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Reproduction in *Anthelia glauca* (Octocorallia: Xeniidae). II. Transmission of algal symbionts during planular brooding

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Abstract The soft coral *Anthelia glauca* Lamarck, 1816, of the family Xeniidae, is found on the reefs of KwaZulu-Natal, South Africa. Its gastrodermal cells contain numerous endosymbiotic unicellular algae (zooxanthellae). *A. glauca* is a gonochoric species that simultaneously broods its planulae within the pharyngeal cavity of the polyps. Symbiotic algae appear with zygote formation within the pharynx, embedded in amorphous material. The algal cells adhere to the ciliated ectodermal surface of immature planulae and are most probably endocytosed by them. Zooxanthellae are translocated towards the basal part of the ectoderm. Gaps are subsequently opened in the mesoglea into which symbionts surrounded by ectodermally derived material, including plasma membrane, pass. The basal membrane of endodermal cells disintegrates, and the algae bulge into spaces formed in the underlying endoderm. Throughout the process, each zooxanthella resides within a vacuolar membrane in the detached ectodermal cytoplasm. The acquisition process is essentially one in which zooxanthellae are translocated from the pharyngeal cavity into the ectoderm and then through the mesoglea into the endoderm, culminating in the final symbiotic state. The direct transmission of symbiotic algae to the eggs or larvae probably provides the most efficient means whereby zooxanthellae are acquired by the host progeny.

Introduction

Octocorals of the family Xeniidae are common throughout the Indo-Pacific reef system and constitute a major faunistic component in many areas (e.g. Roxas 1933; Gohar 1940; Benayahu 1985, 1990). Life-history features and various ecological aspects of several xeniid species have been extensively examined (e.g. Gohar 1940; Benayahu and Loya 1984a, b; Dinesen 1985; Alino and Coll 1989; Benayahu 1991). To date, all members of this family are known to brood planulae (Gohar 1940; Benayahu 1991, 1997). However, both the mode and the site of brooding vary among genera. In *Xenia* species, the planulae develop inside brooding pouches located below the anthocodia among the polyp cavities (Benayahu and Loya 1984b; Benayahu et al. 1988; Achituv et al. 1992). *Heteroxenia* species are elaborate in their brood care, with early embryogenesis occurring inside the gastrovascular cavities of either the anthocodia or the tentacles, and subsequent development and maturation of planulae taking place in the intersiphonozooid spaces (Benayahu 1991). *Efflatounaria* sp. is an external-surface brooder (Dinesen 1985; Alino and Coll 1989). In *Sympodium caeruleum*, planulae have been found within the gastrovascular cavities (Benayahu 1991). To date, no information is available on the reproduction of the other xeniid genera, C*espitularia* and *Fungulus*. An early study by Gohar (1940) noted that the xeniid *Anthelia glauca* reproduces by planular release. This finding has recently been confirmed, and, in addition, a unique brooding mode within the pharyngeal cavity of the polyps has been described for this species (Kruger 1996; Schleyer et al. 1997; Kruger et al. 1998).

Numerous reef cnidarians, including all known xeniid soft corals, harbor numerous endosymbiotic algae (zooxanthellae) in their gastrodermal tissue (Gohar 1940; Schlichter 1982; Benayahu et al. 1988). Utilization of the products of algal photosynthesis and even the symbionts themselves is of major nutritional importance for *Heteroxenia fuscescens* of the family Xeniidae (Schlichter 1982). Several studies have addressed the question of the developmental stage at which the sexually produced offspring of symbiotic hosts acquire their algal symbionts (e.g. Muscatine 1974; Fitt 1984; Trench 1987). Trench (1987) proposed two modes of acquisition

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of the symbiotic algae: either by direct transmission via the egg or brooded larvae (maternal inheritance), or from the ambient environment (open system), by post-larval stages. Direct transmission of symbionts has been documented in species of the cnidarian classes Hydrozoa (e.g. Trench 1987; Campbell 1990), Scyphozoa (Montgomery and Kremer 1995) and Anthozoa (e.g. Glynn et al. 1991; Benayahu et al. 1992; Benayahu 1997). Acquisition of symbionts from the ambient environment is far more common than maternal inheritance, and occurs both in scleractinian corals (Babcock et al. 1986; Harrison and Wallace 1990; Shlesinger and Loya 1991) and octocorals (Kinzie 1974; Benayahu et al. 1989). Eggs broadcast by the vast majority of species of both these groups lack algal symbionts upon release, as opposed to brooders which release mostly zooxanthellate larvae (Babcock et al. 1986; Harrison and Wallace 1990). Among the Xeniidae, *Xenia* species release zooxanthellate planulae, whose symbionts are incorporated at an early embryonic stage (Benayahu and Loya 1984b; Benayahu et al. 1988). *Heteroxenia* species release azooxanthellate planulae, and algal uptake occurs at an early primary polyp stage (Benayahu et al. 1989).

