BioOne COMPLETE

Early Development of Zooxanthella-containing Eggs of the Corals Porites cylindrica and Montipora digitata: The Endodermal Localization of Zooxanthellae

Authors: Hirose, Mamiko, and Hidaka, Michio

Early Development of Zooxanthella-containing Eggs of the Corals *Porites cylindrica* and *Montipora digitata*: The Endodermal Localization of Zooxanthellae

Mamiko Hirose* and Michio Hidaka

Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

We studied the early development of zooxanthellae-containing eggs of the scleractinian corals *Porites cylindrica* and *Montipora digitata* to elucidate how zooxanthellae become localized to the endoderm of planulae during the course of development. In both species, zooxanthellae were distributed evenly in the oocytes and delivered almost equally to the blastomeres during cleavage. In *P. cylindrica,* gastrulation occurred via delamination or ingression, and blastomeres containing zooxanthellae dropped into the blastocoel during gastrulation. Thus, zooxanthellae were restricted to the endodermal cells at the gastrula or early planula stage in *P. cylindrica.* In *M. digitata*, gastrulation occurred by a combination of invagination and epiboly to form a somewhat concave gastrula. Zooxanthellae were present in both endodermal and ectodermal cells of early planulae, but they disappeared from the ectoderm as the planulae matured. In our previous study on two species of *Pocillopora*, we found that zooxanthellae were localized in eggs as well as in embryos, and that blastomeres containing zooxanthellae later dropped into the blastocoel to become restricted to the endoderm (Hirose *et al.*, 2000). The timing and mechanism of zooxanthella localization and types of gastrulation differed among species belonging to the three genera. These results suggest that zooxanthella localization in the embryos reflects the timing of the determination of presumptive endoderm cells and/or specificity of zooxanthellae toward presumptive endoderm cells.

Key words: coral, development, endoderm, symbiosis, zooxanthellae

INTRODUCTION

Reef-building corals harbor intracellular symbiotic dino-flagellates called zooxanthellae in their endodermal cells. Although the vast majority of reef-building corals spawn gametes that lack zooxanthellae (Babcock *et al.*, 1986), some hermatypic corals spawn eggs containing zooxanthellae (Kojis and Quinn, 1982; Babcock and Heyward, 1986; Heyward *et al.*, 1987; Tomascik and Sander, 1987; Yeemin, 1988; Glynn *et al.*, 1991, 1994; Kinzie, 1993, 1996; Sier and Olive, 1994; Kruger and Schleyer, 1998; Neves, 2000; Hirose *et al.*, 2001). It is not known how zooxanthellae delivered to oocytes become restricted to the endodermal cells during the course of development. Although the early development of scleractinian corals has been described in various species (*e.g.*, Szmant-Froelich *et al.* 1980, 1985; Babcock and Heyward, 1986; Harrison and Wallace, 1990), the early development of zooxanthella-containing eggs has been reported only in spawning species, such as *Montipora effusa* (Yeemin, 1998), *M. verrucosa* (Maté *et al.*, 1998), and *Pocillopora verrucosa* and *P. eydouxi* (Hirose *et al.*, 2000), and in a brooder, *Porites porites* (Tomascik and Sander, 1987).

Although zooxanthellae are generally restricted to endodermal cells in adult corals, zooxanthellae are at least temporarily observed in the ectoderm of planulae in some stony and soft corals (Szmant-Froelich *et al.*, 1985; Benayahu *et al.*, 1988; Benayahu, 1997; Benayahu and Schleyer, 1998; Schwarz *et al.*, 1999). This is probably because algal infection first occurred in the ectodermal cells of embryos or early planulae (Szmant-Froelich *et al.*, 1985) or because dividing cells at these stages transferred the multiplying symbionts to their daughter cells, including presumptive ectodermal cells (Benayahu, 1997; Benayahu and Schleyer, 1998). In the latter case, zooxanthellae were transferred from the ectoderm to the endoderm across the mesoglea before the larvae developed into mature planulae (Benayahu, 1997; Benayahu and Schleyer, 1998). In the scyphozoan *Linuche unguiculata*, Montgomery and Kremer (1995) observed symbiotic algae mainly in the ectodermal cells of early embryos, but the number of algae in the endoderm increased with time. They suggested that ectodermal cells containing symbiotic algae might migrate to the endoderm of planulae.

The corals *P. verrucosa* and *P. eydouxi* release eggs containing zooxanthellae. In these corals, zooxanthellae are concentrated in one hemisphere of the egg and are not equally delivered to all daughter cells. Furthermore, blastomeres containing zooxanthellae drop into the blastocoel during gastrulation, and zooxanthellae become restricted to the endoderm in the gastrula or early planula (Hirose *et al.*,

---

* Corresponding author. Phone: +81-98-895-8577;
              Fax   : +81-98-895-8576;
              E-mail : mhirose@zenno.jp

2000). In a temperate zooxanthellate sea anemone, *Anthopleura ballii*, blastomeres containing zooxanthellae were observed to invaginate at one end of the embryo, leading to the restriction of zooxanthellae to the planula endoderm (Davy and Turner, 2003). Thus, the larvae of these corals and this sea anemone do not transfer the algae from the ectoderm to the endoderm.

The corals *Porites cylindrica* and *Montipora digitata* spawn zooxanthellate eggs, but the zooxanthellae are distributed evenly within the eggs. We studied the early development of these corals and compared the timing and mechanism of zooxanthella localization to the endoderm of planulae in these two corals with those reported for two *Pocillopora* species, in which a localized distribution of zooxanthellae occurs in oocytes (Hirose *et al.*, 2001). We describe differences in the timing and mechanism of zooxanthella localization among the embryos of species belonging to the three genera. We also discuss the way in which gastrulation and the distribution pattern of zooxanthellae in eggs are related to the timing of zooxanthella localization to the endoderm in coral larvae.

MATERIALS AND METHODS

Collection of coral colonies

Branches were collected from colonies of *M. digitata* and *P. cylindrica* a few days before the full moon in May, June, and/or July from 1995 to 2000, from the reefs at Sesoko Island and Bise, northern Okinawa Island, Japan. The branches were maintained in an outdoor tank supplied with unfiltered running seawater.

