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REPRODUCTION IN THE PHYLUM PORIFERA: A SYNOPTIC OVERVIEW

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RESUM

Les esponges són organismes importants des del punt de vista ecològic, evolutiu i biotecnològic: *a*) tenen un paper ecològic rellevant en moltes comunitats marines i participen en passos crucials en els cicles dels nutrients solubles i la matèria orgànica, *b*) els seus trets cel·lulars i genètics encara reflecteixen i proporcionen informació sobre la transició entre la condició unicel·lular i l'organització multicel·lular dels animals i *c*) les esponges i els seus simbionts són prometedores fonts de compostos amb interès per a la biomedicina i diversos processos industrials. Per aquestes raons, enginyers, químics, microbiòlegs, ecòlegs, genètics i biòlegs evolutius, generalment amb escassa formació en esponges, necessiten apropar-se professionalment a la complexa i distintiva biologia reproductiva d'aquest grup. Aquest repàs sinòptic, que no pretén ser una revisió total, intenta respondre les necessitats d'aquesta audiència heterogènia. Es resumeix el procés general de reproducció sexual i asexual en el fílum, combinant dades tant ecològiques com citològiques. Es fa èmfasi en els processos d'espermatogènesi, oogènesi i fecundació. A més de l'esquematització dels processos generals, es mencionen les excepcions més destacables, així com els punts febles en el coneixement actual amb intenció de promoure investigacions futures.

Paraules clau: reproducció d'esponges, reproducció asexual, gametogènesi, desenvolupament d'invertebrats, larva d'esponges.

SUMMARY

Sponges (phylum Porifera) are important organisms from an ecological, evolutionary and biotechnological point of view: i) they play relevant ecological roles in many marine communities and participate in crucial steps of the cycle of dissolved nutrients and organic matter; ii) their cellular and genetic traits still reflect and inform about the ancient transition between the unicellular condition and the multicellular organization of animals, and

iii) they and/or their symbionts are a promising source of compounds of interest in biomedicine and some industrial processes. For these reasons, engineers, chemists, microbiologists, general ecologists, geneticists, and evolutionary biologists, who usually have little expertise with sponges, need to professionally approach the complex, unique reproductive biology of this group. This synoptic overview, which does not intend to be a comprehensive review, attempts to fulfill the needs of such a heterogeneous potential audience. It summarizes the general process of sexual and asexual reproduction in the phylum, combining both ecological and cytological data. Emphasis is made on the processes of spermatogenesis, oogenesis, and fertilization. In addition to outlining general processes, a brief mention of exceptions, recent relevant findings and the weak points in current knowledge is provided with the aim of encouraging future research.

Key words: sponge reproduction, asexual reproduction, gametogenesis, invertebrate development, poriferan larva.

INTRODUCTION

The phylum Porifera (sponges) comprises more than 8,000 species distributed in 3 taxonomic classes: Hexactinellida (10%), Calcarea (5%), and Demospongiae (85%). Members of Hexactinellida show a distinct syncytial organization. Calcarea and Demospongiae posses a conventional cellular histology, but are distinguished from each other on the nature of their skeletal pieces (spicules), being calcareous in the former and siliceous in the latter.

Sponges are sessile, mostly marine, with only a small cosmopolitan demosponge family (Spongillidae) inhabiting fresh waters. The body is organized around an intricate system of chambers interconnected by canals through which ambient water is pumped in and out. This water flow through the body is crucial for most physiological functions, facilitating food uptake (bacteria, microalgae, dissolved organic mater), elimination of digestion residuals and excretes, respiration and gas exchange, release and intake of gametes and other reproductive products, etc. Sponges lack true organ systems, having instead several lines of pluripotent cells which are able to undergo transient trans-differentiations and de-differentiations into diverse cell types to accomplish a wide variety of functions (e.g., Simpson, 1984; Harrison and Vos, 1991). The body of adult sponges is externally limited by a pseudo-epithelium (cells or syncytia) that usually lacks sealing permanent junctions and a basement membrane. The walls of the aquiferous canals are lined by a similar internal pseudo-epithelium, except in regions where the tubes expand as chambers and are lined by characteristic monoflagellated collar cells, the choanocytes (figure 1*a-b*). These cells beat their flagellum to promote water circulation through the canal system and use their microvilli to entrap bacteria and other feeding materials suspended in the inflow. Between the external and the internal epithelial systems, there is a mesohyl which consists of a dense intercellular matrix rich in collagen fibrils, many large macromolecules and symbiotic «microorganisms» (i.e., bacteria, cyanobacteria, dinoflagellates, yeasts, etc.), and several types of amoeboid cells responsible for food delivery and energy storage, secretion of skeletal materials, phagocytosis of invading organisms and basic immunity, nursing of embryos, etc. Because of its lax nature and simple organization, the mesohyl has never been interpreted as a real tissue.

