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Reproductive Cycle and Developmental Processes During Embryogenesis of *Clavularia hamra* (Cnidaria, Octocorallia)

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Abstract

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Clavularia hamra Gohar, 1948 is a common octocoral on the reefs of the Gulf of Eilat (northern Red Sea). Reproductive biology of *C. hamra* was studied for two years. Direct observations of spawning were conducted *in situ* and in aquaria. Cleavage of eggs and further embryo metamorphosis into mature planulae were examined by scanning electron microscopy and histological sections.

Clavularia hamra is dioecious. Young oocytes appear annually in September, gradually grow in size and attain maturity within 11 months. The main spawning event of the population is highly synchronized, occurring on a single night each year between the last quarter and the new moon in mid summer.

The released orange eggs are held together by mucus and remain attached amongst the polyps on the outside of the female colonies. Twelve hours after spawning, the young embryos have blastomeres of equal size with numerous microvilli on their external surface. Due to unequal cleavage, bizarre embryos are also formed. By 48 h a blastopore is visible, indicating that a gastrula is developing. Eight days after spawning mature planulae are observed.

The external mode of embryo development on the surface of the parent colony reduces dispersal of the planulae. However, this reproductive feature enhances formation of locally dense populations of *C. hamra*, with distinct habitat preferences.

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Introduction

Broadcasting of gametes is the most common mode of reproduction among hermatypic corals (Fadlallah 1983; Babcock *et al.* 1986; Chornesky & Peters 1987). This type of spawning facilitates the study of their early developmental stages (Babcock & Heyward, 1986). In contrast, there are only a few reports on spawning of alcyonacean soft corals (Yamazato *et al.* 1981; Babcock *et al.* 1986; Benayahu & Loya 1986). Other studies on soft corals have demonstrated external brooding of eggs (Benayahu & Loya 1983; Dinesen 1985; Farrant 1985, 1986). Our knowledge on morphogenesis of alcyonacean embryos developing from eggs to planulae is concerned only with very few species (Mergner 1971; Benayahu & Loya 1983; Uehara *et al.* 1987).

Clavularia hamra Gohar, 1948 (Fig. 1) is an abundant species at the shallow coral reefs of the northern Red Sea and reproduces sexually by spawning (Gohar 1948). This paper presents results of a two-year study on aspects of sexual reproduction and embryogenesis of *C. hamra* and deals with its annual gonadal development, timing and mode of reproduction. Morphological changes associated with the cleaved embryos and their subsequent development into mature planulae were examined by scanning electron microscopy and light microscopy.

Materials and Methods

The study was conducted in the Coral Nature Reserve at Eilat, Red Sea. In this area, colonies of *C. hamra* are found in shallow crevices on the reef flat and on hard substrates in the back reef lagoon (0.5–1.0 m depth). A remarkably dense population is also found on the back reef, where the highest living coverage occurred on poorly illuminated surfaces, especially on overhanging substrates.

The encrusting and patchy growth form of *C. hamra* (see Gohar 1948) made it difficult to distinguish between individual colonies. Therefore, the sampling procedure followed a random colony collection from a large reef area (800–1000m^2), rather than repeated sampling of tagged corals. Samples of 10 large colonies were taken at random monthly from August 1985 to August 1987. Specimens were routinely fixed in 4% sea water formalin for 24–48 h, rinsed in fresh water and transferred into 70% alcohol. The preserved material was examined under a binocular dissecting stereoscope for gonadal development and sex determination. Wet preparations of gonads from 10 polyps of each colony were studied under a microscope. The diameter of the oocytes and spermaries was measured with a calibrated ocular. In addition, gonad structure was examined in histological sections stained with haemotoxilin (for methodology, see Benayahu & Loya 1983).

During observations in August 1985 orange spawned eggs of *C. hamra* remained on the outer surface of the colonies. In the summer of 1986 and 1987, freshly collected fragments were examined for large orange coloured oocytes in the polyp cavities. A few weeks prior to the spawning date, predicted on the basis of the 1985 observations, colonies were carefully removed from the sea and transferred to running sea water aquaria for spawning observations. In addition, during this period dives were conducted at dusk and early in the morning to look for gamete release.

After spawning, fertilized eggs were reared in aerated aquaria. Embryogenesis of these planulae was compared to *in situ* material collected successively until mature larvae were obtained. Early cleavage

