Unusual Pattern of Embryogenesis of *Caryophyllia inornata* (Scleractinia, Caryophylliidae) in the Mediterranean Sea: Maybe Agamic Reproduction?

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ABSTRACT While knowledge of the reproductive biology of tropical scleractinian corals is extensive, information from temperate zones is limited. The aim of this study is to describe the reproductive biology of *Caryophyllia inornata*, a temperate species, and to increase the understanding of the reproductive strategies of Mediterranean corals. Samples of *C. inornata* were collected during SCUBA surveys at Elba island. Sexually active individuals displayed either male or female germ cells, showing a gonochoric sexuality. *C. inornata* exhibited an unusual pattern of embryogenesis. Embryos appeared throughout the whole year in males and in sexually inactive individuals, and they did not show a seasonal pattern of development, as usually expected for sexual reproduction. This observation suggests the possibility of asexual origin. These embryogenetic sexually inactive individuals were larger in size than the embryogenetic sexually active ones, and they might be senile polyps that preserve the ability to produce embryos only by agamic reproduction. J. Morphol. 273:943–956, 2012.

KEY WORDS: embryo development; gametogenesis; sexual inactive polyps; sexuality; reproductive mode

INTRODUCTION

Knowledge of pattern of sexuality and mode of reproduction are fundamental for the understanding of the macroevolutionary processes of all multicellular organisms (Kerr et al., 2011). Knowledge of the reproductive biology of corals (Scleractinia), gained by studying their sexuality (hermaphroditic or gonochoric), reproductive mode (broadcasting or brooding), embryonic (coeloblastula or stereoblastula), and larval development (benthic or planktonic), is the first step to understanding the population dynamics of marine organisms (e.g., Goffredo et al., 2005).

Despite in-depth studies over the last three decades, which have greatly increased understanding of the reproductive biology of scleractinians, the wide range of reproductive strategies of this group remains enigmatic (Loya and Sakai, 2008). Of the more than 1,500 recognized coral species, characteristics of sexual reproduction have now been recorded in at least 444 species (Harrison, 2011) mainly from tropical and subtropical zones (Fadlallah, 1983; Heltzel and Babcock, 2002; Neves and Pires, 2002; Mangubhai and Harrison, 2008a,b). Information on scleractinians from temperate zones, however, is limited (Szmant-Froelich et al., 1980; Beauchamp, 1993). Data on corals from the Mediterranean Sea come from observations made more than a century ago by Lacaze-Duthiers (1873), a few observations on *Cladocora caespitosa* (Kruzic et al., 2008) and recent studies on *Balanophyllia europaea* (Goffredo et al., 2002), *Leptopsammia pruvoti* (Goffredo et al., 2005, 2006), and *Astroides calycularis* (Goffredo et al., 2010, 2011).

The variety of reproductive processes and modes among coral species partially reflects the extraordinary ability of their cells to differentiate and to provide their tissues with increased plasticity and evolutionary adaptability (Campbell, 1974; Holstein et al., 2003; Harrison, 2011). The abundance

Contract grant sponsor: European Research Council under the European Union's Seventh Framework Programme (FP7/2007-2013); Contract grant number: 249930-CoralWarm: Corals and global warming: the Mediterranean versus the Red Sea. Contract grant sponsors: Marine and Freshwater Science Group Association (www.msgassociation.net) and the Ministry of Education, University and Research (MIUR).

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Received 16 January 2012; Revised 23 March 2012;
Accepted 6 April 2012

Published online 18 June 2012 in
Wiley Online Library (wileyonlinelibrary.com)
DOI: 10.1002/jmor.20039

of information on the biology and reproductive ecology of scleractinians exceeds that of some other groups of marine invertebrates and therefore provides an important model for the understanding of their evolution and life cycles (Harrison, 2011). Complex and sometimes controversial evolutionary forces are the basis for sexual determination in plants and animals. These may present the same sex throughout their lifetime, or change from one functional sex to another, displaying phenomena of sexual inversion (Loya and Sakai, 2008). Reaching sexual maturity depends on a balance between growth and risk of death, which is linked to the age and size of the organisms. Variations in age and size at the first reproductive event and differences in the "sex ratio" influence population growth rates (Fujiwara and Caswell, 2001). These variations are important, as they may represent the beginning of evolutionary divergences (Richmond and Hunter, 1990).

Various studies performed in the 1970s and 1980s showed that in several ovoviviparous anthozoa planulae production can be derived by asexual reproductive processes (Ottaway and Kirby, 1975; Black and Johnson, 1979; Stoddart, 1983; Ayre and Resing, 1986), contradicting the assumption that these are only of sexual origin (Hyman, 1940; Connell, 1973). The selective advantages of sexual versus asexual reproduction change in different conditions, and the energy allocation intended for each reproductive strategy can reflect changes in the environment (Bradshaw, 1965; Jackson and Coates, 1986; Stearns, 1992). Reproductive flexibility and its effect on the structure of the population are often generalized in life-history theory. Theoretical models suggest under favorable conditions and low stress levels, energy investment in asexual propagation predominates (Williams and Mitton, 1973; Warner, 1975; Williams, 1975). Such asexual reproduction would generate a clonal line that might contribute to keeping populations inside the area of the parental habitat, thus propagating well-adapted genotypes at the local level. On the other hand, when local conditions are unfavorable and stress levels are high, more energy will be invested in sexual reproduction and dispersion (Warner, 1975; Williams, 1975; Carvalho, 1994). This produces a genotypically different lineage, which might enable a wide dispersion or recolonization of more heterogeneous habitats (Williams, 1975; Maynard Smith, 1978), thus contributing to an increase in the fitness and survival of the species (Harrison, 2011). Sherman et al. (2006) state that the relationship between stability of the habitat and genetic diversities might be far more complex than has been theorized. According to these authors, asexual reproduction may be an adaptation that allows the exploitation of newly available substrata after a disturbance event. The availability of suitable space after a disturbance event may allow for the rapid recolonization of these areas by the localized recruitment of asexually generated larvae from surviving colonies (Sherman et al., 2006). Gilmour (2002a) observed that the Australian population of the *Fungia fungites* coral, exposed to a high rate of chronic sedimentation, shows up to 30% of asexually derived polyps. The population of *Fungia scutaria*, common in very rough shallow water, shows a more marked asexual budding compared with populations of *Fungia granulosa*, common in calm, deep water, suggesting that the evolution of distinct reproductive strategies in closely correlated species might in part be the consequence of different environmental constraints (Kramarsky-Winter and Loya, 1998; Goffredo and Chadwick-Furman, 2003).

Changes in several biological processes, for example, the consequences of climate changes, are already evident in several ecosystems (Harley et al., 2006). Increases in temperature may cause alterations in gamete release into the environment (Lawrence and Soame, 2004), as well as in the quality of the eggs and survival of the larvae (McClanahan et al., 2009; Randall and Szmant, 2009). The repercussions of climate change are expected to be greater in the temperate and high-latitude zones (Solomon et al., 2007), with marked consequences in organisms that display seasonality in gonadic development (Lawrence and Soame, 2004). Therefore, sexual reproductive processes are sensitive to a wide range of natural and anthropogenic stress factors, which impair or block the critically important phases of reproduction and recruitment required to maintain and replenish coral populations (Harrison, 2011). Without sexual recombination, these populations have little chance of adapting to changes in environmental conditions and, in particular, ocean warming (van Woesik, 2009). Considering that the reproduction of coral seems more sensitive to stress in comparison with other vital functions (Harrison and Wallace, 1990), the presence of ecologically appropriate environmental conditions is essential to ensure reproductive success (Harrison, 2011).