Likewise, there is controversy as to whether the internal and external epithelial systems of sponges can be equated to the ectodermal and endodermal layers of the other animals.

SEXUAL REPRODUCTION

General features

Sponges use a variety of strategies for reproduction including sexual and asexual processes. Gametogenesis in sponges unfolds following the same basic sequence of processes as seen in other animals. The only noticeable difference is that, in the absence of a predetermined germ line, some lines of somatic cells became gonial cells at the time of gametogenesis. However, this situation is not exclusive to sponges and has also been reported in other lower invertebrates, such as some cnidarians, acoel flatworms, etc (e.g., Extavour, 2007). Regarding gamete production, sponges can be gonochoristic or hermaphroditic (table 1). Gonochoristic species do not exhibit sexual dimorphism. Reported sex ratios usually depart from 1:1 values, with frequent cases of female overabundance. Among hermaphroditic sponges, contemporaneous hermaphroditism is more common than successive hermaphroditism. In some species, contemporaneous hermaphroditism in most members of a population co-exists with the occurrence of a few gonochoristic individuals (e.g., Meewis, 1938). Inversely, populations consisting of mostly gonochoristic sponges have been reported to contain a minority of successive-hermaphroditic individuals (Baldacconi *et al*., 2007).

Regarding development, sponges can be either oviparous or viviparous (table 1). Embryonic development is always external in oviparous sponges, leading to formation of a free-swimming larval stage. In contrast, viviparous sponges brood their embryos in the mesohyl until their release through the aquiferous canals as free-swimming larvae. Larvae disperse for days to weeks in plankton before settling and giving rise to a sessile juvenile. In a few demosponges, the free-swimming larval stage has been lost and the embryos develop directly into a juvenile (table 1).

Regarding reproductive timing, sponge populations may produce gametes and embryos either during a short period or over the year. This variability appears not to depend on phylogenetic constraints only, but it is modulated by environmental variables, with the longest periods of active reproduction reported from relatively stable environments and characterized by attenuate changes in temperature, food availability, etc over the annual cycle. The onset of gametogenesis is thought to be triggered by environmental factors, with temperature the most clearly influential parameter identified by correlation approaches. In latitudes subjected to recognizable seasonal changes, rising temperatures have widely been reported to trigger and/or accelerate gametogenesis, also synchronizing embryonic growth rates and larval release. For some species, declining rather than rising temperatures appear to trigger the reproductive process (e.g., Fromont and Bergquist, 1994; Corriero *et al*., 1998; Ereskovsky, 2000; Riesgo and Maldonado, 2009). In those habitats with only subtle temperature variations over the year, gametogenesis may instead depend on stimuli undergoing more intense changes over the annual cycle, such as photoperiod, wave height, salinity variations, peaks in food fluxes, etc (e.g., Elvin, 1976; Witte, 1996; Corriero *et al*., 1998).

TABLE 1. *Summary of the taxonomic distribution (at the family level) of gametic, developmental and larval features in the phylum Porifera*

Taxa	*Gametic status*	*Development*	*Larval stage*	*Taxa*	*Gametic status*	*Development*	*Larval stage*
O. Homosclerophorida				F. Tedaniidae	?	IT(w)	ntP
F. Plakinidae	H V	IT	C	F. Cladorhizidae	H V	IT(w)	stP
O. Spirophorida				F. Desmacellidae	?	IT(w)	?
F. Tetillidae	H/G O	IT/ET	D; ?	F. Guitarridae	?	?	?
F. Samidae	?	?	?	F. Esperiosidae	?	IT(w)	ntP
F. Spirasigmidae	?	?	?	F. Hamacanthidae	?	?	?
O. Astrophorida				F. Mycalidae	H V	IT(w)	ntP
F. Ancorinidae	G? O	ET(1)	?	F. Merliidae	?	?	?
F. Calthropellidae	?	?	?	F. Podospongiidae	?	IT(w)	stP
F. Geodiidae	G O	ET(2)	?	F. Isodictyidae	?	IT(w)	?
F. Pachastrellidae	G O	ET(3)	?	F. Latrunculiidae	H V	IT(w)	stP
F. Thrombidae	?	?	?	**O. Halichondrida**			
F. Alectonidae	?	IT, w	Hp	F. Axinellidae	G O	IT(w)	? (8)
O. Hadromerida				F. Bubaride	?	?	?
F. Clionidae	G/H O/V	ET	Cl	F. Desmoxyidae	?	?	?
F. Hemiasterellidae	G O?	ET(4)	?	F. Dictyonellidae	?	IT(w)	ntP
F. Placospongiidae	?	?	?	F. Halichondriidae	H/G O/V	IT(w)	ntP / tP
F. Polymastiidae	G O	ET(w)	Cl	**O. Agelasida**			
F. Spirastrellidae	G?	?	?	F. Agelasiidae	G O	ET	ntP *
F. Stylocordylidae	G? V	IT(w)	D; ?	F. Astroscleridae	?	IT(w)	stP
F. Suberitidae	G/H O/V	ET	?	**O. Haplosclerida**			
F. Tethyidae	G O	ET(w)	Cl	F. Callyspongiidae	H V**(9)**	IT(w)	tP
F. Timeidae	?	?	?	F. Chalinidae	GH/ V	IT(w)	tP
F. Trachycladidae	?	?	?	F. Niphatidae	G V	IT(w)	tP
F. Acanthochaetetidae	?	?	?	F. Phloeodictyidae	?	ET(w)	?