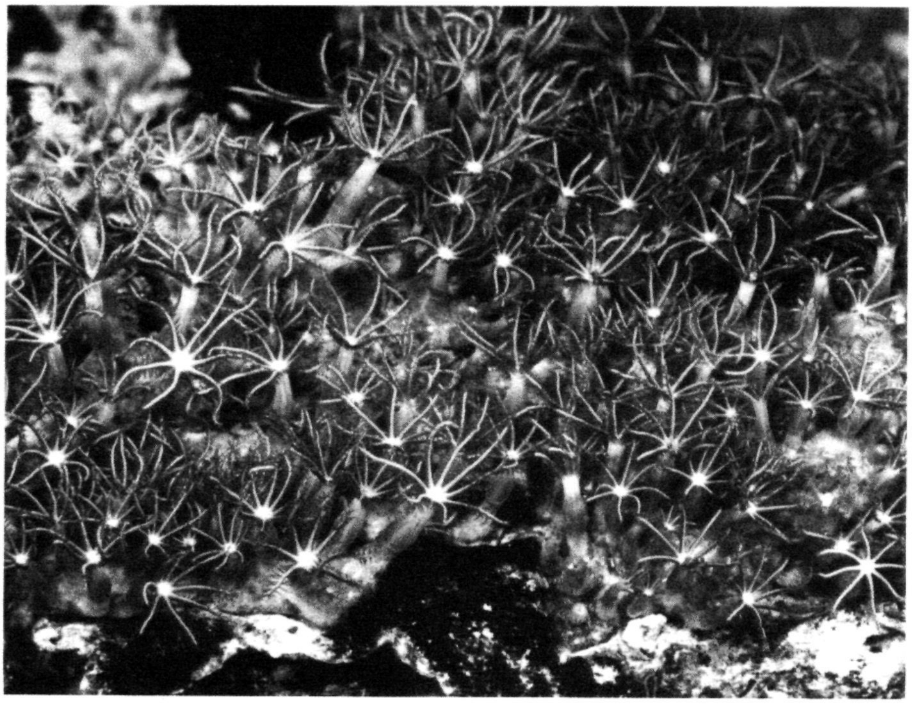


Fig. 1. A colony of *Clavularia hamra.*

embryos and later developmental stages were prepared for regular histology (see above) and scanning electron microscopy (SEM) fixed in 2.5% glutaraldehyde. Samples were dehydrated through a graded series of ethyl alcohols and then critically point dried from liquid CO_2. The preparations were coated with gold and examined with a Jeol-S35 SEM, operated at 25 kV.

Results

Gonads and their annual development

Clavularia hamra is a dioecious octocoral. Male and female gonads develop on the six ventral and lateral mesenteries of the polyps. Noticeably, no gonads were recorded in polyps found in coenenchyme thinner than 1.5–2 mm. Young oocytes were observed in September. During the following 11 months they grew in size and attained maturity in August, with a maximal diameter of 650–750 μm (Fig. 2). Prior to maturation, the number of ripe oocytes per polyp ranged from 14 to 26. However, by May some additional, smaller oocytes were also found among the prominent oocytes. These oocytes attained a size of 180–220 μm and disappeared, most probably through reabsorption by the larger ones, and are therefore excluded from Fig. 2.

A nucleus resides at the periphery of the developed oocyte (Fig. 3). Each oocyte is surrounded by a follicular layer consisting of a columnar epithelium (Figs 3, 4). During oogenesis the oocytes are an opaque-white colour, whereas a few weeks prior to maturation they become orange. The sperm sacs (Fig. 5) commenced development between October and December and their build-up took 8–10 months until August (Fig. 2). In mid summer mature white spermaries of a wide size range (250–550 μm) fill the male polyp cavities (Fig. 6).

Spawning

Maturation of male and female gonads is highly synchronized (Fig. 2). The main spawning event was observed directly in midsummer of 1985, 1986 and 1987, both in the field and in aquaria. Spawning occurred in the sea and in the laboratory during the same single night each year between 21.00–24.00 h, a few days prior to the new moon. A sporadic gamete release by 5–10% of the population preceded the main spawning event by 2-3 weeks. During spawning the ripe spermaries released sperm through the polyp mouth to the ambient water (Figs 7, 8). After spawning, the orange eggs were held together by mucus and remained attached amongst the polyps on the outside of the female colonies (Fig. 9). Neither embryos nor sperm were ever found in female polyps. Thus fertilization is most probably external and occurs shortly after spawning.

Embryogenesis

Embryogenesis of *C. hamra* takes place externally, on the outside of female colonies (Fig. 10). Spawned eggs (Fig. 11) start to cleave within 5–8 h after spawning. Cleavage of many eggs is holoblastic, equal and synchronous, resulting in embryo formation with a regular blastomere arrangement (Fig. 12). However, 35–40% of the embryos exhibit bizarre shapes (Fig. 13), due to a pronounced, unequal and asynchronous cleavage. Microvilli are uniformly scattered on the surface of the blastomeres (Fig. 14).

Cell divisions are rather rapid for 5–16 h after spawning. A solid blastula is developed, with an incipient endoderm forming by delamination (Fig. 15). As delamination proceeds (Fig. 16) a round morula is formed 24–36 h after fertilization (Fig. 17). At this stage, all embryos, both regular and bizarre-shaped have blastomeres of an equal size. By the second day of development, the embryos are smooth and a blastopore is apparent (Fig. 18), indicating a gastrula stage. The cells in the blastopore area are large and demonstrate a certain irregularity. During gastrulation the embryos change their shape and dimensions.