Observation of early development

While the gonochoric colonies of *P. cylindrica* released individual eggs or sperm, the hermaphroditic colonies of *M. digitata* released egg–sperm bundles at night a few days after the full moon (Heyward *et al.*, 1987). To collect gametes, the branches were placed separately into plastic containers before the expected spawning time, at about 1930 h (*M. digitata*) and 2230 h (*P. cylindrica*). Released gametes were collected by sucking up seawater from the container with a large plastic pipette. The gametes from each species were placed in separate plastic beakers, with gametes from two or three colonies mixed in a single beaker (100–300 ml suspension each). Filtered (0.45 μm) seawater (FSW) was added to the beaker to a final volume of 1 or 2 L. For *M. digitata*, the concentration of gametes was about 1 bundle/ml (sperm: 10^{-4}–10^{-5} cells/ml). Fertilized eggs were kept in FSW at room temperature (28–30°C). Eggs and embryos were sampled and observed under a light microscope at intervals of 30 min to 1 h, and photomicrographs were taken (Nikon Microphot; Nikon, Tokyo, Japan).

Histology and transmission electron microscopy

Eggs and embryos were put into a microtube and allowed to sink to the bottom. The supernatant was discarded, and the specimens were fixed for at least 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 3% NaCl. The specimens were rinsed three times in the same buffer and post-fixed in 1% osmium tetroxide in the same buffer for 1 h on ice. The specimens were then dehydrated in a graded series of acetone, immersed in n-butyl glycidyl ether (QY1), and embedded in Spurr's resin. For light microscopic observation, sections (0.5–1 μm thick) were stained with 1% methylene blue and 1% azur II in 1% borax. For electron microscopy, silver or gold sections were stained with uranyl acetate and lead citrate and observed under a JEM-2000EX electron microscope (JEOL, Tokyo, Japan) at an acceleration voltage of 100 kV.

RESULTS

Early development of Porites cylindrica

Fifteen of 16 branches of *P. cylindrica* spawned gametes repeatedly for 2 to 6 days after the full moon in June 2000. The branches began releasing gametes 3 h after sunset (2230 h), and spawning persisted for 1 to 1.5 h. Eleven of these 15 branches were female. The female colonies released buoyant eggs, which were about 220 μm in diameter and contained about 400 zooxanthellae (Table 1). Zooxanthellae occupied about 2.6% of the egg volume. Male colonies released sperm at almost the same time.

Zooxanthellae were located evenly in spawned eggs of *P. cylindrica* (Fig. 1A). Cleavage occurred at intervals of 30 to 40 min. The zooxanthellae in the embryo were delivered almost equally to the blastomeres (Fig. 1B). At the morula stage, each daughter cell contained only a few zooxanthellae (Fig. 1C). At 6 h after fertilization, a hollow blastula was formed (Fig. 1D). During gastrulation, blastomeres possessing zooxanthellae accumulated around the blastocoel, and the surface layer of the embryo was occupied by relatively large, columnar cells without zooxanthellae (Fig. 1E, F). At

Table 1. Various parameters of spawned eggs of *Pocillopora verrucosa, P. eydouxi, Porites cylindrica* and *Montipora digitata* in 1998–2000. Means±SD.

	P. verrucosa*	P. eydouxi*	P. cylindrica	M. digitata
Day of spawning	after new moon	after full moon	after full moon	after full moon
Timing of spawning	1 h after sunrise	1 h after sunrise	3 h after sunset	1 h after sunset
Sex character	hermaphrodite	hermaphrodite	gonochoric	hermaphrodite
Released gamete form	individual	individual	individual	bundle
Egg buoyancy	sink	sink	buoyant	buoyant
Diameter of eggs (μm)[a]	145.2±6.9 (11)	136.0±1.6 (3)	223.8±5.9 (6)	383.5±18.8 (9)
Number of zooxanthellae per egg[a]	123.7±18.7 (9)	84.6±4.1 (3)	402.2±32.5 (9)	1359.3±245.8 (3)
Diameter of zooxanthellae in spawned eggs (μm)[b]	10.0±0.5 (11)	10.6±0.1 (3)	9.0±0.5 (3)	9.3±0.3 (6)
Number of zooxanthellae per egg volume (zoox / μm³)	$9.7×10^{-6}$	$8.0×10^{-6}$	$8.6×10^{-6}$	$5.8×10^{-6}$
Volume occupied by zooxanthellae (%)	4.3	4.1	2.6	2.0

* Hirose *et al.* (2000)

[a] Ten eggs were measured for each colony to calculate the average diameter of eggs and the average number of zooxanthellae per egg.

[b] Ten zooxanthellae were measured for each of at least three eggs to calculate the average diameter of zooxanthellae contained in spawned eggs for a colony.

