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Planulae brooding and acquisition of zooxanthellae in *Xenia macrospiculata* (Cnidaria: Octocorallia)

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ABSTRACT: Brooding in the octocoral *Xenia macrospiculata* is described. Young planulae of *X. macrospiculata* were found in brooding pouches located below the anthocodia among the polyps' cavities. These cavities are connected by and lined with ectoderm. Detached zooxanthellae were present within the brooding pouches, and are most probably acquired later by the planulae. The zooxanthellae enter into ectodermal ameboid cells by phagocytosis, and are then transferred to the endoderm.

INTRODUCTION

Xenia macrospiculata is a common xeniid species of the coral reefs of the Gulf of Elat (Benayahu & Loya, 1984a). The annual dynamics of gonadal development, planulation and post-larval development of this species have been described by these authors (1984a, b). The colonies are diecious and the gonads develop along four lateral and two sulcal mesenteries, except for the anthocodial part of the polyp cavity. Fertilization probably occurs inside the polyp; fertilized eggs pass to special brooding pouches situated near the anthocodial bases. Planulae are found within these pouches from June through August. Embryogenesis takes place in these endodermal chambers and mature planulae are shed via temporary openings found among the polyps.

Soft corals of the family Xeniidae host innumerable symbiotic dinoflagellates (zooxanthellae) in their endodermal cells (Gohar, 1940). The density of zooxanthellae is high mainly in the anthocodial part of the polyp. In the stalk of the colonies most of the zooxanthellae are found in the peripheral solenia. The released planulae of *X. macrospiculata* already harbour zooxanthellae in their endoderm (Benayahu & Loya, 1984b). Gohar (1940) has stated that mature planulae of *X. macrospiculata* acquire the symbionts by direct parental transmission. This observation was confirmed by Benayahu & Loya (1984b). The method of transmission of the symbiotic algae from the mother colony to the sexually produced propagules has remained obscure.

There are two major methods of acquisition of zooxanthellae by the developing cnidarian host. In many species, the zooxanthellae are passed from one generation to the next by direct parental transmission. In other cnidarians the early developmental stages lack zooxanthellae and the juvenile must acquire the symbiotic algae from the ambient sea water. In the cases where algal infection occurs at a juvenile stage, the algae must enter the gastrovascular cavity and penetrate the endodermal cells by phagocytosis, or via an intermediate zooplankter (Trench, 1979). Muscatine (1961) found that the eggs as well as the buds of *Chlorohydra viridissima* carry zoochlorellae which subsequently pass to the following generation. Campbell (1990) described the pathway by which symbiotic *Chlorella* move from the parent endoderm into the developing oocytes of *Hydra viridissima*. The algae are released from the digestive cells into the basal membrane (mesolamella) and then move as individual cells into the intercellular spaces of the ectoderm. The oocytes take up the particular algae by phagocytosis.

Fitt (1984) summarized the mode of acquisition of algae by sexual progeny of various invertebrate hosts. In symbiotic cnidarians, where the infection is not through parental transmission, the zooxanthellae are taken directly into the endoderm of the progeny, in most cases by endocytosis. Fitt (1984) demonstrated that capture and digestion of brine shrimps by aposymbiotic scyphistomae of the jelly fish *Cassiopeia xamachana* led to their infection by zooxanthellae, when the brine shrimps had been pre-fed with zooxanthellae. The zooxanthellae were transferred via the *Artemia* nauplii to the endodermal cells of the scyphistomae. This author also observed that infection of scyphistomae by zooxanthellae can be accomplished by random contact between the two partners.

Benayahu et al. (1989) confirmed that in the soft coral *Heteroxenia fuscescens* the symbiosis is reestablished in each new generation of the colony. The symbiont acquisition coincides with the development of the mouth opening in the metamorphosed primary polyp.

The present study was initiated in order to describe the structural components of brooding and the mode of zooxanthellae acquisition by the developing planulae of *X. macrospiculata*.