This study, which is part of the European project FP7-IDEAS ERC "Corals and global warming: the Mediterranean versus the Red Sea," aims to investigate the reproduction of *Caryophyllia inornata* (Fig. 1) in the northern Tyrrhenian Sea to increase knowledge on Mediterranean scleractinian corals, key organisms of the brother project. We describe the morphological aspects of gametogenesis and embryonic development, defining the sexual condition, sex-ratio and reproductive mode of *C. inornata*.

The Caryophylliidae family is ubiquitous, formed both by solitary and colonial corals and includes 296 living species divided into 51 genera (Cairns, 1999; Kitahara et al., 2010). Nine species live in the Mediterranean Sea, grouped into five genera

Fig. 1. *Caryophyllia inornata*. Specimens photographed at Elba isle (Leghorn, 42°45′N and 10°24′E). [Color figure can be viewed in the online issue, which is available at wileyonline library.com.]

(Minelli et al., 1995); four of these (*Caryophyllia*, *Ceratotrochus*, *Paracyathus*, and *Trochocyathus*) are solitary (Baird et al., 2009). *Caryophyllia* Lamarck, 1801 is exclusively an azooxanthellate genus and contains 66 species (Kitahara et al., 2010), including *C. inornata* (Cairns, 1999).

The distribution of *C. inornata* is found in the eastern and western part of the Mediterranean sea (Zibrowius, 1980) and extends up to the north-eastern Atlantic coasts (Cairns, 1999), from the Canary Islands to the North Sea (Zibrowius, 1980). It colonizes caves, walls, and wrecks, from the surface down to 100 m deep in dimly lit environments. It is one of the main species that populate the walls and the vaults of caves and in some cases is the dominant species (Zibrowius, 1978).

MATERIALS AND METHODS

Sampling

Samples of *C. inornata* were collected from the aircraft wreck of Elba Isle (Leghorn, Tuscany, 42°45′N and 10°24′E), during 18 monthly collections from May 2009 to October 2010. Using SCUBA, 20 polyps were collected each month, at a depth of 12–15 m. In this study, over 315 polyps were collected in 18 monthly dives. Water temperature was continuously recorded in the field by underwater digital thermometers, and at the time of each specimen sampling with mercury thermometers. The mean population density in the sampling site was 6025 ± 898 individuals m^{-2}, corresponding to 1.669 ± 358 kg m^{-2} of calcium carbonate. Bed coverage was 15.3 ± 2.5%. The color of the polyps varied from pink to brownish. Photoperiod was obtained from the online database EuroMETEO® (http://www.eurometeo.com). Polyps were fixed in saturated formalin solution (10% formaldehyde and 90% seawater; the solution was saturated with calcium carbonate) and transferred to the laboratories for histological analysis.

Biometric Analysis

Histological analysis was performed on 72 polyps (Table 1). The biometric analysis of each polyp was performed by measuring the length (L, major axis of the oral disk), the width (w, minor axis of the oral disk), and height (h, oral–aboral diameter; Fig. 2). The body volume (V) was calculated using the equation: $V = h * (L/2) * (w/2) * \pi$ (Goffredo et al., 2002).

Histological Analysis

Polyps were postfixed in Bouin solution. After decalcification in ethylenediamine tetra acetic acid and dehydration in a graded alcohol series from 80 to 100%, polyps were embedded in paraffin, and serial transverse sections were cut at 7 μm intervals along the oral–aboral axis, from the oral to the aboral poles. Tissues were then stained with Mayer's hematoxylin and eosin.

Cytometric Analysis

Histological observations were made using a light microscope NIKON Eclipse 80i. Cytohistological readings were made with a two image analysis systems: NIKON NIS-Elements D 3.1 and LEICA Q500IW. The maximum and minimum diameters of the spermaries and oocytes in nucleated sections were measured and classified into developmental stages in accordance with earlier studies on gametogenesis in scleractinians (Goffredo et al., 2005, 2010). The presence of embryos in the gastrovascular cavity and mesenterial septa were recorded, and their stage of maturation was identified (Goffredo and Telò, 1998; Goffredo et al., 2005). The size of each reproductive element was determined as the mean of the two diameters (Goffredo et al., 2005, 2010).

The following definitions were used: sexually active polyps, individuals that display gametogenetic activity; spermatogenetic polyps, individuals that display spermaries; oogenetic polyps, individuals that display oocytes; embryogenetic polyps, individuals that display embryos; sexually inactive polyps, individuals that do not display gametogenetic activity.

RESULTS

Sexuality

The sexually active individuals had either oocytes or spermaries; no individual displayed both types of germ cells. Sexual dimorphism was not observed, significant differences were not found in the mean size of spermatogenetic and oogenetic individuals (Student's t-test for L: $t = 1.423$, df = 35, $P = 0.255$; Student's t-test for V: $t = 2.705$, df = 35, $P = 0.073$; Table 2). The sex ratio of sexually active polyps was significantly different from 1 with a 1:3.1 ratio that favored spermatogenetic individuals (chi-square test, $\chi^2 = 9.76$, df = 1, $P < 0.01$). Embryos were found in the coelenteric cavity and/or inside mesenterial septa of 76.4% of the polyps analyzed, suggesting internal development (Table 1). Embryos were identified in all monthly samples and inside oogenetic, spermatogenetic, and inactive individuals. All nine oogenetic polyps had embryos (L = 7.7 ± 0.4 mm; V = 347.1 ± 45.1 mm^3; means ± SE; Table 2). Of the 28 spermatogenetic polyps, 24 had embryos (L = 7.2 ± 0.2 mm; V = 251.5 ± 16.0 mm^3; means ± SE; Table 2) and four were without embryos (L = 6.7 ± 0.5 mm; V = 256.7 ± 19.8 mm^3; means ± SE; Table 2). Of the 35 inactive polyps, 28 were embryogenetic (L = 8.4 ± 0.3 mm; V = 394.64 ± 0.8 mm^3; means ± SE; Table 2) and 7 did not show embryos (L = 6.9 ± 0.6 mm; V = 317.7 ±

TABLE 1. Caryophyllia inornata—Size, sex condition, and reproductive state of analyzed polyps