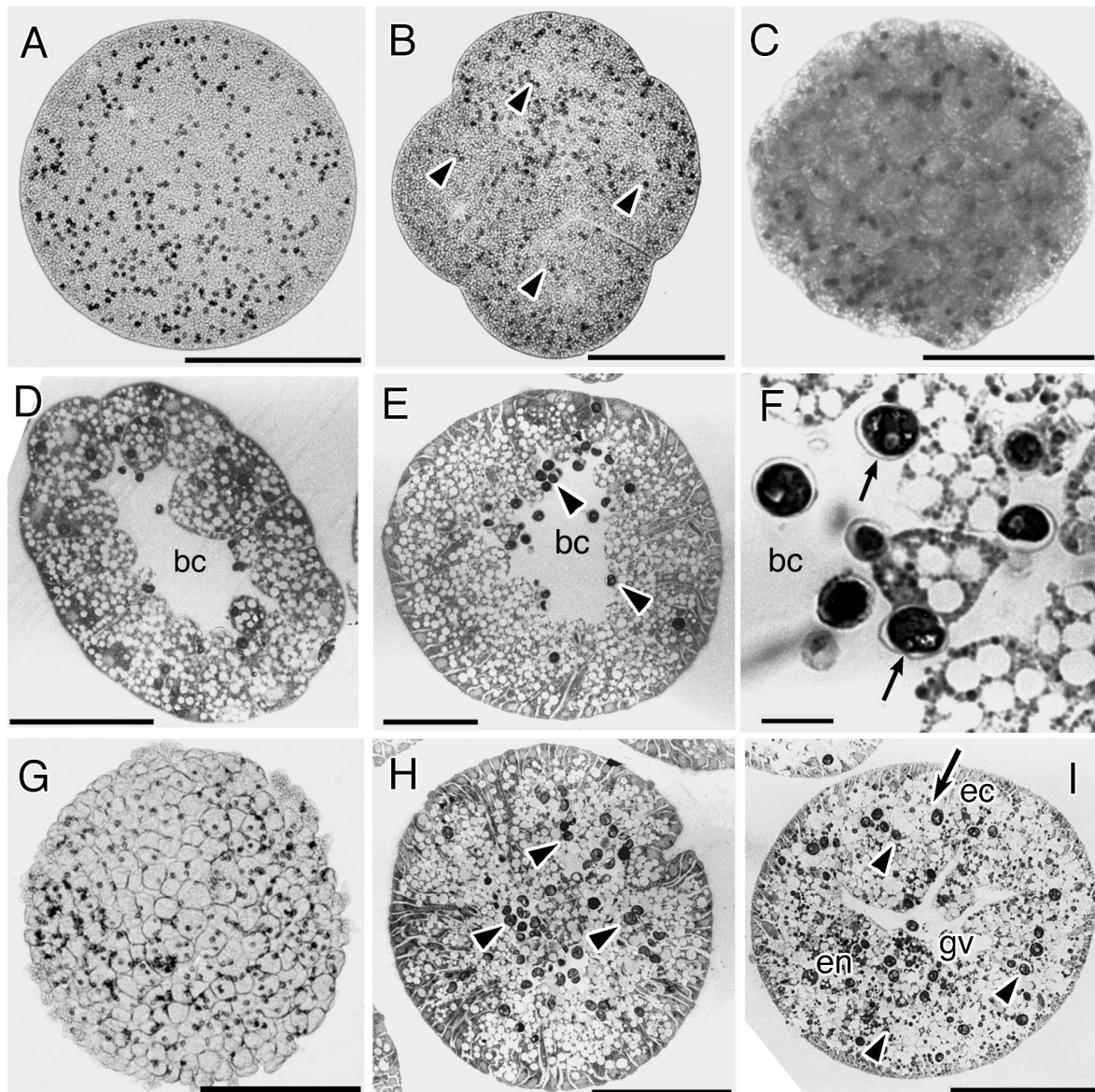The number in the parentheses represents the number of colonies examined.

Fig. 1. Early development of *Porites cylindrica*. **(A)** Spawned egg. Zooxanthellae are seen scattered throughout the cytoplasm. **(B)** Four-cell stage. The blastomeres contain an almost equal number of zooxanthellae (arrowheads). **(C)** Blastula. Each daughter cell contains a few zooxanthellae. **(D)** Section of an early blastula. A blastocoel has formed. **(E)** Section of a blastula showing multiple ingression of blastomeres containing zooxanthellae (arrowheads). **(F)** Section of part of a blastula during gastrulation. Blastomeres containing zooxanthellae (arrows) are in the process of ingression. **(G)** Gastrula. **(H)** Section of a gastrula. Blastomeres containing zooxanthellae (arrowheads) and those containing lipid droplets fill the inner space of the gastrula, forming a stereogastrula. **(I)** Section of a planula. Zooxanthellae (arrowheads) and lipid droplets are in the endodermal cells. Arrow indicates the mesoglea. **bc**, blastocoel; **ec**, ectoderm; **en**, endoderm; **gv**, gastrovascular cavity. Bars represent 100 μm in (A), (B), (C), and (G); 50 μm in (D), (E), (H), and (I); and 10 μm in (F).

about 10 h after fertilization, a stereogastrula was formed (Fig. 1G). Blastomeres containing zooxanthellae and/or lipid droplets filled the inner part of the embryo and the endoderm, and the ectoderm without zooxanthellae appeared to differentiate, although the mesoglea was not clearly seen (Fig. 1H). A mouth opening was observed as an invagination of the ectoderm (Fig. 1H). Ciliated larvae started to swim 24 h after fertilization. At this stage, the ectoderm and endoderm were demarcated by the mesoglea, and zooxanthellae were located in the endoderm (Fig. 1I). A gastrovascular cavity was formed.