MATERIAL AND METHODS

Colonies of *Xenia macrospiculata* were sampled during the breeding seasons of 1989 and 1990 in Elat (Gulf of Aqaba) at a depth of 15–20 m using SCUBA gear. The colonies were dissected longitudinally and their sex was determined under a dissecting microscope. Female specimens were fixed in 2 % glutaraldehyde in sea water. Samples for histology were then decalcified in a mixture of 1:1 formic acid (50 %) and sodium citrate (15 %) for 30 min, and embedded in Paraplast (Monoject Scientific); 8μ sections were cut and stained in hematoxylin-eosin and examined by light and epifluorescence microscopy. For TEM examination, planulae were removed from pouches of the fixed colonies, embedded in Epon, sectioned, stained with uranyl acetate and lead citrate, and examined with a JEOL TEM 1200. Hand sectioned planulae were prepared for SEM examination following the GTGO procedure (Gamliel et al., 1983). Samples were observed using the JEOL JSM 840.

RESULTS

Figure 1 presents a sagittal section in a mature female colony. Ova at different stages of development are found along the mesenteries of the polyp. The ova are located in the gastrovascular cavities but not in the anthocodia. The youngest ova are found only at the upper part of the polyp cavity. The ova are attached to the mesentery by a pedicel made

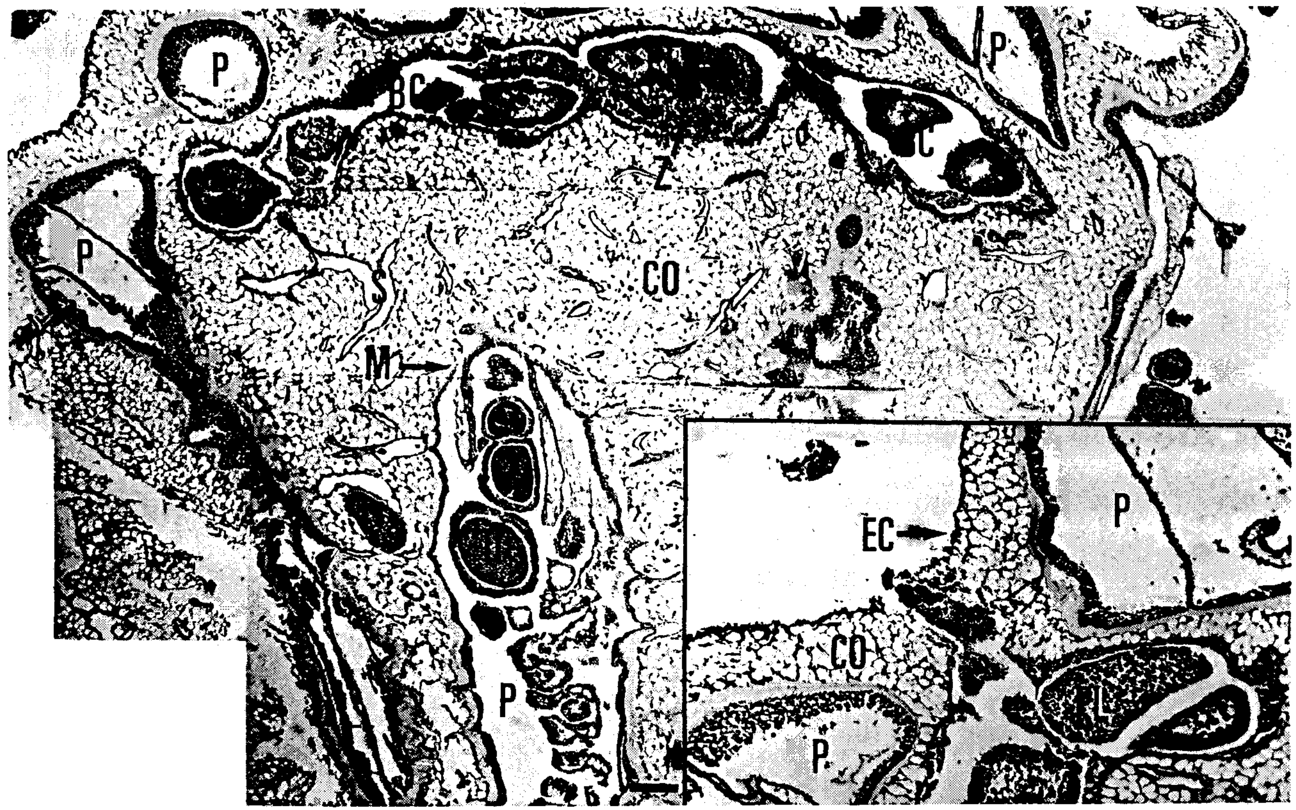

Fig. 1. *Xenia macrospiculata*. Sagittal section in a ripe colony showing brooding cavity and polyp cavity; insert: opening of brooding cavity. BC brooding cavity; CO coenecheme; EC ectoderm; M mesoglea; O oocyte; P polyp cavity; PL planula; S solenia; Z zooxanthellae. Scale bar = 100 μm