Species of the xeniid genus *Anthelia* are less abundant on Indo-Pacific reefs than those of *Xenia* or *Heteroxenia* (Benayahu 1985; personal observations). *A. glauca* has been recorded on many Indo-Pacific reefs and seems to be the most abundant species of its genus (e.g. Roxas 1933; Gohar 1940; Benayahu 1990, 1993). This soft coral is gonochoric, bears gonads throughout the year and reproduces by releasing planulae over a period of 3 to 4 months a year (Kruger et al. 1998). The current study focuses on the mode of transmission of algal symbionts to brooded planulae of *A. glauca*. We describe the stepwise process of algal infection and movement into endodermal cells. Uptake of zooxanthellae is apparently initiated at an immature planula stage, where symbionts are translocated from seawater in the pharyngeal cavity into the planular ectoderm. Symbionts are subsequently detached, surrounded by ectodermally derived plasma membrane and some cellular debri, and translocated through the mesoglea into the endodermal layer. The implications of these features are discussed in relation to previously described modes of symbiont acquisition, and their developmental consequences are considered.

Materials and methods

The study was conducted on the reefs of Sodwana Bay on the KwaZulu-Natal Coast, South Africa. These reefs are at their southernmost distribution on the east African coast and consist of a thin veneer of scleractinian corals growing on submerged beach rock formed from fossilized sand dunes (Schleyer 1995). The reefs have a markedly dense and diverse assemblage of soft corals (Benayahu 1993; Benayahu and Schleyer 1995; Schleyer 1995). Colonies of *Anthelia glauca* Lamarck, 1816 are found at depths of 8 to 22 m, occasionally forming small clusters. This species was investigated as part of a comprehensive study on the reproduction of several major soft coral species and the scleractinian *Pocillopora verrucosa* on the South African reefs, conducted from September 1991 to September 1994 (Kruger 1996; Schleyer et al. 1997).

The population of *Anthelia glauca* was sampled every 1 to 2 months throughout the years 1992 to 1994 in order to study the gonadal cycle and ascertain the reproductive season (Kruger et al. 1998). In 1993 and 1994, sampling of colonies was intensified during the months January to March, the peak breeding season (Kruger 1996; Schleyer et al. 1997). On each sampling date eight to ten specimens were removed from Nine-mile Reef (27°24.9′S; 32°43.6′E) in the central reef complex of Sodwana Bay by SCUBA divers. Material for light microscopy was fixed for 24 h in 4% formaldehyde in seawater, rinsed carefully in fresh water and then transferred into 70% ethanol. These samples were decalcified in formal-nitric acid solution (Mahoney 1966) and passed through methanol, ethanol and isopropanol in a Biorad tissue processor before embedding in paraplast. Sections, 5 μm thick, were cut on a microtome and stained with Ehrlich's haemalum and eosin (Drury and Wallington 1967). The material for transmission electron microscopy (TEM) was fixed in 4% glutaraldehyde in sea water and decalcified in a mixture of equal volumes of formic acid (50%) and sodium citrate (15%) for 30 min. It was dehydrated in a graded series of ethyl alcohol and embedded in Epon. Sections stained in uranyl acetate and lead citrate were viewed with a Jeol 1200EX electron microscope.

Results

The brood of *Anthelia glauca* is found within the pharyngeal cavity (Schleyer et al. 1997; Kruger et al. 1998), and it thus neighbors an epidermal milieu (Fig. 1a, b). The underlying thin mesoglea delimits the gastrodermis that lines the gastrovascular cavity (Fig. 1a–c). Only the gastrodermal cells of the colonies harbor the symbiotic zooxanthellae in *A. glauca* (Fig. 1b). The mesenteries of the polyp are attached to the pharyngeal wall, and their mesoglea and gastrodermis are continuous with the respective layers of both the pharynx and polyp wall (Fig. 1a, c). The earliest developmental stage observed in the pharyngeal cavity consisted of zygotes (Fig. 1a, b), which lacked both a follicular cell layer and underlying mesoglea found around the oocytes (see also Benayahu 1997). Patches of amorphous material were found among the zygotes (Fig. 1a, b), in which symbiotic algae eventually become embedded (Fig. 1c, d). At this stage,