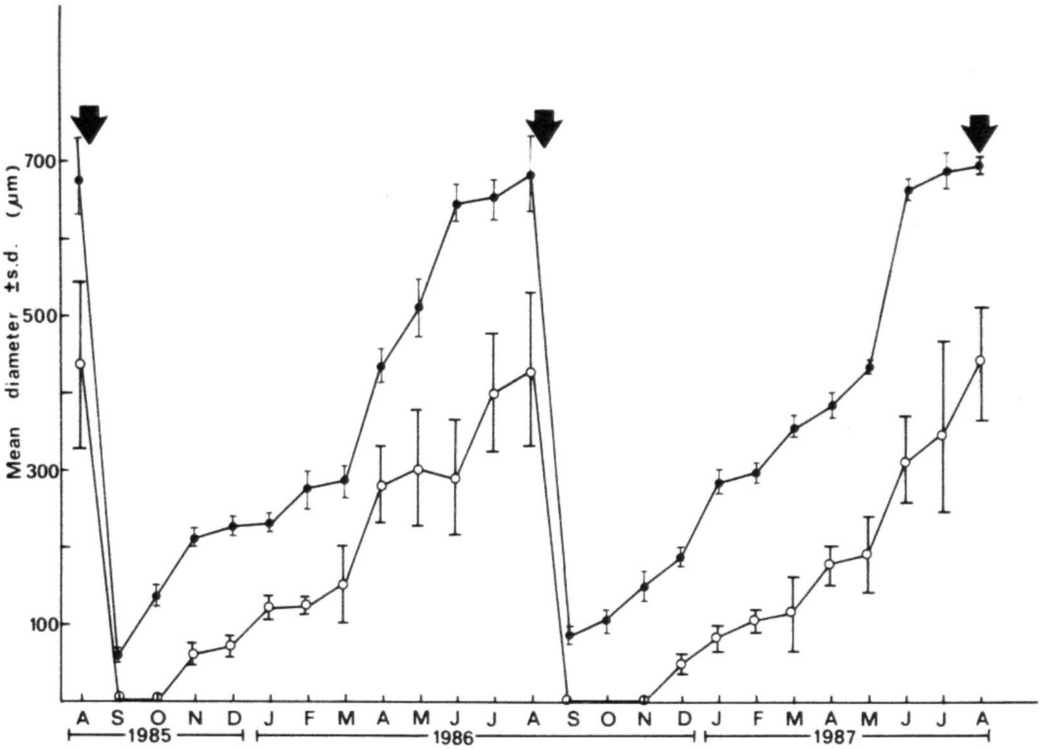


Fig. 2. Monthly changes in mean oocyte and sperm sac diameter of *Clavularia hamra*. Solid circles represent oocytes, open circles represent sperm sacs, arrows indicate timing of annual main spawning event.

Between 48 and 72 h after spawning, the gastrula elongates to form an early planula stage (Fig. 19) measuring *c.* 600 μm in length and 400 μm in width at its anterior pole. The mouth opening is not visible at this stage. The surface of the young planula is densely covered by microvilli and with sporadically scattered short cilia. Numerous clusters of microvilli are arranged on the cells in a collar structure and occasionally a cilium emerges from its center (Fig. 20).

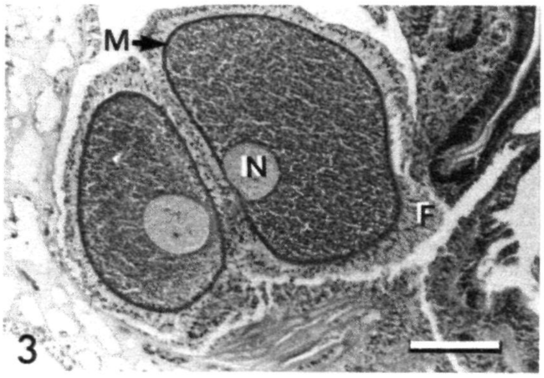
Some four days after spawning the planula has an ectodermal layer, a thin mesogloea and yolk-laden material fills its interior (Fig. 21). During the next 2–3 days the planulae grow in size. Numerous planulae are found on the surface of the parent colonies. However, clusters of larvae are gradually detached and sink down to the bottom, still coated with mucus. Most of the planulae tend to change shape by body contractions from pear-like to rounded, and vice versa (Fig. 22). By eight days mature planulae are obtained, mostly scattered on the surrounding substrates and exhibiting slow movements by crawling. Their bodies are covered by microvilli and numerous long cilia, some of which project from a collar structure (Fig. 23). Mature planulae have a remarkable orange colour. The planula larvae are elongated and when fully extended their maximal length reaches 1.8–3.0 mm (Fig. 24).

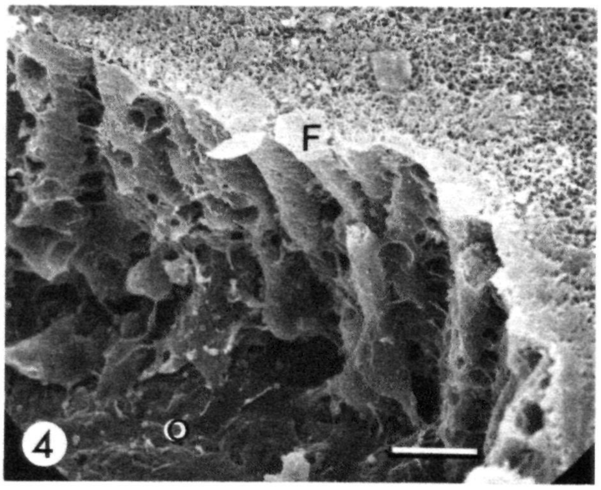
Discussion

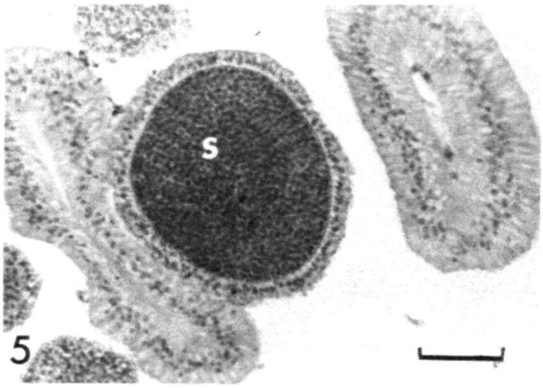
Clavularia hamra is dioecious with an annual pattern of gonadal development. The structure and development of gonads are similar to that of other studied soft corals (Gohar 1948; Farrant 1986). *C. hamra* has large oocytes (600–750 μm) resembling those found in other alcyonaceans (Benayahu & Loya 1986). Development and maturation of such oocytes demand substantial space within the polyp cavities, which are size limited, particularly in encrusting colonies. Consequently, gonads of *C. hamra* are developed only in fleshy and thick colony crusts which provide enough space for oocytes. Young polyps might be sexually immature, while the sterile thin stolons of the studied species are probably adapted for rapid growth and, hence, do not produce eggs.