Early development of Montipora digitata

Seven of 29 branches of *M. digitata* spawned egg–sperm bundles on the third to fifth night after the full moon during May–June 1999, and 11 of 40 branches spawned for 7 days after the full moon in June–July 1999. In 2000, all 12 branches collected spawned gametes for 10 days, beginning 1 day before the full moon in June. The spawning started about 1 h after sunset (between 2000 and 2100 h) and persisted for 1 to 1.5 h. Each egg–sperm bundle consisted of 10 to 15 eggs and sperm. The eggs were about 380 μm in diameter and contained about 1,400 zooxanthel-
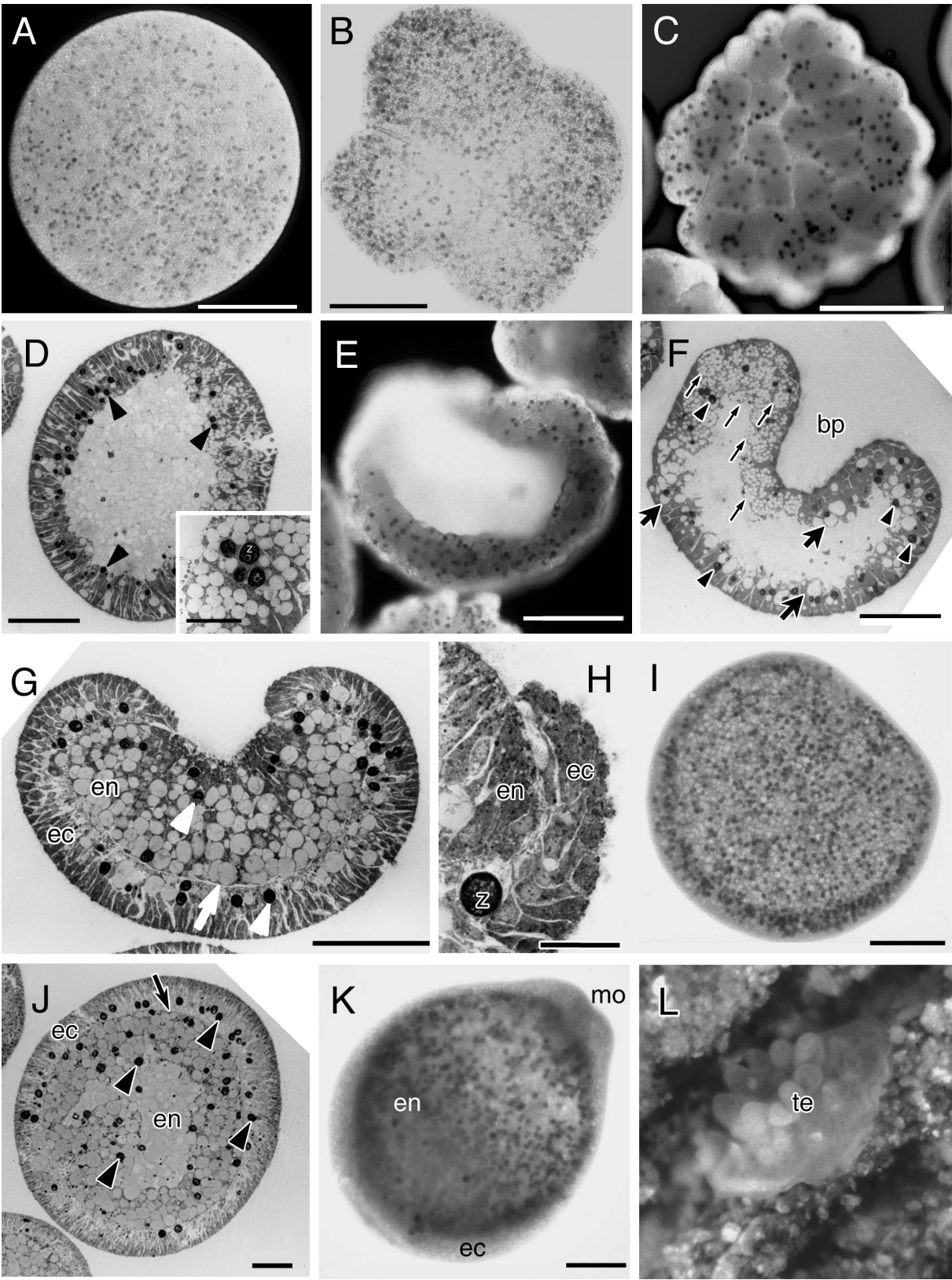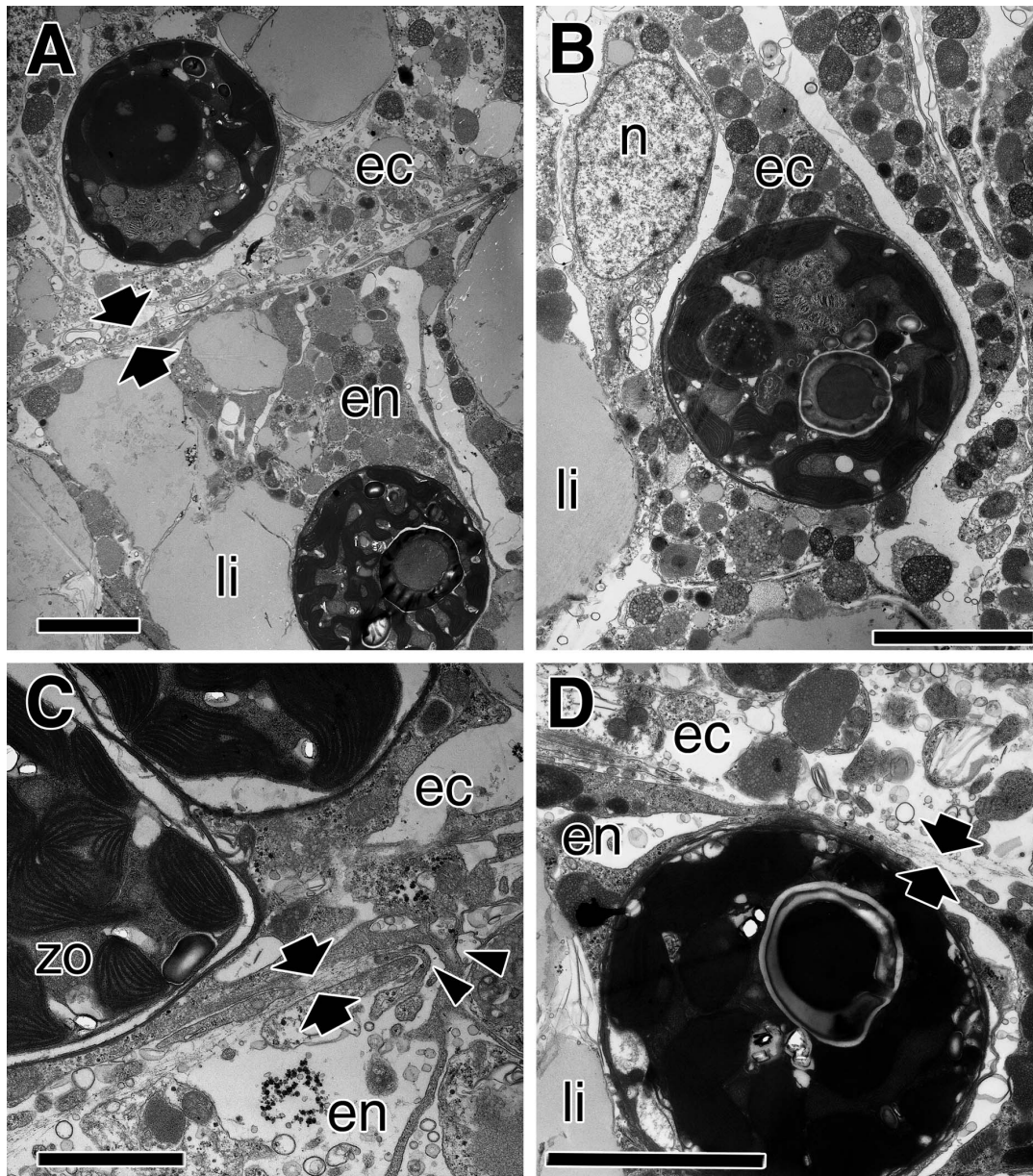
Fig. 3. Transmission electron micrographs of early planulae in *Montipora digitata*. **(A)** Zooxanthellae residing in the ectoderm and endoderm of an immature planula. Arrows indicate the mesoglea. **(B)** Zooxanthella in the cellular process of an ectoderm cell. The zooxanthellae are located at the basal end of the cell. **(C)** Zooxanthella in an ectoderm cell near the mesogleal layer. A cellular process (arrowheads) of an ectoderm cell has entered the endoderm through a gap in the mesogleal layer (arrows). **(D)** Zooxanthella in the endoderm near the mesogleal layer. The mesogleal layer (arrows) near the zooxanthella is obscured. **en**, endoderm; **ec**, ectoderm; **li**, lipid droplet; **zo**, zooxanthella. Bars represent 5 μm in (A), (B), and (D) and 2 μm in (C).

