unipolar invagination is common in actinarians (Megner 1971), though multipolar invagination has also been reported (Chia and Spaulding 1972).

Substrate selection and settlement in *S. didemon* stand in contrast to that behavior exhibited by *Tealia*. From behavior, it might be inferred that there is a definite substrate preference which may be related to an apical organ. The nature of this selection remains unknown, however, as no interaction of the type demonstrated for *Tealia* planulae and other invertebrate larvae with the tubes of various polychaetous annelids was observed for *S. didemon*. The presence of clean sand and gravel favored settlement only if the planulae already had begun actively to seek a favorable site for attachment. If they had not, then no actual induction of substrate selection or settlement occurred.

Lack of fertilization and cleavage in the cross-fertilization experiments suggests that the gametes of the two species are highly incompatible. This could indicate a long history of specific divergence; however, as no previous attempts at producing interspecific crosses of various anemones have been made, a more complete discussion of this is not possible at the present time.

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