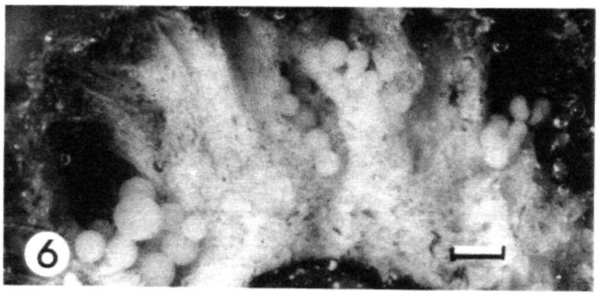
The spermatogenic cycle in *C. hamra* is shorter than the oogenic cycle (Fig. 2), as has been recorded in other soft corals (Yamazato *et al.* 1981; Benayahu & Loya 1983, 1984, 1986). Nevertheless, maturation of eggs and sperm is highly synchronized. Synchronous and brief annual spawning in correlation with lunar phase is widespread among numerous hermatypic corals (Babcock *et al.* 1986). Such reproductive features have been recently described for several alcyonaceans, e.g. *Sarcophyton glaucum* in the Red Sea (Benayahu & Loya 1986), *Alcyonium aspiculatum, Lobophytum compactum, L. microlobatum, L. hirsutum, Sarcophyton* sp., *Sinularia* c.f. *deformis* and *S. polydactyla* on the Great Barrier Reef (Babcock *et al.* 1986).

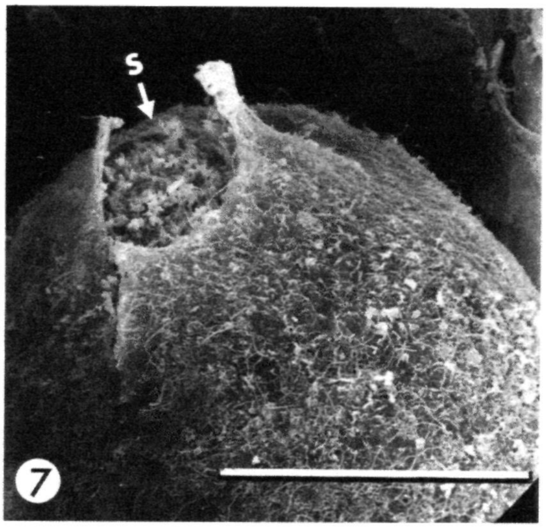
Gohar (1948) observed a case of *C. hamra* spawning white eggs in an aquarium late in May at Ghardaqa, northern Red Sea. However, Gohar did not mention retention of spawned eggs by the female colonies. The present paper demonstrates summer spawning (Fig. 2) of orange eggs in nature and in aquaria. Strikingly, the eggs remained on the surface of the colonies for further embryogenesis (Figs 9, 10). Discrepancies between Gohar's (1948) results and the current findings might well be due to inadequate aquarium conditions in his study that induced early release of immature white eggs. This assumption is substantiated by release of immature genital products by some soft corals subjected to stress (personal observations).