Fig. 2. Early development of *Montipora digitata*. **(A)** Spawned egg. Zooxanthellae are distributed evenly in the egg. **(B)** Four-cell stage. The blastomeres contain an almost equal number of zooxanthellae. **(C)** The 64-cell stage. Zooxanthellae accompany each daughter cell. **(D)** Section of a blastula. The blastula is composed of large, columnar blastomeres, and zooxanthellae (arrowheads) are located in the basal region of the columnar cells. The central region of the blastula is filled with acellular material. Inset: Section of an early blastula at higher magnification. The cells contain a few zooxanthellae (z) and many lipid droplets. Bar=50 μm. **(E)** Early gastrula. One side of the embryo is concave. **(F)** Section of an early gastrula. Two types of blastomeres are seen, those containing small lipid droplets (smaller arrows) and those containing a few large droplets (larger arrows). Zooxanthellae (arrowheads) are present within some blastomeres. **(G)** Section of a gastrula. The ectoderm and endoderm are separated by mesoglea (arrow). The endoderm is discernible as an inner cell mass containing large lipid droplets. Zooxanthellae (arrowheads) are seen in both the ectoderm and endoderm. **(H)** Enlarged view of the ectodermal margin of the gastrula. The ectodermal layer appears to envelop the endoderm. **(I)** Late-stage gastrula. **(J)** Section of a late gastrula or early planula. Zooxanthellae (arrowheads) are distributed in both the ectoderm and endoderm. Arrow indicates the mesoglea. **(K)** Mature planula. The planula is completely ciliated at this stage. The inside of the planula appears dark because of the accumulation of zooxanthella-containing cells. **(L)** Primary polyp with tentacles. **bp**, blastopore; **en**, endoderm; **ec**, ectoderm; **mo**, mouth; **te**, tentacle; **z**, zooxanthella. Bars represent 100 μm except in (H), where bar represents 20 μm.

lae (Table 1), which occupied about 2.0% of the egg volume.

Zooxanthellae were distributed more or less homogeneously in the cytoplasm of *M. digitata* eggs (Fig. 2A). The first cleavage began about 2.5 h after fertilization. From the early cleavage stage to the morula stage, each blastomere contained an almost equal number of zooxanthellae (Fig. 2B, C). A hollow blastula was formed, although the blastocoel was filled with some acellular material (Fig. 2D). The blastula was composed of columnar blastomeres, and zooxanthellae were located in the basal region of the columnar cells. Gastrulation occurred by invagination, and one side of the embryo became concave (Fig. 2E, F). Two types of blastomeres (Fig. 2F) were observed, one containing a few large lipid droplets (arrows) and another containing many small lipid droplets (small arrows). The blastomeres of the latter type were aggregated on one side of the blastopore lip. At a later stage, the blastocoel disappeared, and the ectoderm and endoderm could be discerned (Fig. 2G). The ectoderm consisted of columnar cells, and the endoderm comprised cells containing large lipid droplets. Zooxanthellae were observed in both the ectoderm and endoderm. The ectoderm layer appeared to envelop the endodermal cells (Fig. 2G, H). The embryos then became spherical in shape again and formed a stereogastrula (Fig. 2I). Zooxanthellae were present in both the ectoderm and the endoderm, even in the late gastrula or early planula (Figs. 2J, 3A). Most of the zooxanthellae in the ectoderm were located in the basal region of the ectodermal cells (Fig. 3B). The mesoglea was very thin (about 0.3 μm) and was obscured or lost in some places (Fig. 3C, D). In these cases, cellular processes of ectodermal cells had entered the endoderm through a gap in the mesoglea (Fig. 3C), or zooxanthellae were located very close to the obscured mesoglea (Fig. 3D). As the planulae matured, zooxanthellae disappeared from the ectoderm, and were then seen only in the endoderm of mature planulae (Fig. 3K). Planulae settled and metamorphosed 3–4 days after fertilization. At about 6 days after settlement, the polyps began secreting a skeleton, and tentacles were discernible (Fig. 2L).

DISCUSSION

In adult colonies of hermatypic corals, only endodermal cells contain zooxanthellae, although some hermatypic corals release oocytes that contain them. Hence, zooxanthellae must become restricted to the endoderm at a certain stage of development. We studied the early development of zooxanthellate eggs in *P. cylindrica* and *M. digitata* to investigate how the timing of zooxanthella localization to the endoderm differs among these two species and two species of *Pocillopora* characterized previously (Hirose *et al.*, 2000).