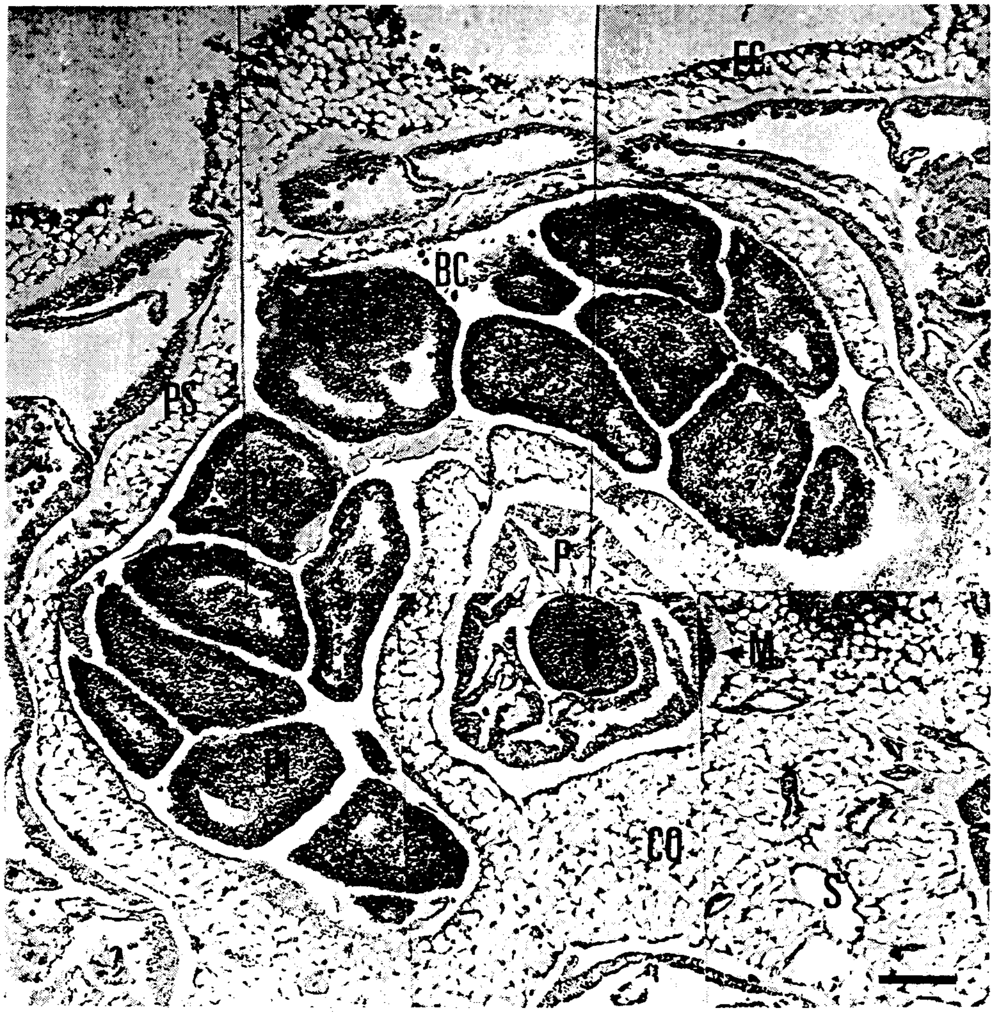

Fig. 2. *Xenia macrospiculata*. Cross section in a colony showing brooding chamber. BC brooding chamber; CO coenecheme; EC ectoderm; M mesoglea; O oocyte; P polyp cavity; PL planula; PS peripheral solenia; S solenia. Scale bar = 100 μm

of endodermal cells, and are enveloped by a thin layer of mesoglea and endodermal follicular cells. Unlike other endodermal cells the pedicel and the follicular cells do not host any zooxanthellae (Figs 1, 3A)