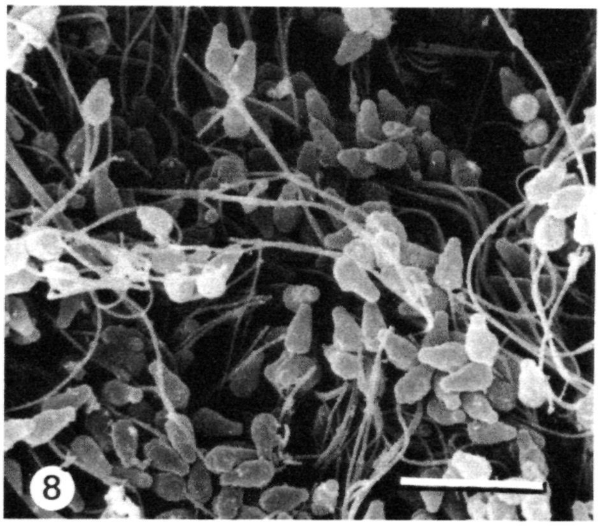










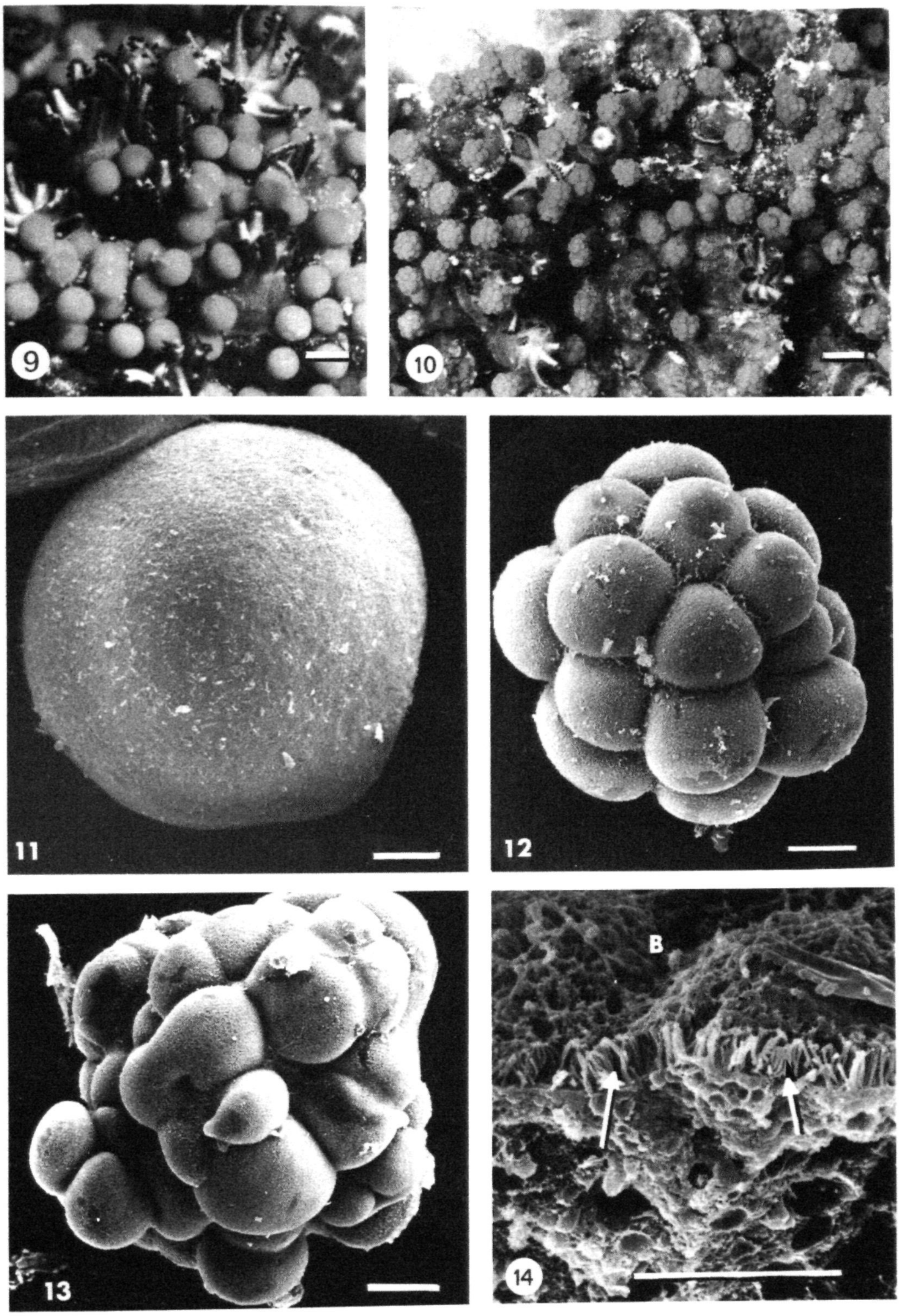


Figs 3–8.—Fig. 3. Photomicrograph of mature oocytes with follicular layer (F), thin mesogloea (*M*) and nuclei (*N*). Bar = 100 µm.—*Fig. 4.* Scanning electron micrograph (SEM) of portion of an oocyte surface (*O*) covered with a follicular cell layer (*F*). Bar = 10 µm.—*Fig. 5.* Photomicrograph of a spermary prior to maturation, with darkly stained sperm (*S*). Bar = 100 µm.—*Fig. 6.* A section through a living male colony prior to spawning with spermaries in polyp cavities. Bar = 500 µm.—*Fig. 7.* SEM of a ripe spermary at spawning. Note the ciliated surface of the sperm sac and the sperm mass (*S*). Bar = 100 µm.—*Fig. 8.* SEM of a cluster of ripe flagellated sperm. Bar = 10 µm.

The external planulae development employed by *C. hamra* resembles the surface brooding exhibited by the alcyonaceans *Parerythropodium fulvum fulvum* (Benayahu & Loya 1983), *Eufflatounaria* sp. (Dinesen 1985) and *Capnella gaboensis* (Farrant 1985, 1986). In contrast to these species, most embryos of *C. hamra* are detached from the colonies and complete their embryogenesis on neighbouring substrates. Other soft corals have also been reported to retain their eggs on external colony surfaces. *Cornularia sagamiensis* and *C. komaii* incubate their spawned eggs in space made by the closed tentacles (Suzuki 1971). Recently, Weinberg (1986) pointed out that released eggs of *Clavularia crassa* are attached under the tentacles on the outside of the polyps. All these findings suggest that alcyonaceans have adopted a variety of modes of external brooding rather than a uniform pattern.