Early cleavage and distribution of zooxanthellae

In *P. verrucosa* and *P. eydouxi*, zooxanthellae moved toward the animal pole 1–2 days before spawning and became concentrated in one hemisphere of the oocytes (Hirose *et al.*, 2001). The first cleavage apportioned zooxanthellae more or less equally between the first two blastomeres. At the second cleavage, however, two of the four blastomeres received almost all of the zooxanthellae, and the other two had few or none. This uneven distribution of

zooxanthellae persisted until the zygotes developed into gastrulae (Hirose *et al.*, 2000). In contrast to the two species of *Pocillopora*, *P. cylindrica* and *M. digitata* exhibited an even distribution of zooxanthellae in their eggs, and the zooxanthellae were delivered almost equally to the blastomeres until blastula formation.

Blastoderm differentiation in the two species

In both *P. cylindrica* and *M. digitata*, fertilized eggs developed into a hollow blastula, as occurs in the two species of *Pocillopora* (Hirose *et al.*, 2000). In *P. cylindrica*, when the blastocoel disappeared and an acoel embryo was formed (Fig. 1G, H), the ectoderm and endoderm appeared to be at least partially differentiated, although they were not clearly demarcated by a mesogleal layer. The embryo at this stage might be considered a gastrula rather than a blastula. A mouth later formed by invagination of the ectoderm, and the ectoderm and endoderm became separated by a mesogleal layer. This process is considered to be planula development rather than gastrulation (Harrison and Wallace, 1990).

In *M. digitata*, the blastocoel was filled with some acellular material, which probably consisted of lipid droplets that had been expelled from blastomeres and accumulated in the blastocoel. This material was likely incorporated later by endodermal cells in the course of invaginating gastrulation. When the blastocoel disappeared upon invagination, the ectoderm and endoderm differentiated, and the endodermal cells became filled with large lipid droplets.

Gastrulation and zooxanthella relocation

In *P. cylindrica* as well as the two species of *Pocillopora*, blastomeres containing zooxanthellae and/or lipid droplets detached from the outer layer and dropped into the blastocoel until it was completely filled, resulting in a stereogastrula. In these corals, zooxanthella localization to the endoderm occurred during gastrulation (Fig. 4). Gastrulation was by multipolar ingression or delamination rather than invagination. Gastrulation through delamination has been suggested in *Astrangia danae* (Szmant-Froelich *et al.*, 1980), *Favia fragum* (Szmant-Froelich *et al.*, 1985), and *M. verrucosa* (Maté *et al.*, 1998).

In *M. digitata*, gastrulation appeared to occur via the combined processes of invagination and epiboly. Zooxanthellae were present in both the endoderm and ectoderm of the late gastrula or early planula. Some presumptive endoderm cells may remain in the ectoderm of *M. digitata* planulae, or the specificity of zooxanthellae toward presumptive endoderm cells may be low or develops at a later stage in *M. digitata* compared to *P. cylindrica* and the two *Pocillopora* species. The zooxanthellae gradually disappeared from the ectoderm as the planulae matured.

The timing of zooxanthella localization differed among *P. cylindrica*, *M. digitata*, and the two species of *Pocillopora* (Fig. 4). This difference in timing may be related to the mode of gastrulation. However, in the temperate sea anemone *Anthopleura ballii*, which produces zooxanthellate eggs, zooxanthellae are localized at one end of the blastula, and the blastomeres containing zooxanthellae invaginate during gastrulation, restricting the zooxanthellae to the endoderm of planulae (Davy and Turner, 2003). The uneven distribu-
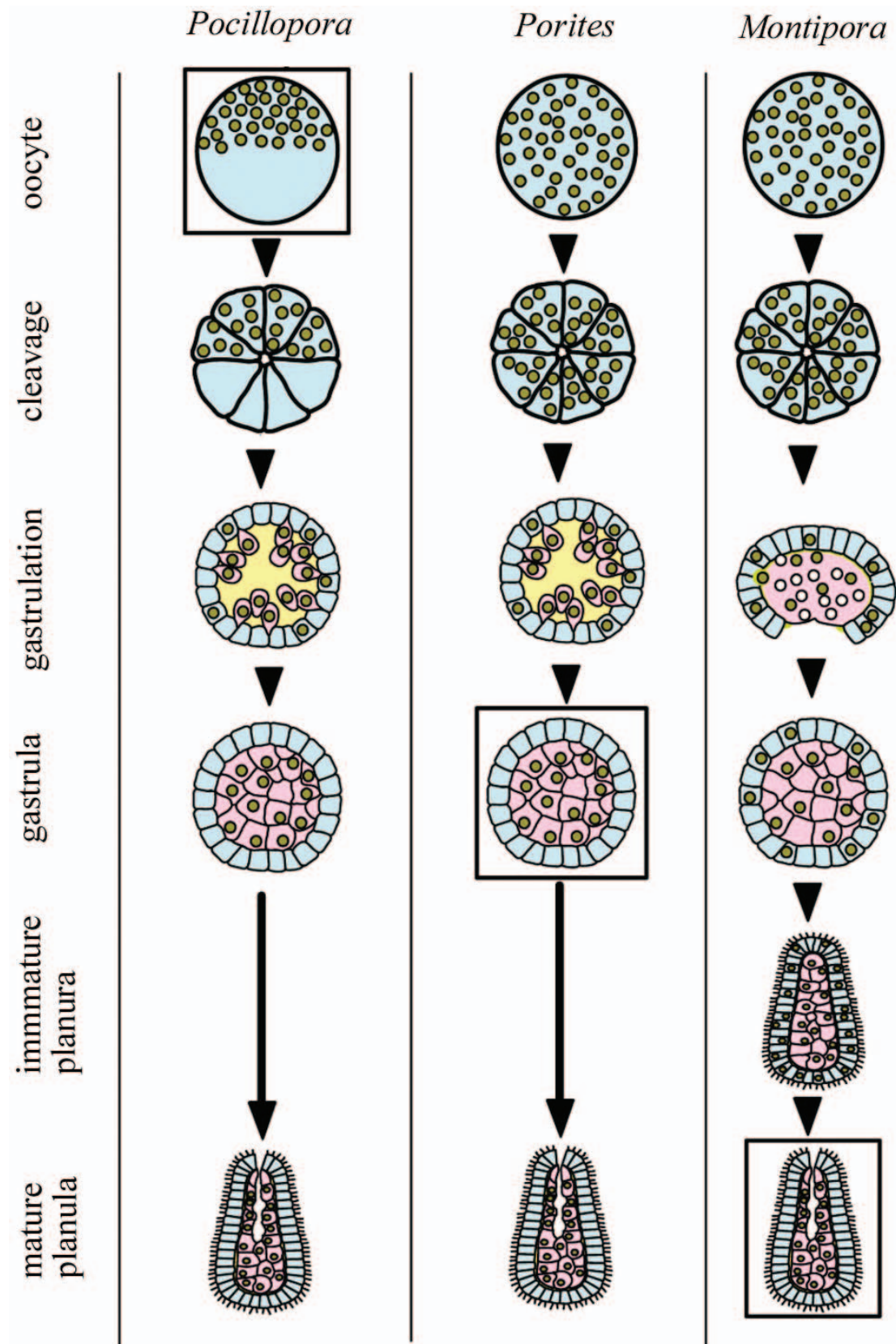
Fig. 4. A schematic illustration showing the timing and mechanism of zooxanthella localization. Developmental stages are indicated on the left. Boxes indicate the first stage during which zooxanthellae are localized in the oocyte or embryo. The timing of zooxanthella localization and type of gastrulation differ among the three genera.