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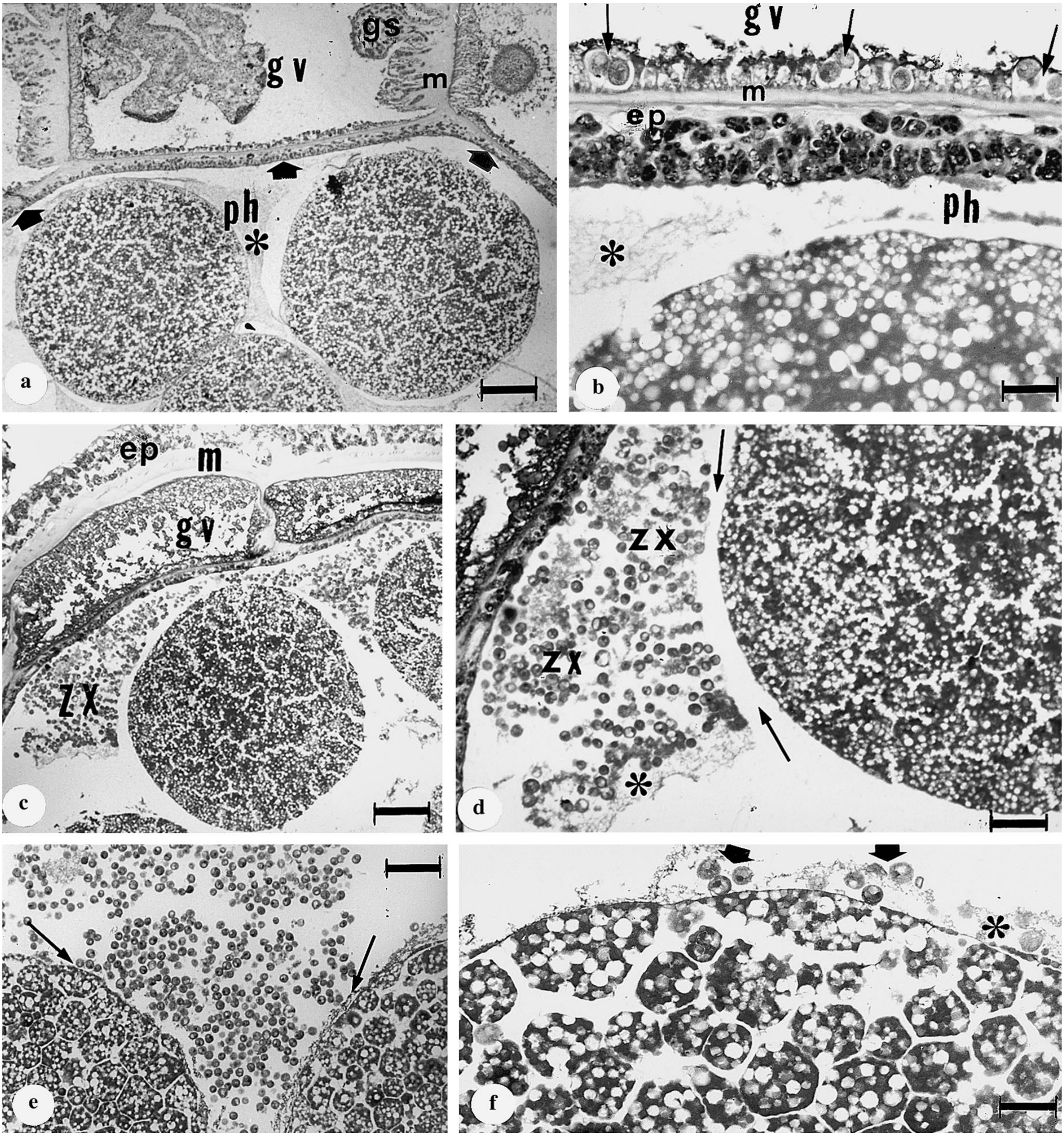
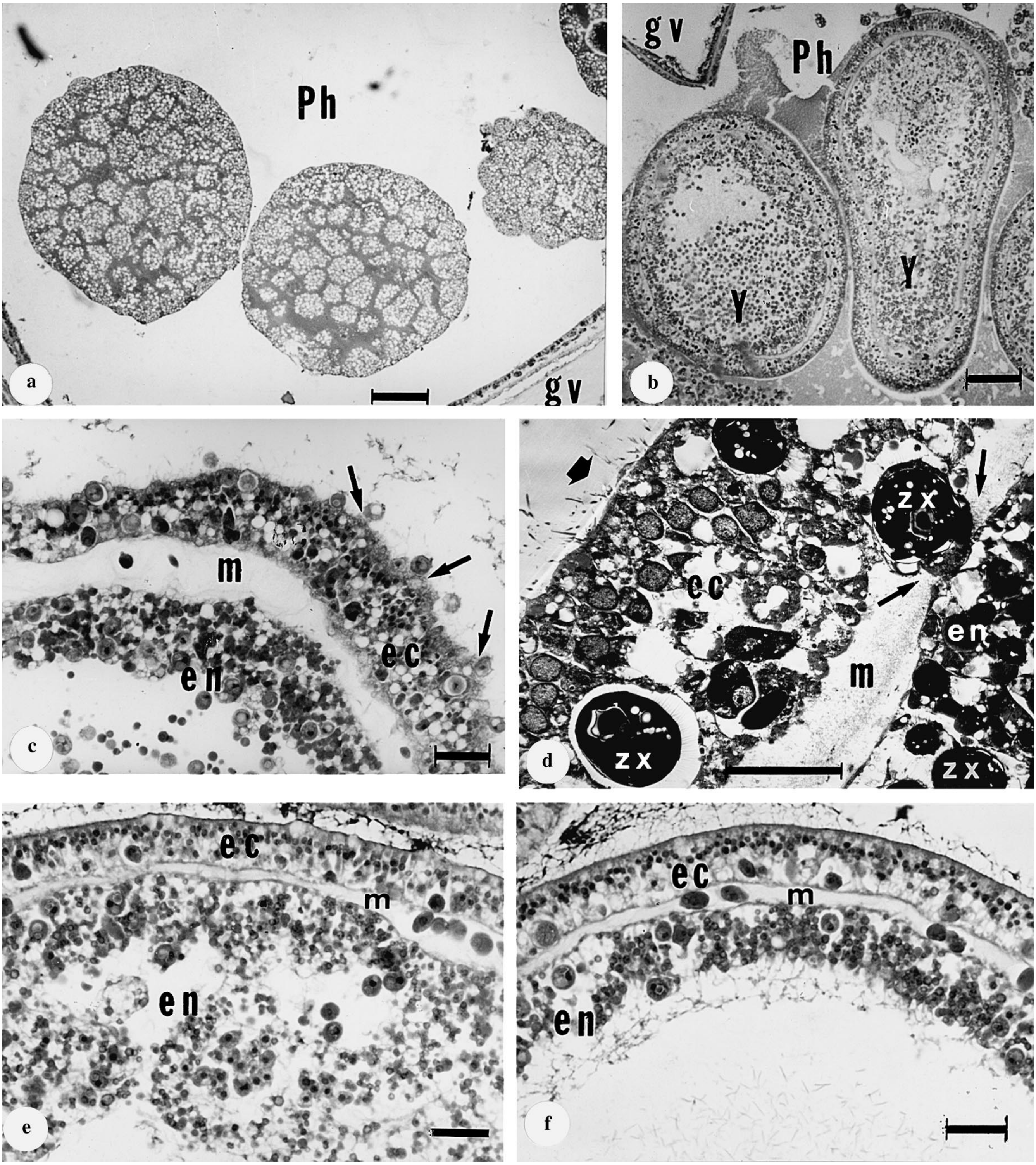
Fig. 1 *Anthelia glauca*. All histological sections. **a** Zygotes adjacent to the ectodermal lining (*arrows*) within pharyngeal cavity (*ph*) and surrounded by amorphous material (*asterisk*). The gastrovascular cavity (*gv*) is lined by gastrodermis (*gs*) and divided by septa attached to the pharyngeal wall, both with a continuous mesoglea (*m*). Scale: 120 μm. **b** Zygote surrounded by amorphous material (*asterisk*) within pharyngeal cavity (*ph*) adjacent to the darkly stained epidermis (*ep*). A thin mesoglea (*m*) borders the zooxanthellae (*arrows*) within gastrodermis that lines the gastrovascular cavity (*gv*). Scale 20 μm. **c** Brooded zygotes surrounded by amorphous material embedded with zooxanthellae (*zx*). Note the peripheral gastrovascular cavity (*gv*) of the polyp, its mesoglea (*m*) and external epidermis (*ep*). Scale: 150 μm. **d** Brooded zygote surrounded by amorphous material (*asterisk*) embedded with numerous zooxanthellae (*zx*). *Arrows* indicate free space around the zygote not occupied by algal cells. Scale: 40 μm. **e** Early embryos surrounded by zooxanthellae; *arrows* indicate points of close contact with the algal cells. Scale: 50 μm. **f** Zooxanthellae (*arrows*) embedded in amorphous material (*asterisk*) in close contact with the surface of an early embryo. Scale: 20 μm neither the amorphous material nor the algal cells are in contact with the zygote surface, and a cell-free zone, 15 to 40 µm in width, encompasses each embryo (Fig. 1c, d). The content of the zygotes is arranged in distinct globules, and close contact is later established between the zooxanthellae and the surface of the young embryos (Fig. 1e, f).