Date	Polyp code	L (mm)	w (mm)	h (mm)	V (mm^3)	Reproductive state	Oocytes	Spermaries	Embroys			Notes
									early	intermediate	late	
	CI-140509-P1	8.40	7.10	9.05	423.91	I	—	—				R
	CI-140509-P2	7.20	6.45	11.20	408.51	S	—	285	—	6	23	
	CI-140509-P3	5.35	5.00	10.00	210.09	S	—	8	—	—	—	
	CI-140509-P4	7.00	6.20	9.00	306.78	S	—	131	—	—	—	
	CI-140509-P5	6.70	6.35	6.10	203.83	S	—	43	5	16	13	
	CI-140509-P6	7.05	6.40	6.65	235.66	S	—	—				R
	CI-140509-P7	6.25	5.30	6.70	174.31	S	—	27	—	8	9	
	CI-140509-P8	7.00	6.10	8.10	271.65	I	—	—	—	27	8	
14th May 09	CI-140509-P10	6.05	5.30	5.80	146.07	S	—	647	—	4	15	
	CI-140509-P12	9.60	8.10	13.50	824.48	I	—	—	—	—	—	
	CI-140509-P13	8.00	7.45	8.55	400.22	O	2016	—	—	14	36	
	CI-140509-P14	5.00	5.80	7.70	175.38	S	—	124	—	—	12	
	CI-140509-P17	9.45	8.35	10.30	638.33	I	—	—	1	2	18	
	CI-140509-P19	7.65	6.70	7.05	283.80	S	—	909	2	11	7	
	CI-140509-P22	7.35	6.30	7.30	265.49	S	—	—				R
	CI-140509-P23	7.00	6.80	8.10	302.82	S	—	1800	—	1	5	
	CI-140609-P1	7.50	6.20	7.25	264.78	I	—	—				R
	CI-140609-P2	8.05	8.00	9.05	457.75	O	865	—	2	—	61	
	CI-140609-P7	7.35	5.30	10.00	305.95	S	—	442	—	1	30	
14th Jun 09	CI-140609-P10	5.85	5.35	7.25	178.21	O	56	—	2	—	1	
	CI-140609-P12	6.90	5.60	5.40	163.88	S	—	770	—	3	8	
	CI-140609-P13	6.45	5.65	8.40	240.42	O	315	—	1	3	25	
	CI-120709-P1	8.15	7.05	10.65	480.60	I	—	—	—	—	4	
	CI-120709-P2	8.20	7.90	10.70	544.40	O	79	—	—	—	3	
12th Jul 09	CI-120709-P5	8.30	7.80	10.40	528.81	I	—	—	—	—	2	
	CI-120709-P6	7.40	5.35	8.35	259.63	S	—	2	—	—	—	
	CI-120709-P14	5.15	4.60	6.40	119.08	I	—	—	—	—	—	
	CI-140809-P1	8.20	7.80	10.00	502.34	I	—	—	—	—	—	
	CI-140809-P2	6.80	7.15	7.20	274.94	I	—	—	—	—	—	
	CI-140809-P3	6.20	7.30	9.40	334.14	I	—	—	—	1	1	
14th Aug 09	CI-140809-P4	7.15	7.40	8.15	338.68	I	—	—	—	—	—	
	CI-140809-P5	7.60	7.00	8.75	365.60	S	—	39	—	1	—	
	CI-140809-P6	7.70	6.30	4.95	188.59	I	—	—	3	2	18	
	CI-170909-P2	10.25	9.00	11.15	807.85	I	—	—				R
	CI-170909-P3	7.25	6.25	8.00	284.71	I	—	—	4	—	2	
17th Sep 09	CI-170909-P4	8.35	7.20	6.70	316.36	I	—	—	1	—	—	
	CI-170909-P5	11.70	9.30	7.35	628.12	I	—	—	—	2	5	
	CI-170909-P6	6.90	6.30	5.90	201.43	I	—	—	—	1	—	
	CI-191009-P1	8.45	6.30	8.10	338.67	I	—	—	2	6	6	
	CI-191009-P3	7.25	6.25	8.00	871.54	I	—	—	—	1	—	
19th Oct 09	CI-191009-P4	8.35	7.20	6.70	297.55	I	—	—	3	1	1	
	CI-191009-P5	11.70	9.30	7.35	142.35	S	—	1	—	3	1	
	CI-191009-P7	5.40	4.70	3.40	67.77	I	—	—	—	—	—	
	CI-181109-P1	5.35	5.00	4.60	96.64	I	—	—	—	—	—	
	CI-181109-P2	7.05	6.25	6.10	211.10	S	—	7	1	—	16	
18th Nov 09	CI-181109-P3	7.55	6.85	7.00	284.33	I	—	—	—	—	8	
	CI-181109-P4	8.10	7.25	7.05	325.16	I	—	—	—	—	4	
	CI-151209-P1	8.45	7.55	6.95	348.24	S	—	71	2	8	21	
	CI-151209-P2	6.65	6.55	6.30	215.52	S	—	5	2	1	2	
15th Dec 09	CI-151209-P3	7.65	6.70	5.65	227.44	S	—	37	—	—	10	
	CI-151209-P4	7.20	6.35	6.20	222.63	I	—	—	1	—	2	
	CI-130110-P2	9.15	6.90	5.55	275.20	I	—	—	—	—	3	
	CI-130110-P4	7.70	6.60	7.30	291.37	I	—	—				R
13th Jan 10	CI-130110-P5	6.45	5.75	5.30	154.38	I	—	—	—	2	3	
	CI-130110-P7	6.00	5.05	4.35	103.52	I	—	—	1	—	5	
	CI-130110-P10	10.00	8.10	7.45	473.95	I	—	—	—	8	51	
	CI-070210-P1	8.20	6.95	6.00	268.56	I	—	—	4	1	1	
	CI-070210-P2	6.60	6.50	7.15	240.91	S	—	4	2	9	12	
07th Feb 10	CI-070210-P3	6.90	5.95	8.50	274.08	O	3	—	—	—	3	
	CI-070210-P8	7.35	6.40	4.65	171.79	S	—	20	—	—	8	
	CI-120310-P1	7.00	6.70	6.80	250.48	S	—	74	—	—	—	
	CI-120310-P3	11.10	8.75	11.55	881.05	I	—	—	—	22	43	
12th Mar 10	CI-120310-P4	11.60	8.55	8.55	666.01	I	—	—	4	45	69	
	CI-120310-P6	10.00	9.00	7.35	519.54	O	110	—	—	3	14	
	CI-180410-P1	7.75	6.70	7.30	297.71	S	—	1285	—	—	11	
	CI-180410-P2	7.35	6.40	4.65	171.79	S	—	913	—	8	45	

TABLE 1. Caryophyllia inornata—Size, sex condition, and reproductive state of analyzed polyps (continued)

Date	Polyp code	L (mm)	w (mm)	h (mm)	V (mm^3)	Reproductive state	Oocytes	Spermaries	early	intermediate	late	Notes
18th Apr 10	CI-180410-P3	6.75	6.20	6.85	225.15	I	—	—	—	1	9	
	CI-180410-P6	8.60	7.15	7.55	364.62	S	—	1922	—	3	28	
	CI-200510-P1	7.05	6.30	7.00	244.18	O	1573	—	1	4	8	
	CI-200510-P3	7.70	6.65	6.25	251.35	S	—	638	—	—	5	
20th May 10	CI-200510-P5	8.55	7.45	5.30	265.15	O	304	—	—	2	60	
	CI-200510-P6	9.40	7.65	6.40	361.46	S	—	2966	—	—	26	

L: major axis of the oral disk; *w*: minor axis of the oral disk; *h*: oral—aboral diameter; *V*: body volume; O: oogenetic polyp; S: spermatogenetic polyp; I: inactive polyp; R: quantitative analysis not performed, presence of embryos confirmed.

102.8 mm^3; means ± SE; Table 2). The mean sizes of the seven inactive polyps without embryos were not significantly different from those of the 37 sexually active polyps analyzed (Student's *t*-test for *L*: $t = 0.793$, df = 42, $P = 0.592$; Student's *t*-test for *V*: $t = 0.746$, df = 42, $P = 0{,}697$; Table 2). The mean sizes of the inactive polyps with embryos were significantly greater than those of the embryogenetic sexually active individuals (Student's *t*-test for *L*: $t = 3.626$, df = 63, $P = 0.001$; Student's *t*-test for *V*: $t = 2.975$, df = 63, $P = 0.010$; Table 2). Quantitative cytohistometric analysis was performed on the nine oogenetic polyps observed, on 26 of the 28 spermatogenetic polyps, and on 31 of the 35 embryogenetic sexually inactive polyps.