Examination of sagittal (Fig. 1) and cross sections (Fig. 2) of ripe colonies of *Xenia macrospiculata* shows a series of brooding pouches, located below the anthocodia among the polyps' cavities. Inspection of serial sections reveals that the pouches are interconnected and adjoining. Embryos and planulae containing zooxanthellae are found within the brooding pouches. In the brooding pouches with immature planulae, dense masses of zooxanthellae embedded in an amorphous material were observed (Figs 3B, 4A). We did

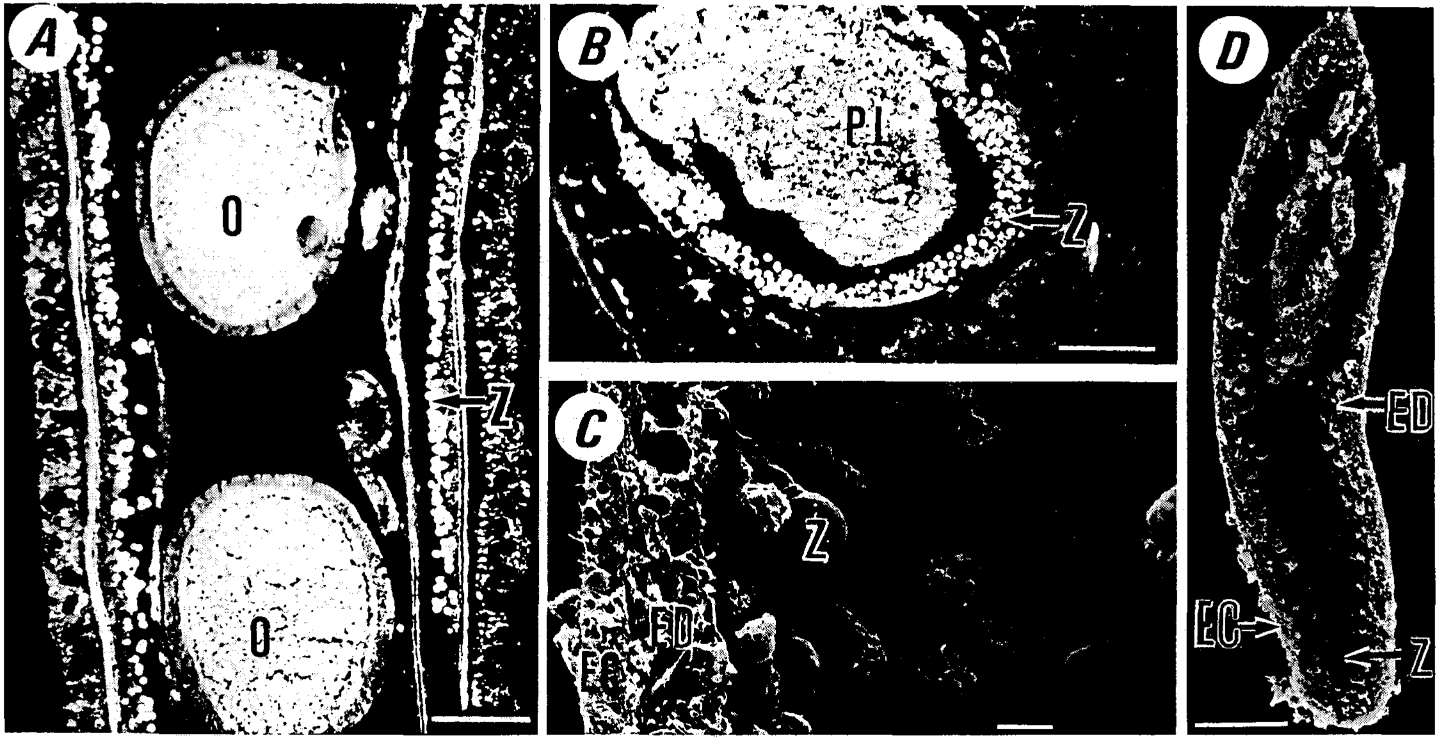

Fig. 3. *Xenia macrospiculata*. A: Epifluorescence micrograph of a polyp cavity of a ripe colony showing oocytes and zooxanthellae within the endoderm. Scale bar = 100 µm. B: Epifluorescence micrograph of a brooding cavity showing planula and loose zooxanthellae. Scale bar = 100µ m. C: SEM micrograph in the body wall of a mature planula, EC ectoderm; ED endoderm; Z zooxanthellae. Scale bar = 10 µm. D: Long section in a planulae showing zooxanthellae within the endoderm. Scale bar = 100 µm. For further abbreviations see Figs 1 and 2