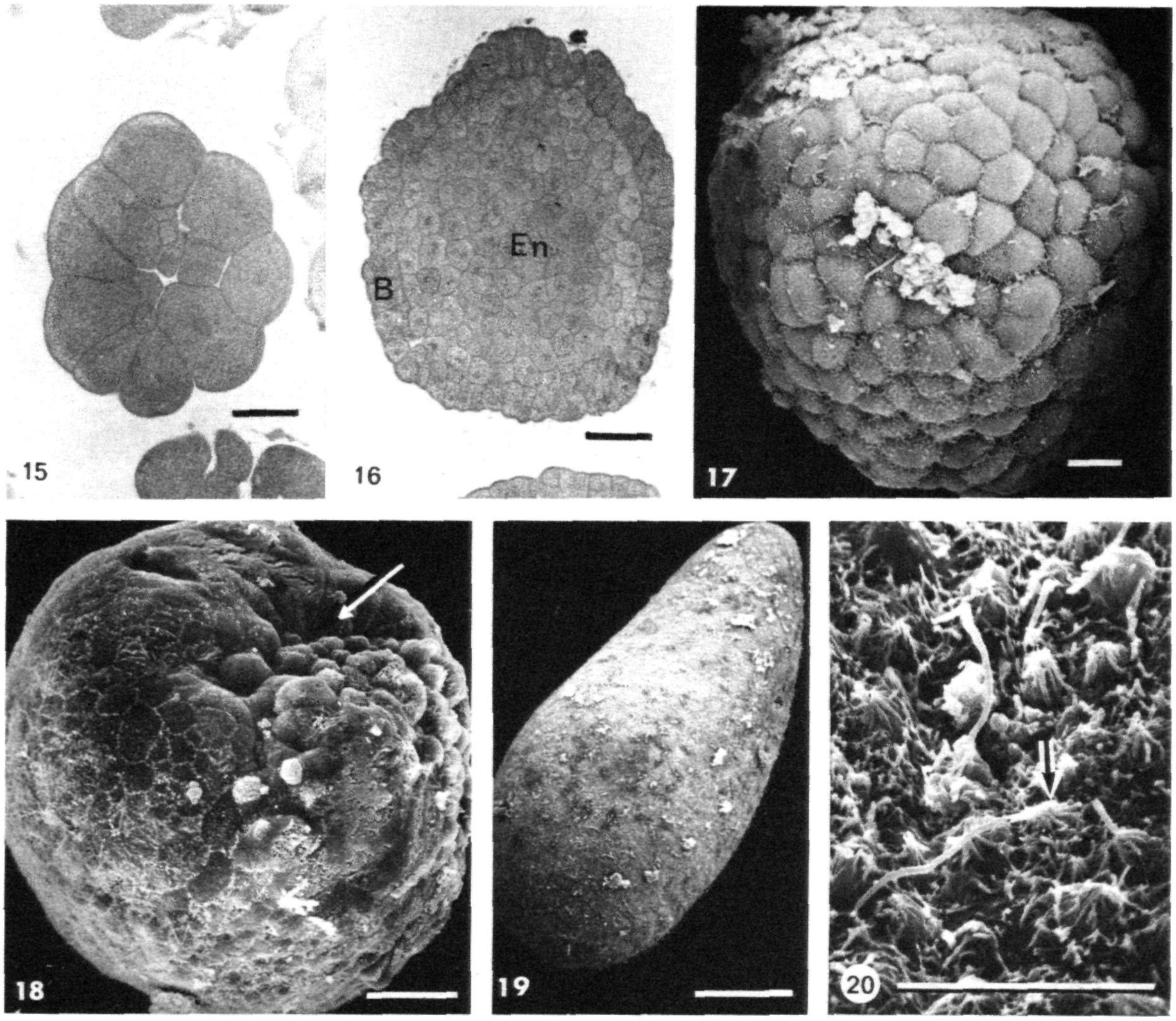
The morphological events associated with cnidarian planular formation have been largely ignored in the past (Martin & Archer 1986). Some old publications described embryogenesis of alcyonaceans (Kowalevsky & Marion 1883; Hickson 1901; Matthews 1917). Based on these data gained by light microscopy, attempts were made to draw generalities concerning morphological events and the development preceding their planulae formation (Mergner 1971; Campbell 1974).

Scanning electron microscopy has been used lately as a powerful tool for examining embryogenesis and mor-



Figs 9–14.—Fig. 9. Spawned eggs among polyps of a living colony of *Clavularia hamra*. Bar = 750 μm.—*Fig. 10.* Early cleaved embryos on a living colony. Bar = 750 μm.—*Fig. 11.* SEM of a freshly spawned egg. Bar = 100 μm.—*Fig. 12.* SEM of a cleaved embryo (6 h after spawning). Bar = 100 μm.—*Fig. 13.* SEM of an irregular and unequal cleavage pattern of embryo (6 h after spawning). Bar = 100 μm.—*Fig. 14.* SEM of a fractured blastula (6 h after spawning). The surface of the blastomeres (*B*) is covered with microvilli (*arrows*). Bar = 10 μm.

phogenetic processes in hydrozoan planulae (Hotchkiss *et al.* 1984; Weis *et al.* 1985; Martin & Archer 1986). This technique has been applied in studying some aspects of embryogenesis of the alcyonacean *P. f. fulvum* (Benayahu & Loya 1983). In the current study scanning electron microscopy enables us to demonstrate for the first time that early cleavage of an alcyonacean results in formation of both regular blastulae along with bizarre-shaped embryos within the same species (Figs 12, 13). The subsequent development of all blastulae produced symmetrical and round gastrulae (Fig. 17). Among alcyonaceans a mouth opening in a young planulae stage has been previously observed in *P. f. fulvum* (Benayahu & Loya 1983). During development of *C. hamra* an indentation representing the blastopore has been observed (Fig. 18). Yet, further studies are required to examine the function of this



Figs 15–20.—Fig. 15. Photomicrograph of a sectioned early blastula (6 h after spawning) with initiation of endoderm formation by delamination. Bar = 100 μm.—*Fig. 16.* Photomicrograph of a sectioned morula (24–30 h after spawning). The peripheral blastomeres (*B*) encompass the inner delaminated endoderm (*En*). Bar = 100 μm.—*Fig. 17.* SEM of a morula (30 h after spawning) with equal sized blastomeres. Bar = 100 μm. —*Fig. 18.* SEM of an early gastrula (36 h old). *Arrow* indicates the blastore. Bar = 100 μm.—*Fig. 19.* SEM of a 3 day old young planula. Bar = 100 μm.—*Fig. 20.* SEM of a surface of 3 day old planula. Microvilli are common, some of them arranged in a collar structure, associated with a cilium (*arrow*). Bar = 10 μm.

indentation (see Thomas *et al.* 1987). Later morphological events of larval development of *C. hamra* closely resemble those of other octocorals (Chia & Crawford 1973; Benayahu & Loya 1983).

During development of *C. hamra* surface specializations appear on the embryo cells. Microvilli occur at an early cleavage stage (Fig. 14), while cilia are not found on the young embryos. Only at a young planulae stage do a few cilia appear (Fig. 20). As development continues, ectodermal cilia grow in length and their density is increased (Fig. 23). Numerous cilia of the planula have a collar of microvilli (Fig. 23) and probably have a sensory or feeding function (Farrant 1986). Ultrastructural evidence, gained by transmission electron microscopy, indicates a high degree of specialization in surface features of the mature planulae of *C. hamra* (in preparation).