Male Gametogenesis

The spermaries were located in the mesenterial septa and were made up of groups of germ cells and delineated by mesogleal envelope (Fig. 3). A total of 13,170 spermaries were identified and measured. Five stages of maturation were identified:

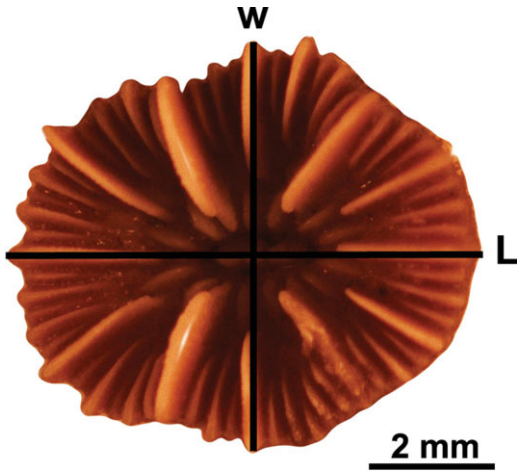

Fig. 2. *Caryophyllia inornata*. Specimens photographed in the laboratory (*L*: major axis, *w*: minor axis of the polyp). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Stage I—undifferentiated germ cells arose in the gastrodermis and then migrated toward the mesoglea of the mesentery where they regrouped forming the spermary. The spermary was made up of an early aggregation of spermatogonia (Fig. 3B). Spermaries had a mean diameter of 28.36 ± 0.77 μm, $N = 152$.

Stage II—the spermary was made up of a group of spermatocytes undergoing meiosis. The mesogleal layer enveloping the spermary had not yet formed a wall. (Fig. 3C). Spermary mean diameter was 51.84 ± 0.88 μm, $N = 530$.

Stage III—the spermary, still made up of a group of spermatocytes undergoing meiosis, was delineated by a clearly differentiated wall formed by the mesoglea (Fig. 3D). Spermary mean diameter was 83.56 ± 0.50 μm, $N = 5{,}791$.

Stage IV—the spermary showed a centripetal maturation gradient: less mature and larger germ cells (spermatocytes) were located at the periphery of the spermary, whereas more mature and smaller ones (spermatids) were located in the center (Fig. 3E,F). Spermary mean diameter was 94.39 ± 0.76 μm, $N = 4{,}310$.

Stage V—the spermary was made up of a mass of spermatozoa with their tails all facing in the same direction (an arrangement known as a "bouquet"; Fadlallah and Pearse, 1982; Fig. 3G). Sper-

TABLE 2. Caryophyllia inornata—mean sizes and standard error of sexually active polyps (S: spermatogenetic, S + E: spermatogenetic with embryos, O: oogenetic, and O + E: oogenetic with embryos) and sexually inactive (I: inactive and I + E: inactive with embryos)

Reproductive state	*L* (mm)	*V* (mm^3)
Sexually active	7.3 ± 0.2 ($N = 37$)	275.3 ± 16.3 ($N = 37$)
S	6.7 ± 0.5 ($N = 4$)	256.7 ± 19.8 ($N = 4$)
S + E	7.2 ± 0.2 ($N = 24$)	251.5 ± 16.0 ($N = 24$)
O	—	—
O + E	7.7 ± 0.4 ($N = 9$)	347.1 ± 45.1 ($N = 9$)
Sexually inactive	8.1 ± 0.3 ($N = 35$)	379.2 ± 38.2 ($N = 35$)
I	6.9 ± 0.6 ($N = 7$)	317.7 ± 102.8 ($N = 7$)
I + E	8.4 ± 0.3 ($N = 28$)	394.6 ± 40.8 ($N = 28$)

L: major axis of the oral disk of the polyp, *V*: body volume of the polyp, and *N*: number of polyps examined.