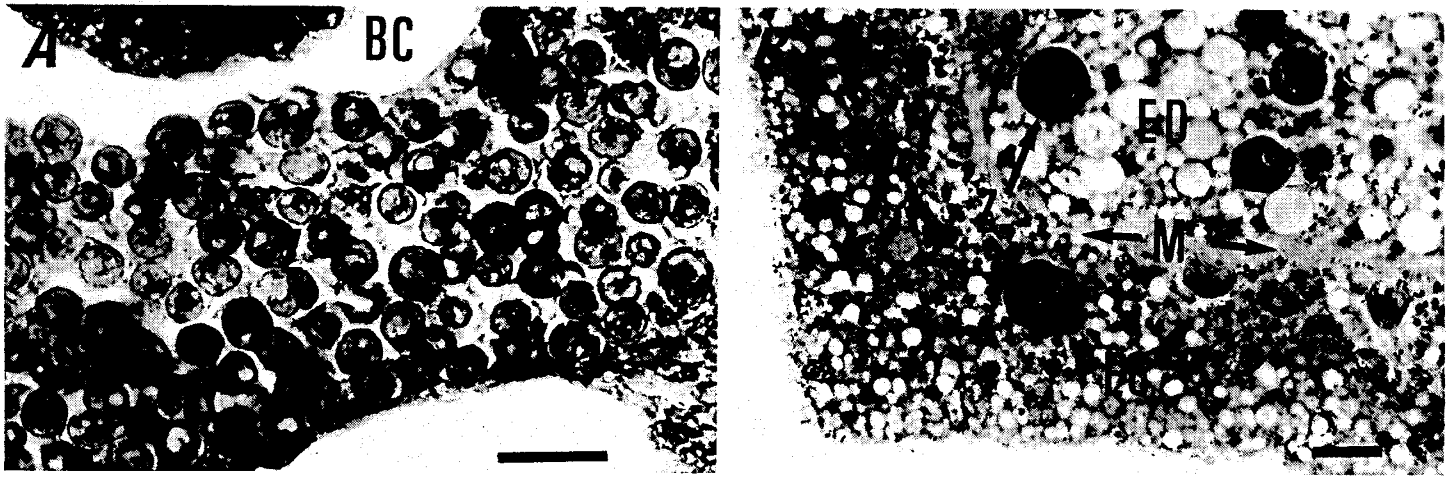

Fig. 4. *Xenia macrospiculata*. A: Free zooxanthellae within a brooding cavity. Scale bar = 20 μm. B: Section in a young planula. Scale bar = 10 μm. For further abbreviations see Figs 1 and 2