Embryos of *Anthelia glauca* undergo equal or unequal divisions, resulting in the development of regular and irregular blastulae, respectively (Fig. 2a), with both forms being found in more-or-less equal proportions. The immature brooded planulae have ectoderm, mesoglea and a largely undifferentiated endoderm with a yolk mass embedded with numerous zooxanthellae (Fig. 2b). This was the most juvenile stage of the sexual offspring found infected with zooxanthellae. Closer examination of these immature planulae revealed that the algal cells in the maternal pharyngeal cavity adhere to



the ciliated ectodermal surface of the immature planulae and most probably become endocytosed (Fig. 2c). The symbionts are located at various levels along the distal–proximal axis in the ectodermis (Fig. 2c, d). They also reside in the mesoglea and become embedded in the inner endodermal-nutritive mass of the immature planulae (Fig. 2c–e). The zooxanthellae disappear from the ectodermal surface during subsequent development of the planulae and reside in the basal part of this layer (Fig. 2e, f). As maturation of the planulae nears completion, the inner nutritive-mass disappears (Figs. 2f, 3a), and the zooxanthellae disappear from the mesoglea and reside entirely within the differentiated endodermal cells (Fig. 3b).



The TEM micrographs shed further light on the residence of zooxanthellae in the larval host-tissues and their mode of relocation in the course of brooding of *Anthelia glauca* planulae. Symbionts surrounded by ectodermal cellular material bulge from the ectoderm through the mesoglea towards the underlying endoderm at an immature planula stage (Fig. 2d). Each translocated zooxanthella is enclosed by ectodermally derived components including vacuolar membrane, cytoplasm and a distinct plasma membrane (Fig. 3c, d). These symbionts are also associated with lipid droplets and cellular debris (see below). They pass through a gap in the mesoglea towards the endoderm (Figs. 3d, 4a). A depression is formed in the mesoglea in the region of translocation. The ectodermally originated symbionts with the associated cellular material subsequently pass through a gap in this layer and encounter the underlying endoderm. The space between the plasma membrane and the vacuolar membrane is filled with cellular debris that varies in thickness around the zooxanthellae. It is composed of cytoplasm and membrane stacks, most probably derived from Golgi apparatus (Figs. 3d, 4). At the area of translocation, reduced mesoglea is noted; its fibers are laminated (Fig. 4b). The endodermal basal membrane disintegrates, and a space appears below the contact region, between the zooxanthella-bearing ectodermalcytoplasm and the underlying endoderm (Fig. 4). The frontal edge of the symbiont as well as the associated ectodermal cellulardebris pass through the mesoglea into the planular endoderm.