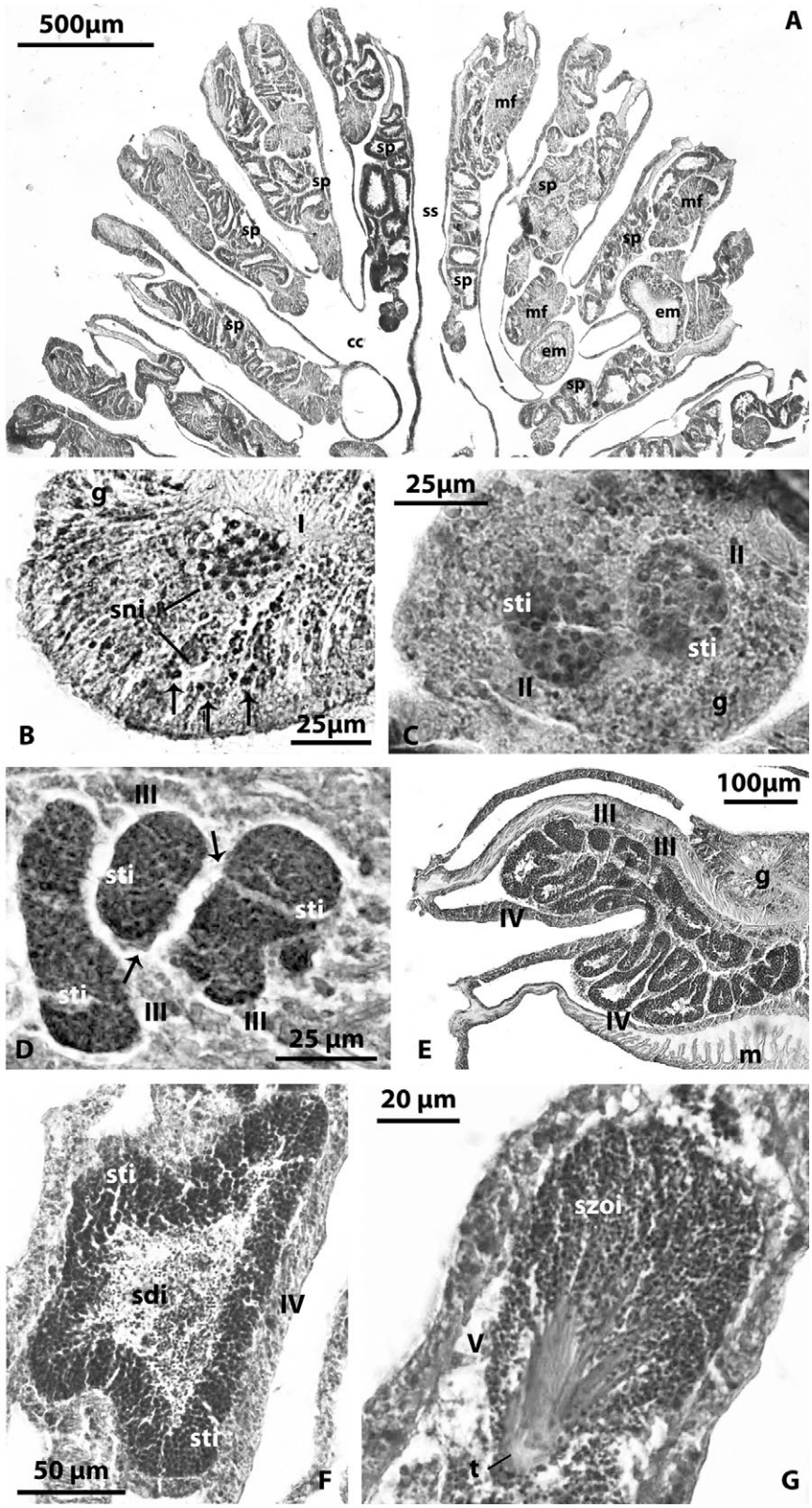

Fig. 3. *Caryophyllia inornata*. Spermatogenesis. **A**: Localization of the spermaries in the mesenterial septa. **B**: Stage I: undifferentiated germ cells disposed in the gastrodermis layers of the mesentery (arrow). The spermaries are made up of a group of spermatogonia. **C**: Stage II: the spermaries are made up by spermatocytes involved in the process of meiosis. **D**: Stage III: the spermary, containing spermatocytes undergoing meiosis, is delineated by a wall that has arisen from the mesoglea (arrows). **E**: Spermaries of stages III and IV located in the mesentery. **F**: Stage IV: the spermary presents an external layer of spermatocytes and an internal mass of spermatids, recognizable by the presence of a tail. **G**: Stage V: the spermary is made up of a mass of spermatozoa. Shortly before leaving the spermary, mature spermatozoa form a bouquet, with their tails all facing in the same direction (arrow). [cc: coelentric cavity; m: mesoglea; ss: skeletal septum; mf: mesenterial filament; sp: spermary; em: embryo; g: gastrodermis; sni: spermatogonia; sti: spermatocytes; sdi: spermatids; szoi: spermatozoa; t: spermatozoa tails; I, II, III, IV, V: spermary developmental stage].

mary mean diameter was 102.19 ± 1.09 μm, N = 2,387.

Female Gametogenesis

The oocytes were oval-shaped and located in the mesenteries (Fig. 4). A total of 5,321 oocytes were identified and measured. The diameter of the oocytes ranged from 11.63 to 141.16 μm, and their mean diameter was 68.75 ± 0.26 μm, N = 5,321. The early stages of oogenesis were visible in the mesentery's gastrodermal layers. Early oocytes had a centrally located spherical nucleus and a high ratio of nucleus to cytoplasm (Fig. 4B). In the intermediate stages, the oocytes still presented a spherical nucleus and the nucleus/cytoplasm ratio decreased due to the accumulation of yolk (Fig. 4C). In the more advanced stages, the nucleus/cytoplasm ratio was further reduced due to the accumulation of yolk (Fig. 4D,E). The nucleus had also migrated to the cell's periphery, and adhering closely to the cell membrane it changed shape, becoming indented and concave (Fig. 4D,E). During oogenesis, the nucleolus was always positioned on the periphery of the nucleus (Fig. 4D,E).

Oral–Aboral Distribution of Gametogenic Processes

Distribution of the germ cells along the oral–aboral axis was significantly different between spermatogenetic and oogenetic polyps (Fig. 5). Although the size of the spermaries was correlated negatively with the distance from the oral pole, that of the oocytes correlated positively (Fig. 6). The mean distance of the spermaries from the oral pole (58.05 ± 0.11%) was significantly greater with respect to that of the oocytes (54.11 ± 0.15%; Student's t-test, t = 20.53; df = 18,489; $P < 0.001$; Fig. 5). Both in spermatogenetic individuals and in oogenetic ones, a third of the polyps corresponding to the oral pole were nongametic.

Embryonic Development

The embryos were located both in the mesenterial septa, inside the mesoglea layer wrapped in the gastrodermis, and in the gastrovascular cavity of oogenetic, spermatogenetic and sexually inactive individuals (Fig. 6A,C,D). A total of 1,056 embryos were identified and measured. Development proceeded through the formation of embryos that did not show a blastocoel cavity (Fig. 6B). Often, early embryos located in the gastrovascular cavity seemed in close morphological continuity with mesenterial septa or detached from it (Fig. 6A detail). Furthermore, early embryos were located also inside the body of embryos in a more advanced stage of development, showing continuity with the host tissues (Fig. 6E). The diameter of the early embryos ranged from 58.49 to 308.08 μm. The mean diameter was 136.27 ± 7.09 μm, N = 44. During the intermediate stage, called stereogastrula, the ectoderm was clearly distinct from the endoderm. The ectodermal layer, formed by multiple layers of cells, seemed clearly differentiated and separate from the endodermal central mass by a well-defined mesoglea layer (Fig. 6F). During the advanced stages of development, the stereogastrula showed an invagination of the ectodermal cells, which led to the formation of the stomodeum (Fig. 6H–J) and the differentiation of the mesenterial septa by the invagination of the mesoglea layer toward the center of the embryo (Fig. 6G). The diameter of the stereogastrula ranged from 82.64 to 853.68 μm. The mean diameter was 307.39 ± 4.21 μm, N = 1,012.

DISCUSSION

Sexuality

All the sexually active polyps examined contained only a single type of germ cell, indicating that *C. inornata* may be gonochoric. Most hermaphrodites are simultaneous (Harrison, 2011), that is, the same organisms develop mature oocytes and spermaries at the same time (Policansky, 1982). Additionally, male and female individuals of *C. inornata* do not display significantly different sizes, which also suggests that the species is gonochoric as, according to Harrison (2011), organisms with gonochoric sexuality do not show any relationship between sex and coral size. Sequential hermaphrodites may exhibit sex change over successive breeding seasons or over their lifetime (Ghiselin, 1974; Policansky, 1982). The direction of sex change (protandrous or protogynous) is determined by the relative reproductive success over the course of a lifetime for the two sexes. The optimal size at sex change is when the potential subsequent lifetime reproductive output as the second sex exceeds that of remaining as the first sex. Charnov's theory of sex allocation (1982) predicts that sex change is favored when reproductive success (fitness) increases more quickly with size or age in one sex than in the other. True protandrous sex change from initial male function in small corals to female function in larger corals has recently been demonstrated for some fungiid mushroom corals (Loya and Sakai, 2008; Loya et al., 2009). In three deep water *Caryophyllia* species, a cyclic hermaphroditic sexuality has been observed. In cyclic hermaphroditism, gonadal development is asynchronous and germ cell maturation does not have seasonality. Male and female germ cells, at different stages of maturation, were identified in the mesentery of the same individual in all the samples of the three species (Waller et al., 2005). In *C. inornata,* mature opposite sex gametes were observed in the same period of the year, always in