Planular maturation of *C. hamra* occurs eight days after spawning. Previous studies indicate that broadcasting octocorals have short embryogenesis (1.5–3 days), while brooding involves longer periods (Benayahu & Loya 1986). The prolonged larval development of *C. hamra* supports the assertion of extended embryogenesis among brooding octocoral species (Benayahu & Loya 1986).

C. hamra exhibits a synchronous and brief annual spawning cued by moon phase. The external mode of embryogenesis on the surface of the parent colony protects the developed planulae, but reduces their dispersal. These reproductive features enhance formation of locally dense populations of *C. hamra* with distinct habitat preferences on Eilat reefs. Further studies on sexual reproduction and embryogenesis of alcyonaceans are required to draw generalities on their morphogenesis and their relation to other cnidarians.

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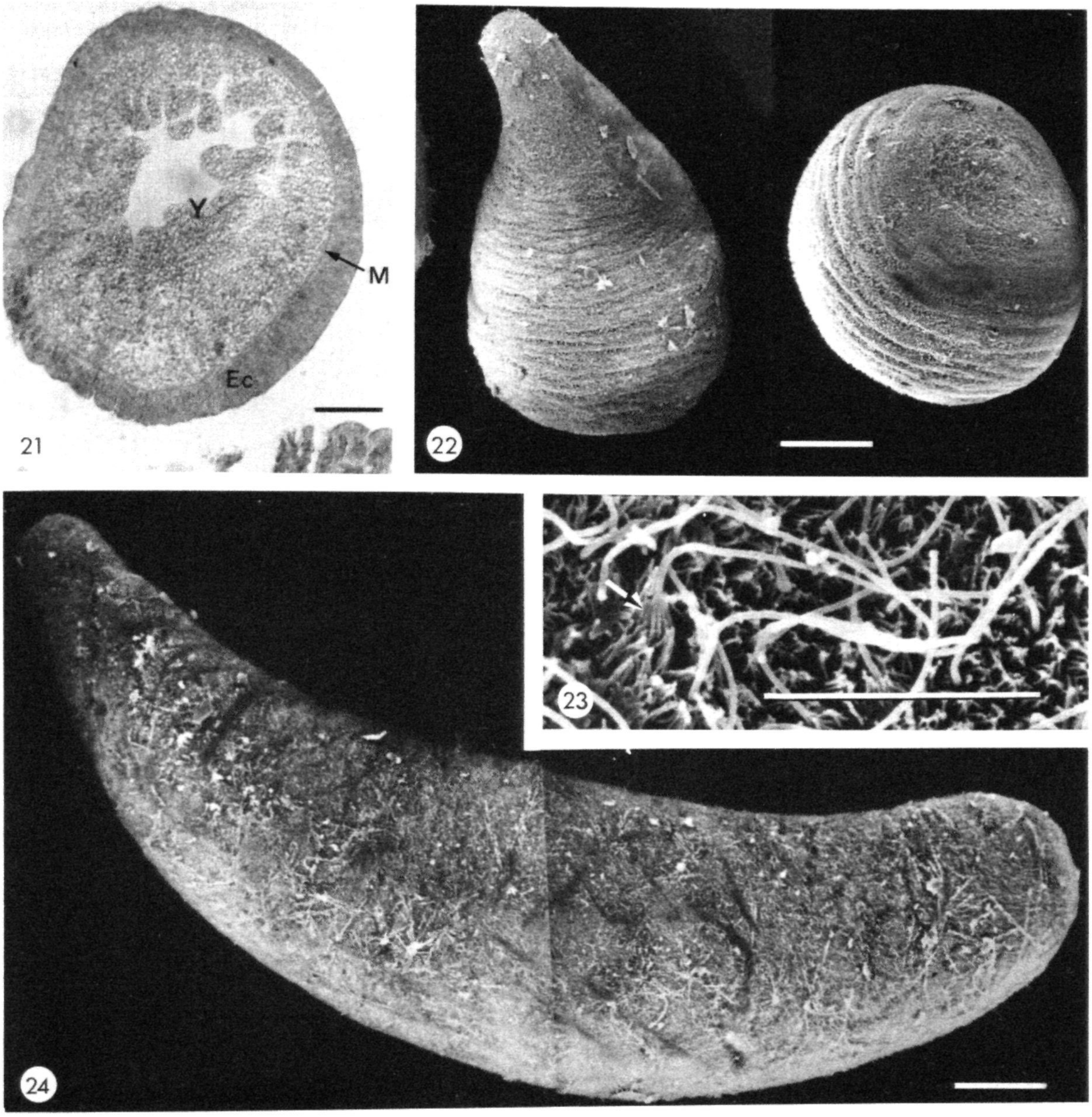
References

Babcock, R. C., Bull, G. D., Harrison, P. L., Heyward, A. J., Oliver, J. K., Wallace, C. C. & Willis, B. L. 1986. Synchronous spawnings of 106 scleractinian coral species on the Great Barrier Reef.—*Mar. Biol.* 90: 379–394.

Babcock, R. C. & Heyward, A. J. 1986. Larval development of certain gamete-spawning scleractinian corals.—*Coral Reefs* 5: 111–116.



Figs 21–24.—Fig. 21. Photomicrograph of a cross-sectioned 4 day old planula with ectoderm (*Ec*), thin mesogloea (*M*) and yolk laden material (*Y*). Bar = 100 μm.—*Fig. 22.* SEM of 6–7 day old planulae exhibiting body contractions. Bar = 100 μm.—*Fig. 23.* SEM of surface specialization of a mature planula (7 day old), covered by numerous microvilli and cilia. Some cilia emerge from a collar of microvilli (*arrow*). Bar = 10 μm. —*Fig. 24.* SEM of 8 day old mature planula. Bar = 100 μm.