Discussion

Among the Cnidaria, direct acquisition of zooxanthellae can be initiated at a variety of developmental stages of the sexual progeny (Fitt 1984; Trench 1987). Eggs of several cnidarians incorporate symbiotic algae into their ooplasm, as has been recorded by Campbell (1990) in the hydrozoan *Hydra viridissima*; by Shäfer (1984) in the sea anemone *Anemonia sulcata*; by Harrison and Wallace (1990), Glynn et al. (1991), and Sier and Olive (1994) in the scleractinians *Montipora*, *Porites* spp. and *Pocillopora damicornis*; and by Benayahu et al. (1992) in the octocoral *Litophyton arboreum*. Acquisition of symbionts at a blastula stage has been described by Benayahu et al. (1988) in the octocoral *Xenia umbellata*, and it probably occurs in the other congeneric species (Benayahu 1990). The oocytes of *Anthelia glauca* and its zygotes (Kruger 1996; present study) lack zooxanthellae. In this investigation, we have demonstrated an intimate surface-contact between young embryos and zooxanthellae (Fig. 1e, f). However, no symbionts were observed internally until the immature planula stage (Fig. 2b, c). Absence of intermediate embryonic stages linking the blastulae and the immature planulae (see "Results") does not exclude the possibility of earlier acquisition. Nonetheless, the current study suggests that algal symbionts found in the pharyngeal cavity may evoke phagocytosis by the ciliated ectoderm of the immature planulae (Fig. 2c). The details and nature of such a process await future research.

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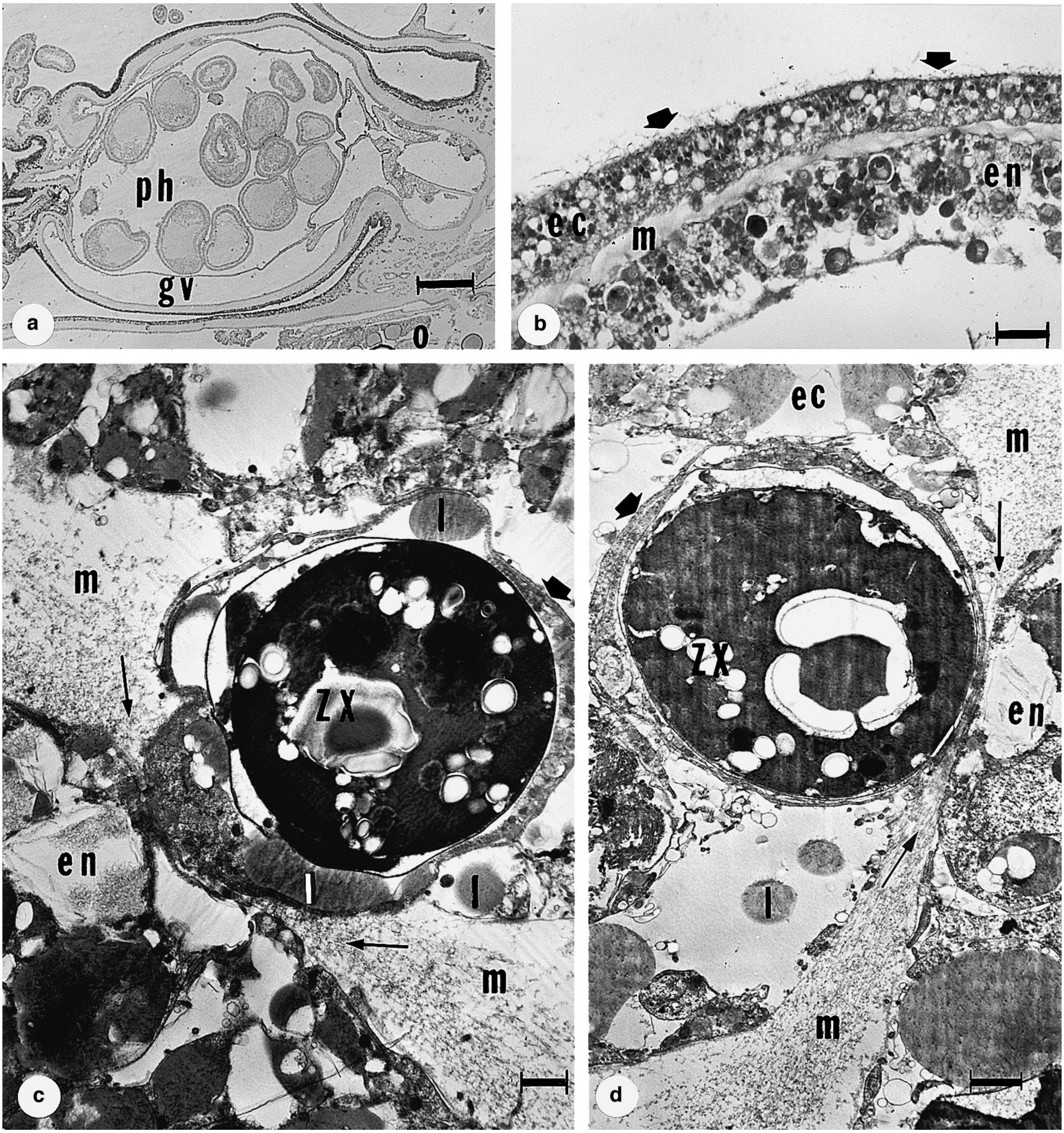
Fig. 2 *Anthelia glauca*. **a** Histological section. Brooded blastulae within the pharyngeal cavity (*ph*). Note the peripheral gastrovascular cavity (*gv*). Scale: 150 µm. **b** Histological section. Brooded immature planulae with yolky material (*y*) within the pharyngeal cavity (*ph*). Note the peripheral gastrovascular cavity (*gv*). Scale: 150 µm. **c** Histological section. Cross section through the body wall of an immature planula. *Arrows* indicate zooxanthellae in close contact with the ectoderm (*ec*). At this stage the entire ectoderm possesses symbionts as does the mesoglea (*m*) and endoderm (*en*). Scale: 20 µm. **d** Transmission electron micrograph. Planular ectoderm (*ec*) with cilia (*wide arrow*) and zooxanthellae (*zx*). Note zooxanthella surrounded by host cellular ectodermally derived material penetrating the mesoglea (*m*) and the underlying endoderm (*en*). The site of contact between the ectodermal cell and the endoderm is indicated by a *pair of arrows*. Scale: 10 µm. **e** Histological section. Body wall of an immature planula. Zooxanthellae are associated with ectoderm (*ec*), mesoglea (*m*) and the yolk laden endoderm (*en*). Note the absence of algal cells in the outer ectodermal surface. Scale: 20 µm. **f** Histological section. Body wall of an immature planula. Zooxanthellae are located in the basal part of the ectoderm (*ec*), mesoglea (*m*) and in the differentiated endoderm (*m*). Scale: 20 µm