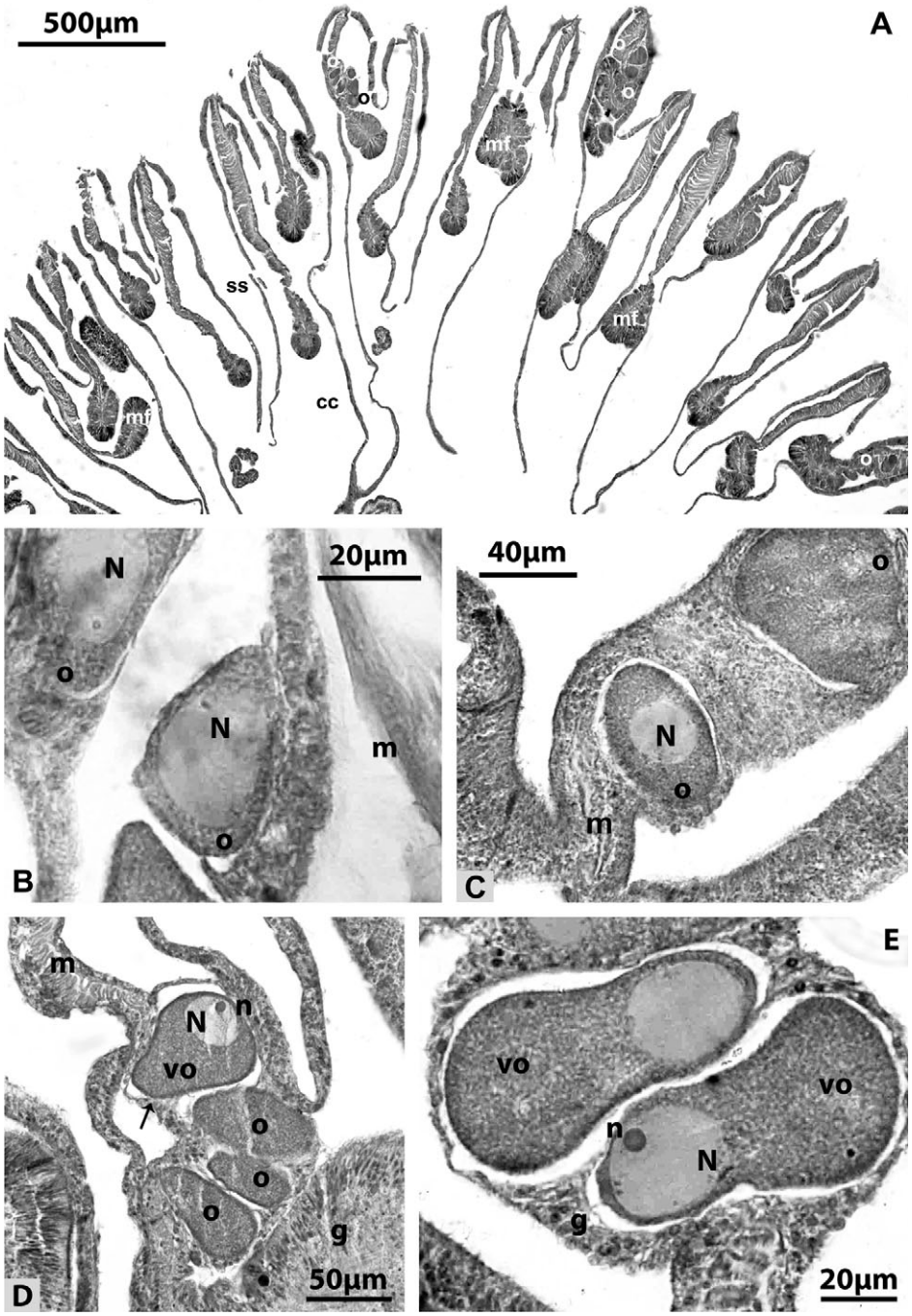

Fig. 4. *Caryophyllia inornata*. Oogenesis. **A**: Localization of the oocytes in the mesenterial septa. **B**: Early stage: two previtellogenic oocytes in the gastrodermis of the mesentery, characterized by a high nucleus/cytoplasm ratio. **C**: Intermediate stage: vitellogenic oocyte located in the mesoglea. The spherical-shaped nucleus is still located in the central portion of the cell. The nucleus/cytoplasm ratio is reduced. Note a larger vitellogenic oocyte in the same section. **D**: Late stage: the nucleus of the oocyte has started to migrate toward the cell's periphery. Note the distinct membrane of the oocyte (arrow). **E**: Late stage: two mature oocytes, located in the mesentery; the nucleus/cytoplasm ratio is greatly reduced. [cc: coelentric cavity; mf: mesenterial filament; ss: skeletal septum; o: oocyte; m: mesoglea; N: nucleus; n: nucleolus; vo: vitellogenic oocyte; g: gastrodermis].

different individuals, showing a marked seasonality and synchronicity, according to the pattern expected for gonochoric conditions.

In the Caryophylliidae family, the gonochoric condition is predominant (7 species out of 11 studied; Baird et al., 2009; Harrison, 2011). Whereas in the genus *Caryophyllia* only one other case of gonochorism is known, *Caryophyllia smithii* (Hiscock and Howlett, 1977; Tranter et al., 1982), three cases of hermaphroditism are known for the deep

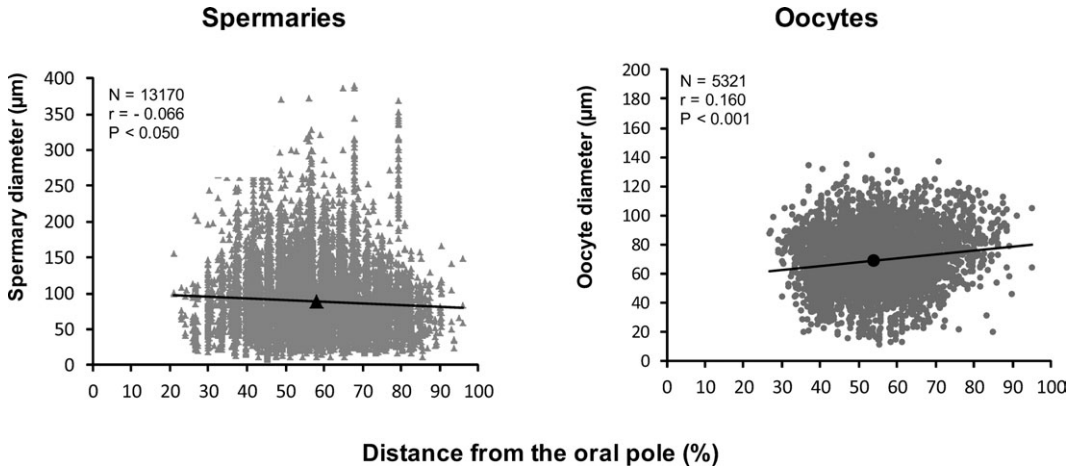

Fig. 5. *Caryophyllia inornata.* Distribution according to size along the oral–aboral axis of spermaries in male polyps and oocytes in female polyps. The distance from the oral pole is expressed as a percentage: 0% = at oral pole level and 100% = at aboral pole level. ▲: the point at which mean spermary distance (58.05 ± 0.11%; mean ± SE) and mean spermary size (88.57 ± 0.40 µm) intersect; •: the point at which mean oocyte distance (54.11 ± 0.15%) and mean oocytes size (68.75 ± 0.26 µm) intersect. Note that the value ranges on the ordinate axes are different.

species, *Caryophyllia ambrosia, Caryophyllia sequenzae*, and *Caryophyllia cornuformis* (Waller et al., 2005). The data available for scleractinians indicate that the sexual condition tends to be constant within genera or families (Harrison, 2011). Of the 105 genera of scleractinians where data of sexual condition are available, 50 contain only hermaphroditic species, 38 only gonochoric species, whereas the remaining 17 genera presented species with both sexual conditions. The genus *Caryophyllia,* displaying mixed sexuality, falls into a particularly interesting group. Szmant (1986) observed that most scleractinians are hermaphroditic, thus suggesting that hermaphroditism is an ancestral condition. Nevertheless, in a more recent morphological and molecular analysis, Daly et al. (2003) found that gonochorism is the ancestral condition of anthozoa hexacorallia, including scleractinians. Supporting these considerations a phylogenetic analysis of the reproductive properties concluded that gonochorism is the ancestral sexual condition of scleractinians. The reproductive mode, instead, evolves faster than sexual condition, and it is too variable between taxa to find the ancestral state among scleractinians (Baird et al., 2009; Kerr et al., 2011).