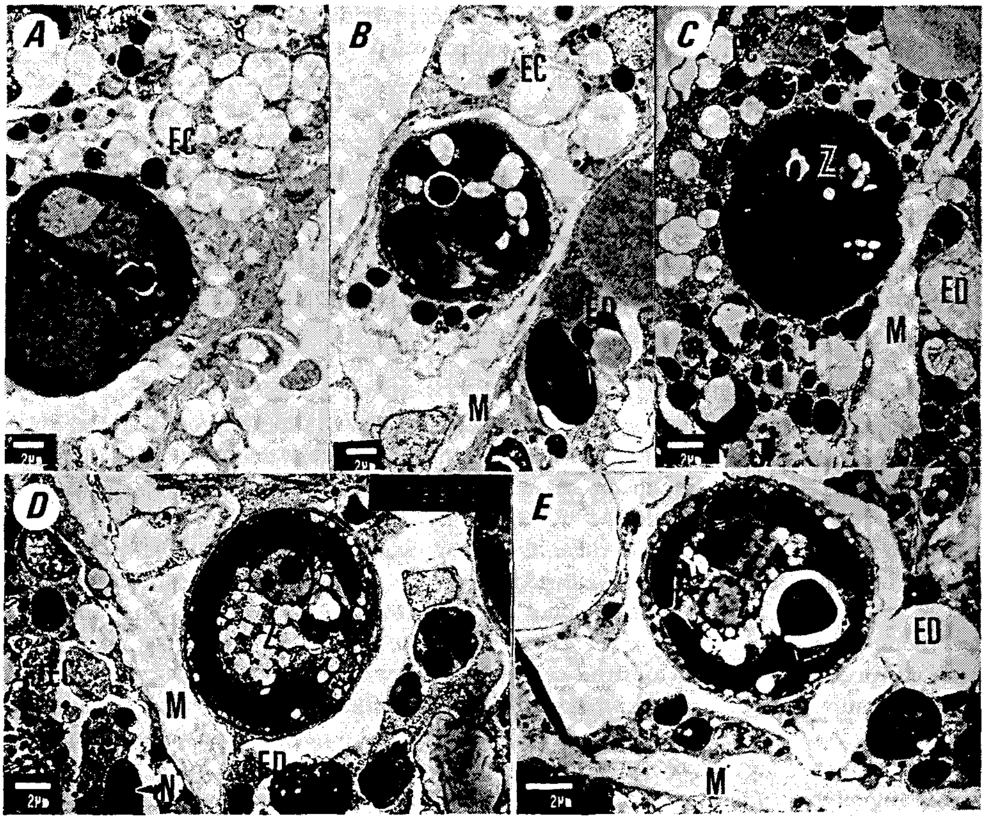

Fig. 5. *Xenia macrospiculata*. A, B, and C: TEM micrographs of zooxanthellae within ectodermal cells, zooxanthellae in A and C are undergoing mitosis. D and E: Zooxanthellae within endodermal cells. EC ectoderm; ED endoderm; N nematocyst; M mesoglea; Z zooxanthellae. For further abbreviations see Figs 1 and 2

not observe zooxanthellae in the lining of the pouches and there is no mesoglea underneath the epithelium of the pouches. The brooding pouches open to the ambient water via openings found in the capitulum among the polyps; the epithelial tissue of the capitulum is contiguous to that of the brooding pouches (Fig. 1, insert).

The young planulae are solid. The ectoderm encloses a mass of endodermal cells rich in lipids, and between their endoderm and ectoderm there is a thin layer of mesoglea; their ectoderm lack nematocyts (Figs 3B, 4B). The mature planulae are hollow (Figs 4C, D) and nematocyts are found in the ectoderm (Fig. 5E). In immature planulae, symbiotic algae are found within the ectoderm as well as the endoderm (Figs 4B; 5A, B, C). In the mature planulae, zooxanthellae were found only in the endoderm (Figs 3C, D). In 4 out of 13 young planulae examined zooxanthellae of the ectoderm were observed at different mitotic stages, while only in one case was mitosis observed in zooxanthellae found within the endoderm.

Examination of transmission electron microscopy sections shows algal symbionts at various levels within the ectoderm and the endoderm. (Figs 5A, B, C). Figure 5A presents a zooxanthella within an ectoderm cell. Pseudopodia are present at the outer surface of the ectoderm (Fig. 5A); this is probably the site of penetration of the zooxanthellae. Figure 5C presents an algal cell at mitosis in an ectoderm cell in juxtaposition with the mesoglea. In Figure 5C the cell with its zooxanthella is close to the mesoglea, and the mesoglea at that point is rather thin compared to adjacent mesoglea. Figure 5D presents an algal cell within the endoderm in juxtaposition with the inner side of the mesoglea. A cell which was detached from the outer layer of the endoderm is presented in Figure 5E; the algal cell is already within the endoderm, but the endoderm cell is still connected to the mesoglea at one point.

DISCUSSION

The present study indicates that the young planulae are transferred from the polyp cavity to brooding pouches found on the capitulum of the colony. Fertilization and cleavage were not observed; however, we assume that it occurs within the gastrovascular cavity of the polyp, followed by a rapid embryogenesis. Babcock (1990) stated that fertilization in *Heliopora* occurs within the polyp. Szmant-Froelich et al. (1980) demonstrated that in *Astrangia danae* embryogenesis could be a quick process and ciliated larvae were formed six to eight hours after fertilization. We assume that in *Xenia macrospiculata* there is an internal fertilization; however, we have no direct evidence for such a procedure.