While embryogenesis is rapid in externally developed planulae (1.5 to 3 d: Benayahu and Loya 1986), it is more protracted during surface brooding (6 to 7 d: Benayahu and Loya 1983; Benayahu 1989). The intimate relationship between the host-parent and offspring (Montgomery and Kremer 1995) has prevented direct observations on the time frame of the sequence of embryonic events in *Anthelia glauca,* as is the case with other brooding corals. The embryogenic cycle in the brooder *Xenia macrospiculata* was estimated to be 14 d (Benayahu and Loya 1984b), and in *A. glauca* at least 14 d (Kruger et al. 1988) by inference from the appearance of brooded embryos in female colonies to the time of planulation. The sexual progeny of these two species acquires zooxanthellae during these respective time spans; hence we propose that the various steps of zooxanthella uptake are highly synchronized with the embryogenesis of planulae. Although the temporal sequence of algal infection by *A. glauca* is unknown, the developmental stages at which the various acquisition phases occurred were deduced in the present study from embryogenic stages that have been formerly described for other octocorals (e.g. Benayahu et al. 1988; Benayahu 1989; Achituv et al. 1992).

The images obtained in the current study indicate that immature planulae of *Anthelia glauca* acquire zooxanthellae through a stepwise process, involving the translocation of ectodermally derived symbionts associated with cellular material of the same origin into the endoderm (Figs. 3, 4). The presence of symbionts in various positions in the ectoderm of planulae (Figs. 2c–f) is interpreted as resulting from the host-cell translocation after the initial surface-infection. Among cnidarians, zooxanthellae are generally restricted to the host endoderm (Muscatine 1974). However, they have been reported in the ectoderm of some planulae by Mont-



gomery and Kremer (1995) in the scyphozoan *Linuche unguiculata*; by Szmant-Froelich et al. (1980) in the scleractinian *Favia fragum*; by Farrant (1985), Achituv et al. (1992) and Benayahu (1997), respectively, in the soft corals *Capnella gaboensis*, *Xenia macrospiculata* and *Litophyton arboreum*. At later stages of development in these species, the algal cells are found exclusively within the host gastrodermis. Recently Benayahu (1997) supplied ultrastructural evidence for passage of zooxanthellae from the ectoderm into the endoderm of the developed planulae of *L. arboreum*. As far as we are aware, the present study describes for the first time the details of the cellular events at the immature planula stage by which the acquired zooxanthellae are translocated from the planular ectoderm into the endoderm (Fig. 2c). They pass from the ectodermal layer into the mesoglea during this process (Fig. 2c–f) and then into the endoderm of the planula (Figs. 3, 4). These findings confirm a suggestion made by Montgomery and Kremer (1995) that the migration of algal symbionts from ec-

Fig 3 *Anthelia glauca* **a** Histological section. Mature planulae brooded within the pharyngeal cavity (*ph*). Note oocytes (*o*) in the adjacent gastrovascular cavity (*gv*). Scale: 1 mm. **b** Histological section. Differentiated body wall of a mature planula. Zooxanthellae are found only within the endoderm (*en*) and there are no algal symbionts in the ciliated (*arrows*) ectoderm (*ec*) or the mesoglea (*m*). Scale: 20 μm. **c** Transmission electron micrograph. Translocated zooxanthella (*zx*) within an ectodermally derived plasma membrane (*wide arrows*) approaching the endoderm (*en*) through a gap (*slender arrows*) in the mesoglea (*m*). Note the lipid droplets (*l*) associated with the algal cell and the large amount of cellular debris in the gap. Scale: 1 μm. **d** Transmission electron micrograph. Translocated zooxanthella (*zx*) approaching the endoderm (*en*) through a gap (*slender arrows*) in the mesoglea (*m*). Note the lipid droplets (*l*) associated with the algal cell and the thin peripheral cellular debris. *Wide arrows* indicate the ectodermally derived plasma membrane. Scale: 1 μm
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toderm to endoderm occurs at a late planula or early polyp stage.