The sex ratio in a population with random mating is normally 1:1 (Maynard-Smith, 1978). A number of additional forces could play an important role in the deviation from this rule, such as errors during sampling or a clonal propagation (Harrison and Wallace, 1990). While sampling errors may have occurred, they are unlikely given large sample size of 15–20 individuals collected randomly from the population for each monthly sample (over 315 polyps in 18 months). The sex ratio skewed, however, in favor of males, which might therefore be explained by clonal propagation. A sex ratio favoring males has also been shown in other solitary scleractinians of the Fungidae family: *F. scutaria, Diaseris distorta, Fungia concinna, F. fungites* where clonal propagation is well known to occur (Kramarsky-Winter and Loya, 1998; Colley et al., 2000; Gilmour, 2002a,b). Agamic propagation is known in other caryophylliids, where 27 genera are colonial and the remaining 24 solitary (Cairns, 1999). Clonal propagation in the Caryophylliidae family has not still been documented. The morphological profiles described in this study suggest that clonal propagation might occur in the formation of new propagules. Eighty-five point seven per cent of spermatogenetic individuals (males) and 80.0% of sexually inactive individuals presented embryos at different stages of maturation and this production of embryos appeared throughout the entire year and was not characterized by a clear seasonal pattern, as would normally be expected in a reproductive model based on an annual cycle of sexual reproduction. The asexual production of brooded planulae has also been shown in some populations of *Pocillopora damicornis* (Stoddart, 1983), sometimes in combination with gametogenetic activity (Ayre and Miller, 2004; Sherman et al., 2006; Yeoh and Dai, 2010), in *Tubastrea coccinea* (Ayre and Resing, 1986; Glynn et al., 2008), and *Tubastrea diaphana* (Ayre and Resing, 1986). *Oulastrea crispata* is of particular interest, as it can also produce embryos asexually in the period when sexual reproduction ends (Nakano and Yamazoto, 1992; Lam, 2000). Embryogenetic sexually inactive individuals of *C. inornata* were larger in size than the embryogenetic sexually active ones. These polyps might be sexually old individuals that preserve the ability

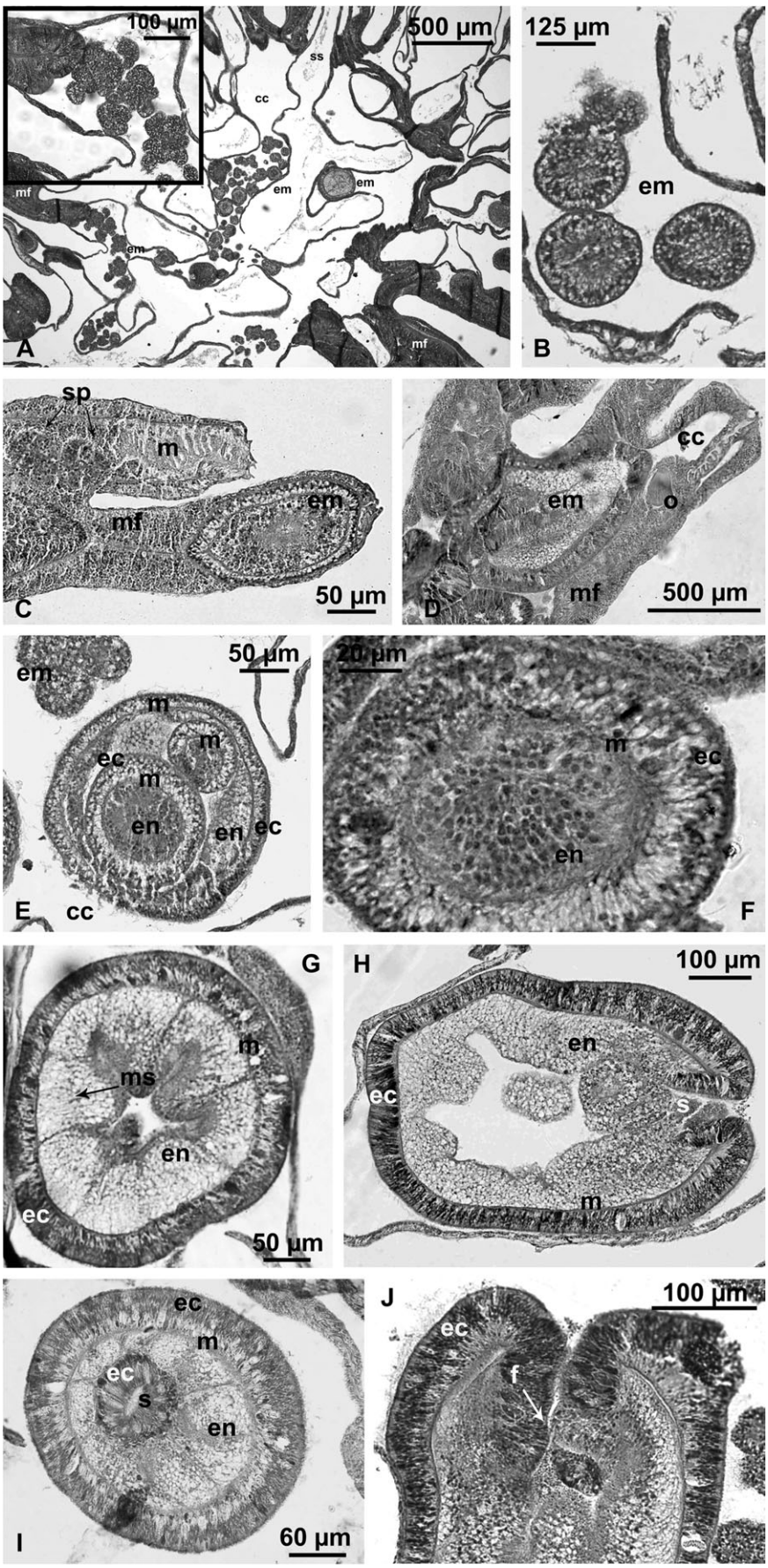
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Figure 6.

to produce embryos agamically. In the scleractinian corals *Stylophora pistillata,* senescence was indicated by the observation of a gradual reduction in the physiological processes of growth and reproduction before the natural death of the colony (Rinkevich and Loya, 1986). Bosch (2009) states that senescence can be observed in metazoa, indicating a progressive decline in physiological functions leading to an increase in the death rate. The same author has observed that degenerative processes in *Hydra* sp., such as decline in reproductive sexual activity, were observed as a clear sign of ageing. To escape population mortality and senescence these organisms reproduce exclusively asexually by budding. The production of asexual brooded planulae by locally adapted genotypes might increase local recruitment and survival in some species (Williams, 1975), whereas sexual reproduction in these species might increase colonization of distant reefs. The high population density of *C. inornata,* in the order of thousands of individuals per m^2, might be the consequence of asexual planulae production whereas the small-sized oocytes and consequent planktotrophic development might promote dispersion and colonization of distant areas. An extensive dispersal minimizes the likelihood of extinction: if local conditions have deteriorated, planktotrophic larvae have an effective means of escape, and this can generally postpone their metamorphosis in the absence of specific environmental cues until not found an appropriate habitat (Pechenik, 1999).