tion of zooxanthellae in embryos could indicate an earlier determination of presumptive endodermal cells. Zooxanthellae might be delivered more or less exclusively to presumptive endodermal cells during early developmental stages in the two *Pocillopora* species and the sea anemone *A. ballii*. It is likely that zooxanthella localization in embryos is related to the timing of the determination of presumptive endodermal cells, with the localization of zooxanthellae to the endoderm occurring no earlier than gastrulation.

Possible mechanisms of zooxanthella translocation from ectoderm to endoderm

Zooxanthellae have been observed in the ectoderm of the early planulae of some corals (*F. fragum*: Szmant-Froelich *et al.*, 1985; *Fungia scutaria*: Schwarz *et al.,* 1999), soft corals (*Xenia umbellata*: Benayahu *et al.*, 1988; *Litophyton arboreum*: Benayahu *et al.*, 1992, Benayahu, 1997; *A. glauca*: Benayahu and Schleyer, 1998), and the scyphozoan *L. unguiculata* (Montgomery and Kremer, 1995). In these cnidarians, zooxanthellae generally reside only in the endoderm in later stages of the life cycle; thus, zooxanthellae in the ectoderm might be translocated to the endoderm or removed from the ectoderm. Several mechanisms have been suggested for the translocation of zooxanthellae from the ectoderm to the endoderm (Montgomery and Kremer, 1995; Benayahu, 1997; Benayahu and Schleyer, 1998). In the jellyfish *L. unguiculata*, ectodermal cells infected by zooxanthellae can migrate to the endoderm of planulae (Montgomery and Kremer, 1995). In the soft coral *A. glauca*, zooxanthellae within a vacuole in the detached ectodermal cytoplasm pass through temporarily opened gaps in the mesoglea toward the endoderm (Benayahu, 1997; Benayahu and Schleyer, 1998). In *M. digitata*, we could not observe zooxanthellae in the process of entering the endoderm through a gap in the mesoglea. Some zooxanthellae in the endoderm, however, were located just below the mesogleal layer, which was more or less obscured, and appeared to have been translocated from the ectoderm (Fig. 3C). Most zooxanthellae in the ectoderm were contained in cellular processes and were located close to the mesogleal layer. It is not clear whether a whole zooxanthella-containing cell migrates to the endoderm, or whether only the zooxanthellae, together with small amounts of cytoplasm, move to the endoderm, as suggested in some soft corals (Benayahu, 1997; Benayahu and Schleyer, 1998).

We observed no degraded zooxanthella particles in the surface layer of embryos or early planulae of *M. digitata*. In contrast, Davy and Turner (2003) only occasionally found zooxanthellae in the ectoderm of the late gastrula or early planula and suggested that these stray zooxanthellae may degrade in the host or be expelled. It has been proposed that degraded zooxanthella particles are produced by partial digestion of zooxanthellae by the host (Titlyanov *et al.*, 1996, 1998; Mise and Hidaka, 2005). Few zooxanthellae were observed at the bottom of beakers incubating embryos of *M. digitata*. The absence or scarcity of degraded and expelled zooxanthellae may indicate that those in the ectodermal layer of planulae are transferred to the endoderm during the maturation of planulae.

Vertical transmission via zooxanthellate eggs

The three genera *Porites*, *Montipora*, and *Pocillopora*, which contain species that spawn zooxanthellate eggs, belong to different families and occur in different lineages in phylogenetic trees of scleractinian corals (Veron, 1995; Romano and Palumbi, 1996, 1997; Romano and Cairns, 2000; Fukami *et al.*, 2000; Kerr, 2005). Furthermore, the same family can contain some species that release zooxanthella-containing eggs and others that produce zooxanthella-free eggs. For example, most *Montipora* species produce eggs containing zooxanthellae, whereas *Acropora* species, which belong to the same family, release eggs without zooxanthellae (Fadlallah, 1983). This together with the present finding that the timing of zooxanthella localization and types of gastrulation were different among the three genera (*Pocillopora*, *Porites,* and *Montipora*) suggests that vertical transmission of symbionts via zooxanthellate eggs evolved independently in the three genera studied.