The initial appearance of zooxanthellae within the planular ectoderm can result from the infection of oocytes (e.g. *Litophyton arboreum*, see: Benayahu et al. 1992), embryos (e.g. *Xenia umbellata,* see: Benayahu et al. 1988) or immature planulae (present study). In all these developmental stages the zooxanthellae show no specificity towards the presumptive endodermal cells. It has been suggested that, as the cells of the infected embryos undergo mitotic divisions, they transfer the multiplying symbionts to all their daughter cells. However, at this stage the number of infecting zooxanthellae, as well as their growth and division rates, are not known. The presence of zooxanthellae in immature planular ectoderm as well as in the mesoglea of *Anthelia glauca* (Fig. 2c–f) thus represents a transitional stage in establishing the mature gastrodermal symbiotic state. We further conclude that the transfer of symbiotic algae to the endoderm and their retention within it are associated with the completion of cellular differentiation and maturation of tissues of the sexual offspring.

Throughout all the phases of translocation, each zooxanthella is sheathed by membranes (Figs. 3c, d, 4). These membranes are probably the periplast, which includes elements of the algal cell, and its plasma membrane (Muscatine et al. 1986). The cellular debris associated with the algal cells is located between the vacuolar membrane and the ectodermally derived plasma membrane. The presence of lipid droplets both in the symbionts and in their vicinity (Fig. 3c, d) implies an intense lipid metabolism (Kellogg and Patton 1983), the

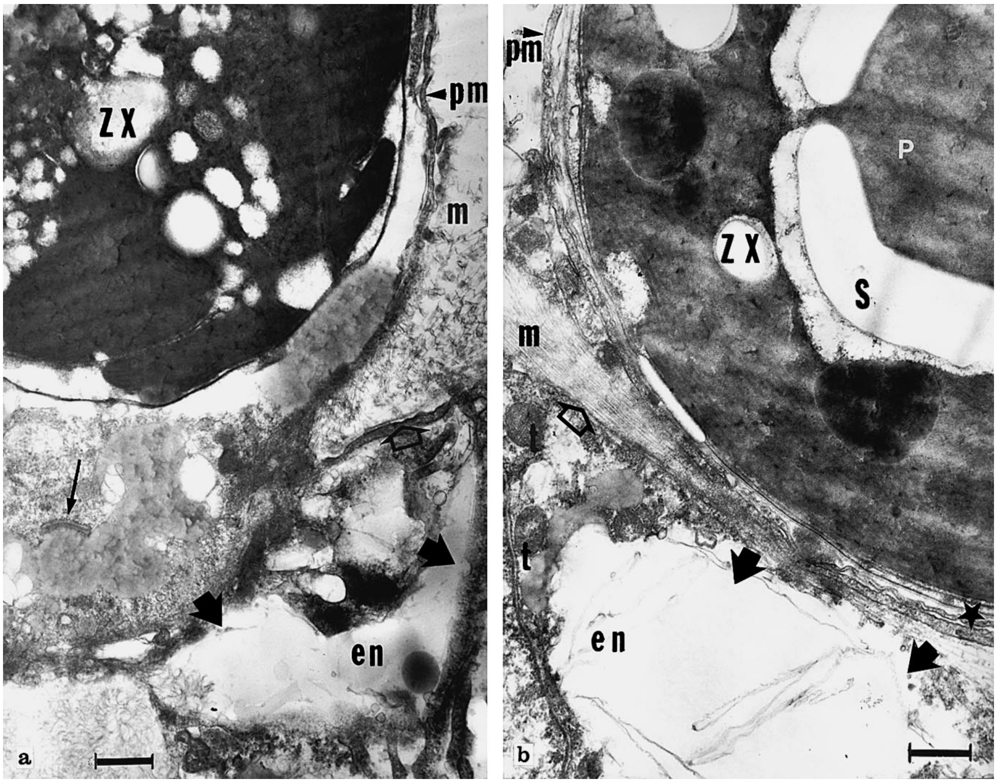

Fig. 4 *Anthelia glauca.* Transmission electron micrographs. **a** Translocated zooxanthella (*zx*) within ectodermally derived cellular material, surrounded by a plasma membrane (*pm*) and an inner vacuolar membrane. There is a large amount of cellular debris with a Golgi apparatus (*thin arrow*) between the two membranes. The advancing zooxanthella within the ectodermal entity passes through a gap opened in the mesoglea (*m*) into the endoderm (*en*). *Wide open arrow* indicates the basal membrane of the endodermal cell and *Wide closed arrows* indicate the space forming in the endoderm to accommodate the translocated algal cell. Scale: 1 μm. **b** Translocated zooxanthella (*zx*) within ectodermally derived cellular material, surrounded by plasma membrane (*pm*) and an inner membrane set (*star*). The mesoglea (*m*) is thin and laminated at the translocation site and a space (*wide arrows*) is forming in the endoderm (*en*) to accommodate the translocated algal cell. Apart from this gap, the basal endodermal membrane (*open arrow*) is intact and mitochondria (*t*) are present. Scale: 500 nμm