Male Gametogenesis

The morphological aspects of male gametogenesis in *C. inornata* correspond to those of other species of the genus *Caryophyllia,* described in hermaphroditic corals with external fertilization (*C. ambrosia*, *C. sequenzae*, and *C. cornuformis;* Waller et al., 2005) and to those of gonochoric *C. smithii*, found with external fertilization for some populations (Tranter et al., 1982) and with internal fertilization for others (Hiscock and Howlett, 1977). Within the Caryophylliidae family, *C. inornata* has the morphological profiles of male gametogenesis like those of the colonial gonochoric species, *Lophelia pertusa* (Waller et al., 2005) as well as those of species belonging to other families: for example, *Fungiacyathus marenzelleri* (gonochoric and brooding, Fungiacyathidae; Waller et al., 2002), the colonial *A. calycularis* (gonochoric and brooding, Dendrophylliidae; Goffredo et al., 2010), *Mussimilia hispida* (hermaphroditic and broadcasting, Mussidae; Neves and Pires, 2002) and in species of the genus *Madracis* sp. (hermaphroditic and brooding Pocilloporidae; Vermeij et al., 2004).

Female Gametogenesis

The morphological profiles of female gametogenesis in *C. inornata* are essentially similar to those described for other species of the same genus. Waller et al. (2005) noticed that in the genus *Caryophyllia* the size of the mature oocyte increases with increases in depth characterizing the habitat of the species. Large oocytes and consequent lecithotrophic development are currently recognized as an adaptation to oligotrophic environments such as abyssal ones (Shilling and Manahan, 1994). Most of the deep water scleractinians studied up to now have lecithotrophic larvae (Burgess and Babcock, 2005). *C. inornata* has relatively small oocytes similar to those of *C. smithii,* that has a depth range matching that of *C. inornata.* Other species of the same genus but from abyssal water, *C. cornuformis*, *C. sequenzae*, and *C. ambrosia* display oocytes with maximum sizes that are 2–5 times greater. The small size of the oocytes suggests planktotrophic development (Pechenik, 1999). The size of the oocytes reflects the energetic balance for dispersion, larval settlement, and metamorphosis (Dahan and Benayahu, 1998; Cordes et al., 2001). Planktotrophic larvae generally have a rather long pelagic larval phase, throughout which they feed in the column of water, and a marked ability to disperse. The production of planktotrophic larvae is often combined with high fecundity, thus increasing the probability of recruitment. The fertility estimated for *C. inornata,* despite involving a small number of samples, appears to be relatively high, in the order of thou-

Fig. 6. *Caryophyllia inornata*. Embryogenesis. **A** (detail in the rectangle): Localization of the embryos within of the gastrovascular cavity and in morphological continuity with mesenterial septa. **B**: Early embryos without blastocoel cavity. **C**: Stereogastrula (intermediate stage) located inside a mesenterial septa. Note the presence of two spermaries in the same section (arrow). **D**: Stereogastrula (intermediate stage) in the gastrovascular cavity surrounded by the mesenterial tissues, note the presence of an oocyte. **E**: Early embryos located inside an embryo in a more advanced stage of development. **F**: Stereogastrula (intermediate stage). At this stage of development, the ectoderm is clearly distinct from the endoderm. The two layers are divided by the mesoglea (arrows). **G**: Late stereogastrula, transversal section. Differentiation of the mesenterial septa due to introflexion of the mesoglea (arrow). **H**: Late stereogastrula, longitudinal section. Detail of the oral pole of the embryo showing the stomodeal invagination. **I:** Transversal section. Note the stomodeal opening, surrounded by the ectodermal layer. **J:** Detail of the late stereogastrula. Ectodermal cells have begun to multiply, forming an invagination at the embryo's oral pole. The arrow indicates the pharynx. [cc: coelentric cavity; ss: skeletal septum; mf: mesenterial filament; em: embryo; sp: spermarium; m: mesoglea; o: oocyte; ec: ectoderm; en: endoderm; ms: mesenterial septa; s: stomodeal invagination; f: pharynx.]

sands of oocytes per polyp, similar to that of *C. smithii*.

Oral–Aboral Distribution of Gametogenic Processes

The different distribution observed between spermaries and oocytes might be caused by the migration of these reproductive elements toward the oral and aboral pole, respectively, during their maturation. The oral–aboral distribution of the gametogenetic processes of *C. inornata* is similar to that found in the Dendrophylliidae *B. europaea,* a simultaneous hermaphrodite (Goffredo et al., 2002). We have hypothesized that this type of arrangement, adopted by hermaphroditic species, may decrease encounters between opposite sex gametes produced by the same individual thus serving as a "statistical barrier" to self-fertilization. (Goffredo et al., 2005). Gonochorism ensures cross fertilization. In this case, the stage of maturation and the sizes of the spermaries progressively increase toward the oral pole of the polyp, to allow dispersion of the spermatozoa into the environment. Conversely, the mature oocytes cluster at the base of the polyp, where the embryos develop.

Reproductive Mode

C. inornata is the first certain record of brooder reproductive mode in the genus. Previously, a possible instance of brooding was shown for *C. smithii* (Hiscock and Howlett, 1977) and *Caryophyllia clavus* (Fadlallah, 1983). The other three species whose reproductive mode is known, the deep species *C. cornuformis, C. sequenzae,* and *C. ambrosia* are all broadcast spawners. The reproductive mode appears to be a relatively flexible and variable characteristic in the genus *Caryophyllia* (Harrison, 2011). Shlesinger et al. (1998) suggest that brooding might be the ancestral reproductive mode in hexacorallia, but consider spawning as a derived reproductive characteristic.

Embryonic Development

In this study, oocytes were found only inside the mesentery, including those at the more mature stages of development. Embryos were also observed in the mesenterial septa, within the mesoglea layer and wrapped in the gastrodermis, and in the gastrovascular cavity. The union of the gametes might occur when the mature oocyte is still inside the mesentery. There is no evidence of a blastocoel formation during embryogenesis; embryonic development proceeds via stereoblastulae, and subsequent gastrulation occurs by delamination, giving rise in the last stages of development to fully formed embryos, with clearly differentiated mouth and pharynx and a gastrovascular cavity divided by the mesenterial septa. To the authors' knowledge, this is the first detailed description of embryogenesis in the genus *Caryophyllia*. The morphological profiles are like those observed in other solitary Mediterranean scleractinian corals (Goffredo and Telò, 1998; Goffredo et al., 2000, 2002, 2005). In general, embryonic development might be correlated to the reproductive mode: in brooding corals, physical restrictions might lead to stereoblastulae formation, whereas in the case of broadcast spawners, where there is no physical restriction, celoblastulae development might be possible (Heltzel and Babcock, 2002).

CONCLUSIONS

The population of *C. inornata* from Elba Island is gonochoric, with a sex ratio in favor of males. Mature oocytes are small-sized, suggesting a possible planktotrophic development of the larvae. Embryos, which do not have a blastocoel cavity, develop inside the mesenterial septa and the gastrovascular cavity of females, males, and sexually inactive individuals, suggesting a possible asexual origin.

ACKNOWLEDGMENTS

The authors thank F. Croci and F. Valenti for their underwater assistance in collecting the samples. The diving center Sub Maldive supplied logistic assistance in the field. The Marine Science Group (www.marinesciencegroup.org) supplied scientific, technical, and logistical support. Field coral photographs by G. Neto (www.giannineto.it). Two anonymous reviewers supplied comments that significantly improved the quality of the manuscript.

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