REFERENCES

Babcock RC, Heyward AJ (1986) Larval development of certain gamete-spawning scleractinian corals. Coral Reefs 5: 187–195

Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Mar Biol 90: 379–394

Benayahu Y (1997) Developmental episodes in reef soft corals: ecological and cellular determinants. Proc 8th Int Coral Reef Symp 2: 1213–1218

Benayahu Y, Schleyer MH (1998) Reproduction in *Anthelia glauca* (Octocorallia: Xeniidae). II. Transmission of algal symbionts during planular brooding. Mar Biol 131: 433–442

Benayahu Y, Achituv Y, Berner T (1988) Embryogenesis and acquisition of algal symbionts by planulae of *Xenia umbellata* (Octocorallia: Alcyonacea). Mar Biol 100: 93–101

Benayahu Y, Weil D, Malik Z (1992) Entry of algal symbionts into oocytes of the coral *Litophyton arboreum*. Tissue Cell 24: 473–482

Chornesky EA, Peters EC (1987) Sexual reproduction and colony growth in the scleractinian coral *Porites astreoides*. Biol Bull 172:161–177

Davy SK, Turner JH (2003) Early development and acquisition of zooxanthellae in the temperate symbiotic sea anemone *Anthopleura balli* (Cocks). Biol Bull 205: 66–72

Fadlallah YH (1983) Sexual reproduction, development and larval biology in scleractinian corals. A review. Coral Reefs 2: 129–150

Fukami H, Omori M, Hatta M (2000) Phylogenetic relationships in the coral family Acroporidae, reassessed by inference from mitochondrial genes. Zool Sci 17: 689–696

Glynn PW, Gassman NJ, Eakin CM, Cortés J, Smith DB, Guzman HM (1991) Reef coral reproduction in the eastern Pacific: Costa Rica, Panama, and Galapagos Islands (Ecuador). I. Pocilloporidae. Mar Biol 109: 355–368

Glynn PW, Colley SB, Eakin CM, Smith DB, Cortés J, Gassman NJ,

Guzmán HM, Del Rosario JB, Feingold JS (1994) Reef coral reproduction in the eastern Pacific: Costa Rica, Panama, and Galapagos Islands (Ecuador). II. Poritidae. Mar Biol 118: 191–208

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In " Ecosystems of the World, Vol 25: Coral Reefs" Ed by Z Dubinsky, Elsevier, Amsterdam, pp 133–207

Heyward AJ (1986) Sexual reproduction in five species of the coral *Montipora*. Hawaii Institute of Marine Biology, Technical Report 37: 170–178

Heyward A, Yamazato K, Yeemin T, Minei M (1987) Sexual reproduction of corals in Okinawa. Galaxea 6: 331–343

Hirose M, Kinzie III RA, Hidaka M (2000) Early development of zooxanthella-containing eggs of the corals *Pocillopora verrucosa* and *P. eydouxi* with special reference to the distribution of zooxanthellae. Biol Bull 199: 68–75

Hirose M, Kinzie III RA, Hidaka M (2001) Timing and process of entry of zooxanthellae into oocytes of hermatypic corals. Coral Reefs 20: 273–280

Kerr AM (2005) Molecular and morphological supertree of stony corals (Anthozoa: Scleractinia) using matrix representation parsimony. Biol Rev 8: 543–558

Kinzie III RA (1993) Spawning in the reef corals *Pocillopora verrucosa* and *P. eydouxi* at Sesoko Island, Okinawa. Galaxea 11: 93–105

Kinzie III RA (1996) Modes of speciation and reproduction in archaeocoeniid corals. Galaxea 13: 47–64

Kojis BL, Quinn NJ (1982) Reproductive strategies in four species of *Porites* (Scleractinia). Proc 4th Int Coral Reef Symp 2: 145–151

Kruger A, Schleyer MH (1998) Sexual reproduction in the coral *Pocillopora verrucosa* (Cnidaria: Scleractinia) in KwaZulu-Natal, South Africa. Mar Biol 132: 703–710

Maté JLT, Wilson J, Field S, Neves EG (1998) Fertilization dynamics and larval development of the scleractinian coral *Montipora verrucosa* in Hawaii. Hawaii Institute of Marine Biology, Technical Report 42: 27–39

Mise T, Hidaka M (2005) Degradation of zooxanthellae in the coral *Galaxea fasicularis*. Galaxea 7: 49–55

Montgomery MK, Kremer PM (1995) Transmission of symbiotic dinoflagellates through the sexual cycle of the host scyphozoan *Linuche unguiculata*. Mar Biol 124: 147–155

Neves G (2000) Histological analysis of reproductive trends of three *Porites* species from Kane'ohe Bay, Hawaii. Pac Sci 54: 195–200

Romano SL, Cairns SD (2000) Molecular phylogenetic hypotheses for the evolution of scleractinian corals. Bull Mar Sci 67: 1043–1068

Romano SL, Palumbi SR (1996) Evolution of scleractinian corals inferred from molecular systematics. Science 271: 640–642

Romano SL, Palumbi SR (1997) Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. J Mol Evol 45: 397–411

Schwarz JA, Krupp DA, Weis VM (1999). Late larval development and onset of symbiosis in the scleractinian coral *Fungia scutaria*. Biol Bull 196: 70–79

Sier CJS, Olive PJW (1994) Reproduction and reproductive variability in the coral *Pocillopora verrucosa* from the Republic of Maldives. Mar Biol 118: 713–722

Szmant-Froelich A, Yevich P, Pilson MEQ (1980) Gametogenesis and early development of the temperate coral *Astrangia danae* (Anthozoa: Scleractinia). Biol Bull 158: 257–269

Szmant-Froelich AM, Reutter M, Riggs L (1985) Sexual reproduction of *Favia fragum* (Esper): lunar patterns of gametogenesis, embryogenesis and planulation in Puerto Rico. Bull Mar Sci 37: 880–892

Titlyanov EA, Titlyanova TV, Leletkin VA, Tsukahara J, van Woesik R, Yamazato K (1996) Degradation and regulation of zooxanthellae density in hermatypic corals. Mar Ecol Prog Ser 139: 167–178

Titlyanov EA, Titlyanova TV, Loya Y, Yamazato K (1998) Degradation and proliferation of zooxanthellae in planulae of hermatypic coral *Stylophora pistillata*. Mar Biol 130: 471–477

Tomascik T, Sander F (1987) Effects of eutrophication on reef-building corals. III. Reproduction of the reef-building coral *Porites porites*. Mar Biol 94: 77–94

Veron JEN (1995) Corals in Space and Time. Cornell University Press, New York

Yeemin T (1988) A Comparative Study of Reproductive Biology in Four Congeneric Species of Scleractinian Corals (*Montipora*) from Okinawa. Masters Thesis, Department of Biology University of the Ryukyus, Okinawa