products of which might be transported to the developing planular tissues. In addition, it is possible that glycoproteins secreted by the zooxanthellae (Markell et al. 1992) are present in the periplasmic space and serve as signals that pass between the symbiont and the host (Markell and Trench 1993). We thus suggest that, during translocation, the zooxanthellae are resident within ectodermally derived cytoplasm rather than being "free", and that the translocated entities are ectodermal in origin with their complement of algae (see also: Gates et al. 1992). At this stage we do not exclude the possibility that complete ectodermal cells containing multiple zooxanthellae detach from the outer layer, and are taken up by the planular endoderm. Nonetheless, the validity of such a mechanism awaits further confirmation. The presence of the algal symbionts within vacuoles bounded by a membrane may assist in protecting them against lysosomal activity during the translocation process until the symbionts are settled in the endoderm (Muscatine and McNeil 1989). We further suggest that zooxanthellae housed within host-derived cellular membrane may be protected from an attack similar to an immune response and may be viewed as "self" by the planular endodermal cells.

The movement of the algal cells involves the breakdown and dissolution of histological barriers that are crossed during the journey towards their final residence in the planular endoderm. The translocated zooxanthellae reside temporarily within and pass through the dissociated mesoglea and basal endodermal membrane in the immature planulae of *Anthelia glauca* (Fig. 4). A similar phenomenon has been recorded by Trench (1987) in the oocytes of the hydroid *Myrionema amboinense* and by Benayahu et al. (1992) in the oocytes of *Litophyton arboreum*. The present findings thus imply that the mesoglea is a major barrier to the acquisition and translocation of zooxanthellae by the sexual progeny (e.g. Benayahu et al. 1988, 1992; Campbell 1990; Benayahu 1997).

A question thus arises with regard to the pharyngeal brooding of *Anthelia glauca* (see: Schleyer et al. 1997) and the larval mode of algal acquisition: where do the infective zooxanthellae come from (see also: Benayahu et al. 1992)? Symbiotic algae are known to be extruded by the host gastrodermis as part of a regulatory mechanism in controlling their density (Schlichter 1982; Muscatine and McNeil 1989; Gates et al. 1992). This phenomenon is the suggested source of zooxanthellae for the infection of oocytes of *Litophyton arboreum* while they are still attached within the polyp-gastrovascular cavity (Benayahu et al. 1992). In *Linuche unguiculata*, zooxanthellae are extruded simultaneously with the spawned eggs and are in part acquired by the developing embryos. Further acquisition from the environment is still feasible (Montgomery and Kremer 1995). Thus, a non-pathological and normal regulatory process (sensu Buddemeier and Fautin 1993) may furnish the algae required for infection of the sexual progeny. Zooxanthellae and zygotes were simultaneously found within the pharyngeal cavity of *A. glauca* during the current study (Figs. 1f, 2a–d), suggesting that algal cells are extruded synchronously by the gastrodermis during transfer of the embryos to the pharynx, thus furnishing the epidermal–pharyngeal milieu with symbionts for uptake by the developing planulae. The nature and source of the amorphous material surrounding the zygotes in the brooding chamber (Fig. 1b) remains to be determined, but resembles material observed by Achituv et al. (1992) in the brooding pouches of *Xenia macrospiculata*. It appears to bind the algae in the brooding chamber and prevent their escape through the mouth opening. In addition, it is possible that this material may comprise large-molecular-weight glycoproteins exuded by the zooxanthellae and which function as determinants in specific recognition (Markell et al. 1992; Markell and Trench 1993).

Motile zooxanthellae may also gain access to the brooded planulae from the exterior through the polyp mouth-opening (Kinzie 1974). Planular acquisition of maternal algal symbionts (sensu Trench 1987) may thus be supplemented by infection with non-parental zooxanthellae. Buddemeier and Fautin (1993) hypothesized the presence of a route of entry for extruded zooxanthellae to the host tissues under non-stress conditions. We further propose that, in a situation in which ambient algae are continuously sampled through the parental mouth, the fittest ones could be acquired by the sexual offspring. The combination of this open transmission with direct parental transmission (Trench 1987) would provide the progeny with a wider range of the symbiont gene pool than was previously realized.

In summary, we propose that the acquisition of zooxanthellae is regulated and dictated by the time table of embryogenesis in brooding corals. Such a process prevents the zooxanthellae from multiplying and overgrowing the host (see also: Muscatine et al. 1986). The stepwise translocation process also suggests that surface molecules associated with the zooxanthellae may provide "recognition" signal(s) (Markell et al. 1992) between the symbionts and various embryonic stages. This would involve sequential activation of the developmental stages of the offspring and their cellular constituents, in turn causing the translocation of zooxanthellae in the embryos of *Anthelia glauca* towards the planular endoderm. Studies on the developmental pathways and modes of algal transmission, using molecular techniques, are needed to establish the source of zooxanthellae in cnidarian hosts.

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