Benayahu, Y. & Loya, Y. 1983. Surface brooding in the Red Sea soft coral *Parerythropodium fulvum fulvum* (Forskål, 1775).—*Biol. Bull. mar. biol. Lab., Woods Hole* 165: 353–369.

Benayahu, Y. & Loya, Y. 1984. Life history studies on the Red Sea soft coral *Xenia macrospiculata* Gohar, 1940. I. Annual dynamics of gonadal development.—*Biol. Bull. mar. biol. Lab., Woods Hole* 166: 32–43.

Benayahu, Y. & Loya, Y. 1986. Sexual reproduction of a soft coral: synchronous and brief annual spawning of *Sarcophyton glaucum* (Quoy & Gaimard, 1883).—*Biol. Bull. mar. biol. Lab., Woods Hole* 170: 32–42.

Campbell, D. D. 1974. Cridaria.—In A. C. Giese & J. S. Pearse (eds): *Reproduction of marine invertebrates*, Vol. I. *Acoelomate and pseudocoelomate metazoans*, pp. 133–199. Academic Press, New York.

Chia, F. S. & Crawford, B. J. 1973. Some observations on gametogenesis, larval development and substratum selection of the sea pen *Ptilosarcus guerneyi.—Mar. Biol.* 23: 73–82.

Chornesky, E. A. & Peters, E. C. 1987. Sexual reproduction and colony growth in the scleractinian coral *Porites asteroides.—Biol. Bull. mar. biol. Lab., Woods Hole* 172: 161–177.

Dinesen, Z. D. 1985. Aspects of life history of a stolon-bearing species of *Efflatounaria* (Octocorallia:Xeniidae).—In C. Gabrie, M. Harmelin Vivien, C. La Croix & J. L. Toffart (eds): *Proceedings of the 5th International Coral Reef Congress*, Vol. 6, pp. 89–94. Antenne Museum–EPHE, Moorea, French Polynesia.

Fadlallah, Y. H. 1983. Sexual reproduction, development and larval biology in scleractinian corals.—*Coral Reefs* 2: 129–150.

Farrant, P. A. 1985. Reproduction in the temperate Australian soft coral *Capnella gaboensis*.—In C. Gabrie, M. Harmelin Vivien, C. La Croix, J. L. Toffart (eds): *Proceedings of the 5th International Coral Reef Congress*, Vol. 4, pp. 319–324. Antenne Museum–EPHE, Moorea, French Polynesia.

Farrant, P. A. 1986. Gonadal development and the planulae of the temperate Australian soft coral *Capnella gaboensis.—Mar. Biol.* 92: 381–392.

Gohar, H. A. F. 1948. A description and some biological studies of a new alcyonacean species "*Clavularia hamra* Gohar".—*Publs mar. biol. Stn Ghardaqa* 6: 3–33.

Hotchkiss, A. E., Martin, V. J. & Apkarian, R. P. 1984. A scanning electron microscopic surface and cryofracture study of development in the planulae of the hydrozoan, *Pennaria tiarella.—Scanning Electron Microsc.* 2: 717–727.

Hickson, S. J. 1901. *Alcyonium.—L.M.B.C. Mem. typ. Br. mar. Pl. Anim.* 5: 92–112.

Kowalevsky, A. & Marion, A. F. 1883. Documents pour l'histoire embryogenique des Alcyonaires.—*Annls Mus. Hist. nat. Marseille* Zool.: 1–50.

Martin, V. J. & Archer, W. E. 1986. A scanning electron microscopic study of embryonic development of a marine hydrozoan.—*Biol. Bull. mar. biol. Lab., Woods Hole* 171: 116–125.

Matthews, A. 1917. The development of *Alcyonium digitatum* with some notes on the early colony formation.—*Q. J. microsc. Sci.* 62: 43–94.

Mergner, H. 1971. Cnidaria.—In G. Reverberi: (ed.): *Experimental embryology of marine and fresh-water invertebrates*, pp. 1–84. North-Holland Publ. Co., Amsterdam.

Suzuki, H. 1971. Notes on *Cornularia* (Stolonifera, Alcyonacea) found in the vicinity of Manazuru Marine Biological Laboratory.—*Sci. Rep. Yokohama natn. Univ.*, Sec. II, 18: 1–6.

Thomas, M. B., Edwards, N. C. & Morris, T. A. 1987. Gastrulation in *Halocordyle disticha* (Hydrozoa, Athecata).—*Inter. J. Invert. Reprod. Devl.* 12: 91–102.

Uehara, T., Sato, M. & Yamazato, K. 1987. General description o developmental stages in a soft coral *Lobophytum crassun* Marenzeller.—*Galaxea* 6: 185–193.

Weinberg, S. 1986. Mediterranean octocorallia: description of *Clavularia carpediem* n.sp. and synonymy of *Clavularia crassa* and *C ochracea* on etho-ecological grounds.—*Bijdr. Dierk.* 56: 232–246.

Weis, V. M., Keene, D. R. & Buss, L. W. 1985. Biology of hydractinii hydroids. 4. Ultrastructure of the planula of *Hydractini echinata*.-*Biol. Bull. mar. biol. Lab., Woods Hole* 168: 403–418.

Yamazato, K., Sato, M. & Yamashiro, H. 1981. Reproductive biolog of an alcyonacean coral *Lobophytum crassum* Marenzeller.—I E. D. Gomez, C. E. Birkeland, R. W. Buddemeir, R. E. Johannes J. A. Marsh Jr, R. T. Tsuda (eds): *Proceedings of the 4th International Coral Reef Symposium*, Vol. 2, pp. 671–678. Marine Science Center, University of the Philippines.