Brooding in cnidarians was described in scleractinians as well as alcyonaceans. Planulae of *Sympodium caeruleum* are incubated in the gastrovascular cavities of the parent colony (Benayahu, 1991). Most xeniids brood their planulae; all studied species of *Xenia* retain their planulae within brooding chambers located in the upper part of the coenechyme among the gastrovascular cavities. In *Heteroxenia fuscescens* and *Heteroxenia coheni* the young planulae are retained within the polyp cavities and subsequently among the siphonozooids (Benayahu et al., 1989; Benayahu, 1991). Several species of octocorals brood their embryos in a mucus coat on the surface of the colony (Benayahu & Loya, 1983; Benayahu et al., 1990). In *Heliopora coerulea*, development takes place in superficial temporary brood spaces created by protracted distensions of the polyps.

Following Gohar (1940), Benayahu & Loya (1984b) stated that the brooding pouches of *X. macrospiculata* are lined with endoderm. These brooding pouches are temporary structures and appear only during the breeding season. The present study demonstrates that the brooding pouches are invaginations of the ectoderm and, as such, their lining is a continuation of the ectoderm of the capitulum (Fig. 1, insert). Furthermore, zooxanthellae, which are usually found in the endoderm, were not observed within this epithelial tissue. This is the first evidence for ectodermal brooding pouches among cnidarians. This epithelial tissue differs from the endodermal lining of the inner and the outer solenia by the absence of the thin layer of amorphous mesoglea between the endoderm and the coenechyme. *X. macrospiculata* is, therefore, not a true internal brooder but an external one. External brooding of larvae has been reported in most studied alcyonacean species.

The brooding cavity seems to be homologous with the inter-siphonozooid space occupied by the planulae in *H. fuscescens* (Benayahu et al., 1989). In *H. fuscescens* the fertilized oocytes and the planulae are kept between the siphonozooids before being released. The lining of the brooding cavity of *X. macrospiculata* is similar to the intersiphonozooid spaces of *H. fuscescens.*

Brooding within gastrovascular cavities has been found only in *S. caeruleaum* (Benayahu, 1991) and in *Lithophyton arboreum* (Benayahu et al., 1990). Non-gastrovascular brooding is the rule in alcyonaceans (for review see Babcock, 1990). Our observations agree with this general rule, and we disagree with Gohar's (1940) suggestion that the planulae of *X. macrospiculata* develop within endodermal brooding pouches.

Gohar (1940) described the brooding pouches of *X. macrospiculata* as separate spaces located among the zooids. Our observations indicate that all individual pouches adjoin each other and form a common pouch which is connected to the external environment, the surrounding water by numerous openings situated among the polyps. We therefore suggest that it should be termed brooding cavity.

In order to sustain the population of symbiotic algae, the host must pass them directly from generation to generation, or each generation must be reinfected. The mode of infection and transmission of algae from the ambient water to the endoderm has been described in a few alcyonaceans. In *X. macrospiculata* the uptake of zooxanthellae is most probably by endocytosis by ectodermal ameboid cells which "swallow" the zooxanthellae at the outer surface of the planula. The ameboid cell then traverses the ectoderm and the mesoglea and penetrates into the endoderm. The origin of these zooxanthellae is most probably the zooxanthellae found within the brooding pouches.

Direct evidence was presented by Fitt & Trench (1983) and by Colley & Trench (1983) showing that the method of entry of zooxanthellae into the coelenterate host is by phagocytosis by endodermal cells. Pardy & Muscatine (1973) have demonstrated that during the reestablishment of the symbiosis between aposymbiotic green hydra and *Chlorella*, the algae are taken in by phagocytosis of gastrodermal cells. Hofmann & Honegger (1990) have shown that zooxanthellae are occasionally found in the swimming buds of *Cassiopeia andromeda*. The microalgae are located within the mesoglea or between the bases of the endodermal cells. They suggested that the microalgae start multiplying during the growth phase of the scyphistomae. Kinzie (1974) demonstrated that the planulae and immature polyps of the gorgonian *Pseudopterogorgia bipinnata* are aposymbiotic. Infection of the polyp is through the open mouth.

In *X. macrospiculata* it seems that the infestation of the planulae takes place through the ectoderm, and the zooxanthellae are subsequently transferred to the endoderm. However, we did not observe the first steps of penetration of zooxanthellae into the ectoderm. There are still many unanswered questions concerning reproduction and brooding of *X. macrospiculata*. The means and mechanism by which eggs or embryos pass to the brooding cavity is enigmatic. The strong circumstantial evidence that the origin of the zooxanthellae is the "free" algae found in the brooding cavity still needs sound support.

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