Taxa	*Gametic status*	*Development*	*Larval stage*	*Taxa*	*Gametic status*	*Development*	*Larval stage*
F. Sollasellidae	?	?	?	F. Petrosiidae	G O	ET(w)	stP
O. Chondrosida				F. Calcifibrospongiidae	?	?	?
F. Chondrillidae	G O	ET	Cl	F. Spongillidae	G/H V	IT	ntP
"Lithistids"				**O. Dictyoceratida**			
13 families; 41 genera	G O	ET(5)	?	F. Irciniidae	G/H V	IT	tP
O. Poecilosclerida				F. Thorectidae	?	IT(w)	tP
F. Acamidae	H V	ET(w)	ntP	F. Spongiidae	G V	IT	stP
F. Microcionidae	?	IT	ntP	F. Dysideidae	? V	IT(w)	tP
F. Raspailiidae	G O/V	ET/ IT (6)	ntP *	F. Verticillitidae	?	IT(w)	stP
F. Rhabderemiidae	?	?	?	**O. Dendroceratida**			
F. Chondropsidae	?	IT(7)	?	F. Darwinellidae	?	IT(w)	tP
F. Coelosphaeridae	H V	IT(w)	?	F. Dictyodendrillidae	?	IT(w)	stP
F. Crambeidae	H V	IT(w)	ntP	**O. Halisarcida**			
F. Crellidae	?	IT(w)	ntP	F. Halisarcidae	H V	IT	Di
F. Dendoricellidae	?	?	?	**O. Verongida**			
F. Desmacididae	H? V	IT(w)	ntP	F. Aplysinidae	G/H? O	ET	?
F. Hymedesmiidae	H V	IT(w)	ntP	F. Pseudoceratinidae	?	ET(w)	?
F. Iotrochotidae	?	?	?	F. Ianthellidae	?	ET(w)	?
F. Myxillidae	H V	IT(w)	ntP	F. Aplysinellidae	?	ET(w)	?
F. Phellodermidae	?	?	?				

Abbreviations and symbols are as it follows: H: hermaphroditic; G: gonochoric; O: oviparous; V: viviparous; IT: internal development; ET: external development; C: cinctoblastula; Cl: clavablastula; Hp: hoplitomella; tP: tufted parenchymella; ntP: non-tufted parenchymella; stP: to-be-studied parenchymella; Di: dispherula; D: direct development (absence of larva); ?: unknown or unseen condition; *: published information to be revised. Numbers, letters and symbols in brackets refer to annotations on the condition expressed in the table, as it follows: (w): features extrapolated from studies in very few members; (1): only known in *Stelleta* and *Ancorina*; (2): unknown in most genera, but ET in *Geodia* and *Erylus*; (3): unknown in most genera, external development in *Thenea;* (4): only known in *Adreus*; (5): only known from *Theonella;* (6): internal development in *Eurypon* and *Echinodictyum;* (7): only known in *Batzella*; (8): report on larva of *Axinella cristagalli: Crambe crambe;* (8): only in *Siphonochalina.*

Spermatogenesis

Spermatogonia are reported to be derived from choanocytes in most members of Demospongiae and Calcarea (e.g., Reiswig, 1983; Simpson, 1984; Boury-Esnault and Jamieson, 1999), although totipotent archaeocytes have been suggested as the origin of spermatozoa in some cases (Fincher, 1940; Lévi, 1956). There are also described instances of choanocytes de-differentiating into archaeocyte-like cells before becoming recognizable spermatogonia (Reiswig, 1983). In Hexactinellida, since choanocytes are enucleated cells, spermatogonia are suspected to differentiate from archaeocyte congeries (e.g., Okada, 1928; Boury-Esnault *et al*., 1999).

In most studied cases—nearly all of them belonging to the class Demospongiae—the choanocytes become spermatogonia in a chamber and maintain the flagellum during this differentiation (figure 1*c*) and subsequent stages. This situation is in contrast to the model followed by most animals, in which the flagellum only appears at late stages of gametogenesis. Nevertheless, in some sponges, such as *Suberites massa* (Diaz and Connes, 1980) and *Spongia officinalis* (Gaino *et al*., 1984), choanocytes appear to lose their flagella during differentiation into spermatogonia and then produce new flagella at the spermatocyte stage. The reason for this transient flagellum loss could be due to the fact that spermatogonia need to multiply by mitosis in order to increase their numbers before starting gametogene-

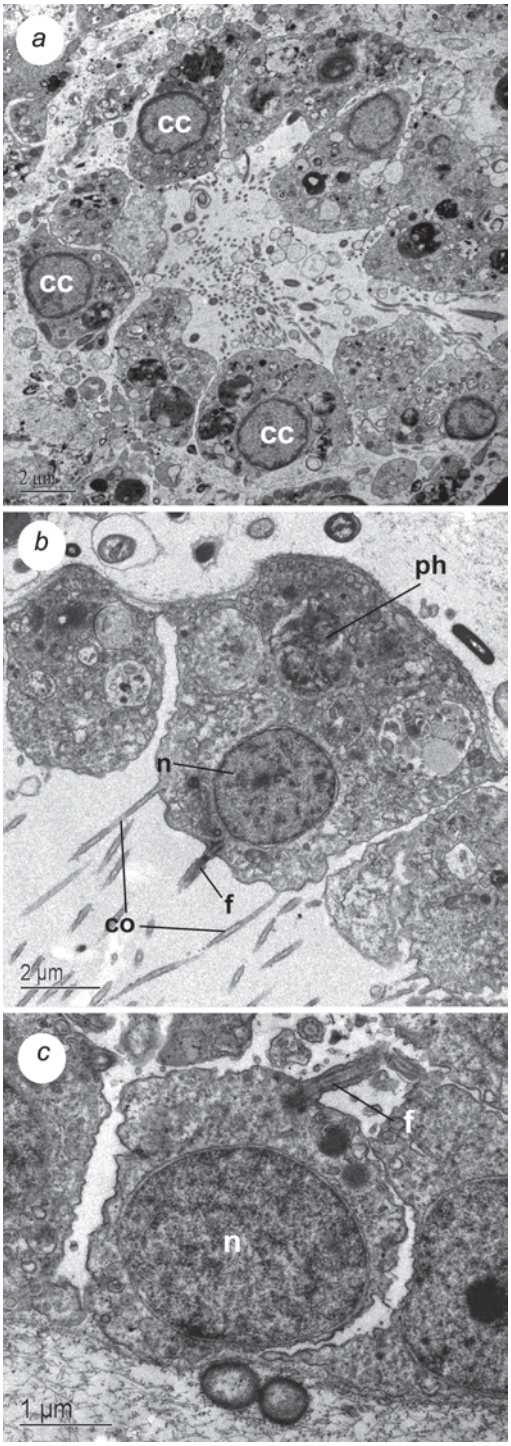


FIGURE 1. *a*) General view of a choanocyte chamber of the poecilosclerid demosponge *Crambe crambe*. *b*) Detail of a choanocyte of the homosclerophorid demosponge *Corticium candelabrum*. *c*) View of a monoflagellated spermatogonia at the basal stratum of a spermatic cyst of *C. candelabrum*. cc: choanocyte; co: microvilli collar; f: flagellum; n: nucleus; ph: phagosome.

sis. Mitotic divisions of spermatogonia prior to the onset spermatogenesis have been described in *Halisarca* (Chen, 1976), *Suberites* (Diaz and Connes, 1980), *Hippospongia* and *Spongia* (Kaye and Reiswig, 1991).

Each group of spermatogonia becomes a spermatic cyst, which in most species—but not always—is enveloped by a simple follicle of flat cells and/or a thin collagen layer (figure 2*a*). The cyst envelope is quite complex in some carnivorous demosponges that have lost their aquiferous canal system, which is the usual via for, not only food intake, but also sperm release (Vacelet and Boury-Esnault, 1995). In carnivorous, canal-lacking sponges, isolated spermatozoa are not released individually. Rather the spermatic cyst develops a thick protective envelope, a structure equivalent to the spermatophore of other invertebrates. This envelope often consists of multiple layers of intertwined cells, collagen, and special spicules (figure 2*b*; Riesgo *et al*., 2007*b*; Vacelet, 2007). When mature, the "spermatophore-like" cysts somehow migrate from the mesohyl to the sponge surface to be released.

Within an individual sponge and depending on the species, spermatogenesis may be synchronized in all cysts or cysts may coexist at different stages of development. The former case is common in oviparous sponges, in which sperm release is a highly synchronous event linked to egg release and often takes place one or only a few days a year. Within a cyst, spermatogenesis may be either synchronous with all cells in the same spermatogenetic stage, or in a maturation gradient, with spermatogonia at the periphery of the cysts and mature spermatozoa towards the lumen (figure 2*c*).

During the first phase of spermatogenesis (i.e., spermatocytogenesis), spermatogonia experience two consecutive divisions: first producing primary spermatocytes which in turn divide into secondary spermatocytes. Similar to that seen in other animals, the sister cells resulting from these

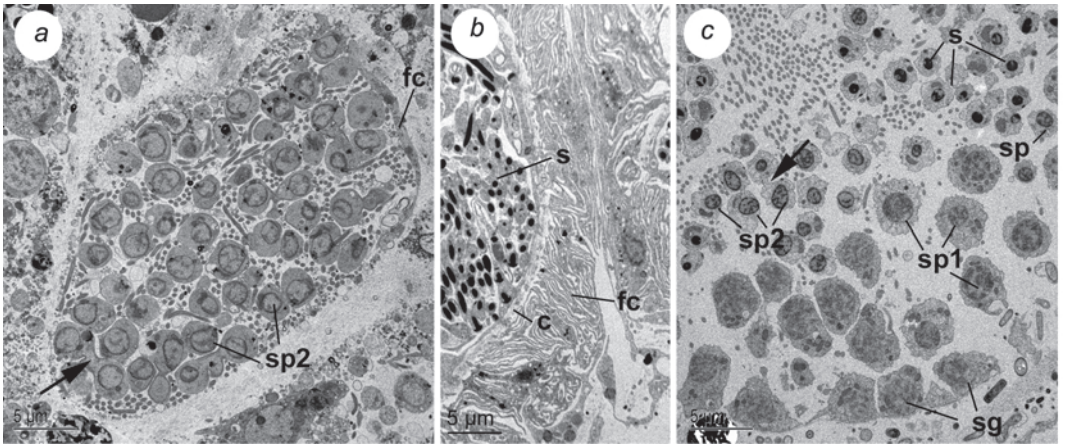
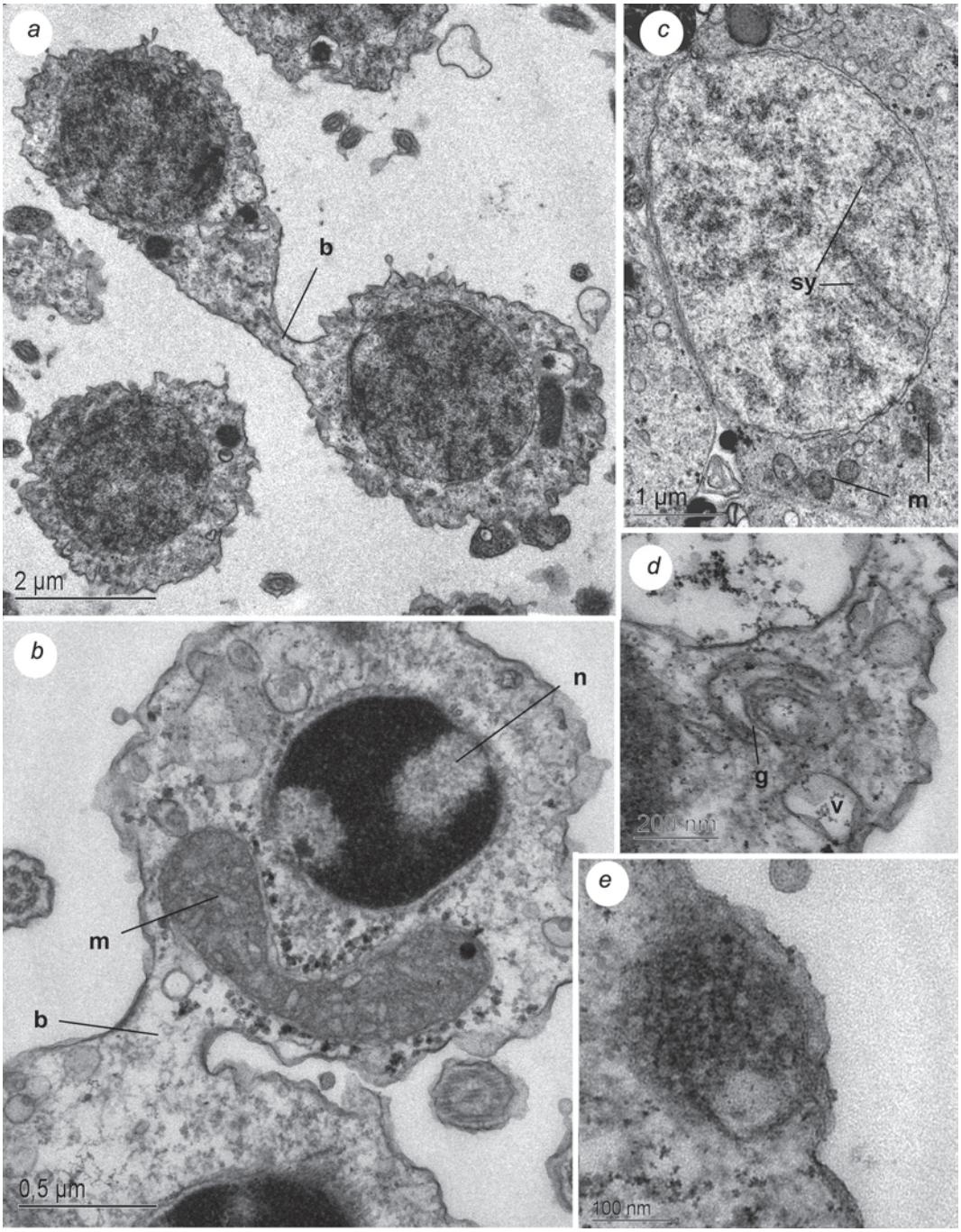


FIGURE 2. *a*) A synchronous spermatic cyst of poecilosclerid demosponge *Crambe crambe* limited by follicle (fc) cells and containing secondary spermatocytes (sp2) only. *b*) The complex envelope of collagen (c) and intertwined follicle cells (fc) in spermatic cyst of carnivorous poecilosclerid *Asbestopluma occidentalis* filled with nearly mature spermatozoa (s). *c*) A spermatic cyst of homosclerophorid demosponge *Corticium candelabrum* showing a maturation gradient from the peripheral spermatogonia (sg) towards the central mature spermatozoa (s), passing through spermatocyte stages (sp1, sp2) and spermatid stages (sp). Note cytoplasmic bridging (arrows) between sister spermatocytes in *a* and *c*.


a
b
2 µm
c
sy
m
1 µm
d
g
v
200 nm
b
n
m
b
0,5 µm
e
100 nm

divisions remain interconnected by cytoplasmic bridges (figure 3*a*) which are maintained until the very end of spermatogenesis (figure 3*b*). Primary spermatocytes are easily recognizable due to their nucleus which exhibits synaptonemal complexes typical of prophase I (figures 2*a*, 3*c*). In both primary and secondary spermatocytes, mitochondria are small and occur in high numbers (figure 3*c*), the Golgi complex consists of large, multiple cisternae, and the basal apparatus shows either a single centriole or a pair. During spermatidogenesis, secondary spermatocytes differentiate into haploid spermatids. This process is rapid and has rarely been documented in detail. The most characteristic feature is that cells diminish to the definitive size of the future spermatozoon by exocytosing most of the cytoplasm matrix and organelles in the form of residual bodies. Finally, spermiogenesis renders mature spermatozoa from spermatids. During this phase, chromatin condenses, several glycogen aggregates appear within the cytoplasm, and the many small mitochondria complete fusion to produce one or a few large mitochondrial units (figure 3*b*). In some sponges, the Golgi complex releases vesicles that will participate in the formation of acrosomal elements (figures 3*d-e*). In some demosponges, such as *Lubomirskia baikalensis* (Efremova and Papkovskaya, 1980), *Spongilla lacustris* (Paulus, 1989), *Halichondria panicea* (Barthel and Detmer, 1990), *Crambe crambe* (Tripepi *et al*., 1984), or *Asbestopluma occidentalis* (Riesgo *et al*., 2007*b*), in which spermatozoa are modified at different degrees, the reorganizations required to produce the definitive morphology (i.e., lengthening, V-shape folding, formation of intracytoplasmic tunnel for the flagellum, etc) consistently take place during this phase.

Consequently, sponge spermatozoa can be classified into two morphological types: "primitive" and "modified" (*sensu* Franzén, 1956; figure 4). Spermatozoa with "primitive" organization have a round head of about 2 μm, with a large round nucleus and several mitochondria (figure 4*a-b*). In most studied cases, a set of proacrosomal vesicles in the cell pole are evident opposite from the flagellum insertion (figure 4*a*). However, proacrosomal vesicles are lacking in some species, such as *Aplysilla rosea* (Tuzet *et al*., 1970), *Halisarca nahantensis* (Chen, 1976), and *Ephydatia fluviatilis* (Leveaux, 1942). Proacrosomal vesicles are usually regarded as the evolutionary prelude of the real acrosomes in higher animals and they are typical in the sperm of many sponges and most of the cnidarians (Franzén, 1970, 1996). Round "primitive" spermatozoa provided with a true acrosome have only been reported in members of the very distinct demosponge order Homosclerophorida (figure 4*b*; table 1; Baccetti *et al*., 1986; Boury-Esnault and Jamieson, 1999; Riesgo *et al*., 2007*a*).

In addition to spermatozoa with "primitive" organization, highly modified types have been found in demosponges such as *Halichondria panicea* (Barthel and Detmer, 1990), *Crambe crambe* (figure 4*c*; Tripepi *et al*., 1984), and *Asbestopluma occidentalis* (figure 4*d*; Riesgo *et al*., 2007*b*). Interestingly,

FIGURE 3 (facing page). *a*) Primary spermatocytes of *Corticium candelabrum* connected by intercellular bridges (b). *b*) A spermatid of *C. candelabrum* showing intercellular bridging (b), a large mitochondrion (m) resulting from previous fusion of smaller units, and a highly condensed nucleus (n). *c*) Detail of the nucleus of a primary spermatocyte in *Crambe crambe* showing synaptonemal complexes (sy) and several small mitochondria (m). *d*) Detail of a small Golgi apparatus (g) and derived vesicle (v) in spermatid of *C. candelabrum*. *e*) Detail of a condensing vesicle from which the acrosome of *C. candelabrum* will be formed.

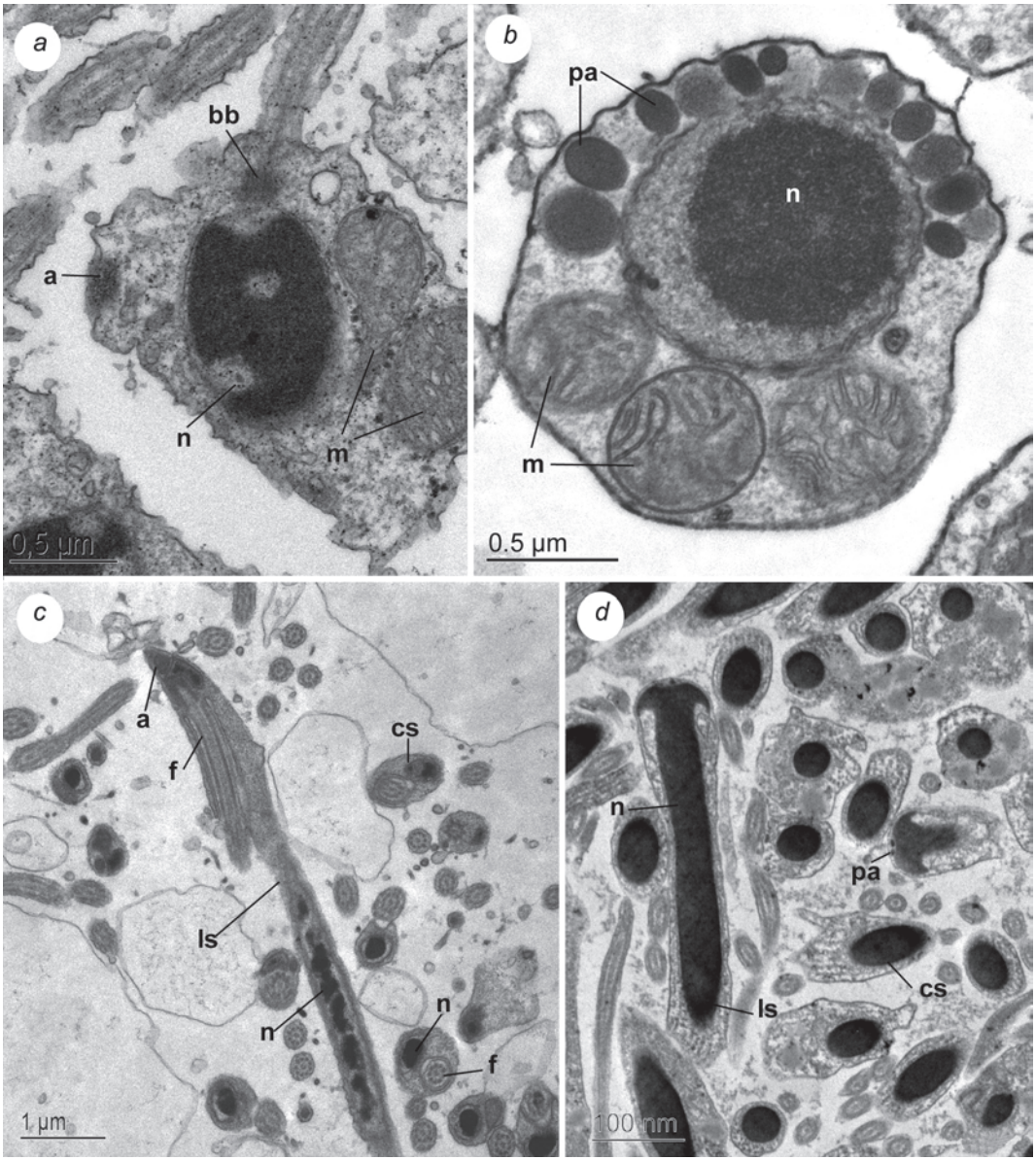


FIGURE 4. *a*) Spermatozoon of *C. candelabrum* showing the basal body of the flagellum (bb), the acrosome (a), 2 large mitochondria (m), and an indented condensed nucleus (n). *b*) Spermatozoon of haplosclerid demosponge *Petrosia ficiformis* showing a battery of proacrosomal vesicles (pa), large mitochondria (m) and a nucleus (n) condensed only at its central portion. *c*) Longitudinal section of a V-shaped spermatozoon (ls) adjacent to several cross-sectioned spermatozoa (cs). These spermatozoa are characterized by a conical acrosomal complex (a) with a perforatorium, a flagellum (f) running through and an intracytoplasmic canal, and a nucleus (n) with helically condensed chromatin. *d*) Cross (cs) and longitudinal sections (ls) of lengthened spermatozoa of the carnivorous poecilosclerid *Asbestopluma occidentalis* showing a hammer-head condensed nucleus (n) and several proacrosomal vesicles at the tip of head.

the V-shaped spermatozoon of *C. crambe* carries a long striated rootlet and an acrosomal complex consisting of a conical acrosome and an adjacent subacrosomal rod. In contrast, the spermatozoon of *A. occidentalis* bears only proacrosomal vesicles and no rootlets, and that of *H. panicea* has neither acrosomal components nor rootlets. An aberrant sperm lacking both flagellum and acrosome has been reported in the Calcarea *Leucosolenia complicata* (Anakina and Drozdov, 2001). At present, both the functional and phylogenetic significance of the diverse morphologies in sponge sperm remain poorly understood.

Oogenesis

Oogenesis has been described in reasonable detail in quite a few demosponges, some calcareous sponges (e.g., Gallissian, 1988; Gallissian and Vacelet, 1990; Anakina and Drozdov, 2001), and, only partially, in 2 hexactinellids, *Farrea sollasii* (Okada, 1928) and *Oopsacas minuta* (Boury-Esnault *et al*., 1999).

Oogonia are thought to be derived from choanocytes in most calcareous sponges and from archaeocytes in most demosponges and hexactinellids. Pinacocytes have been suggested to be the origin of oogonia in the calcareous sponge *Ascandra minchini* (Borojevic, 1969). In some calcareous species, the choanocytes leave the chambers and enter the adjacent mesohyl where they differentiate into oogonium-like cells that experience mitotic divisions before the onset of oogenesis (Fell, 1983). These pre-oogonial divisions are not common in Demospongiae, although they have been documented in *Reniera elegans* (Tuzet, 1947), *Halisarca dujardini* (Lévi, 1956), *Hippospongia communis* (Tuzet and Pavans de Ceccatty, 1958), and *Octavella galangaui* (Tuzet and Paris, 1964).

In some demosponges, particularly oviparous species, all oocytes are produced during only a short period in each annual cycle. They grow during a species-dependent time period and reach maturity more or less synchronously (figure 5*a-b*). Alternatively, other sponges produce new oocytes during many months of the year which leads to an asynchronous extended reproduction (figure 5*c*). Asynchrony is common in viviparous species.

Early-stage oocytes are usually amoeboid cells (figure 5*d*) that may wander through the mesohyl while incorporating symbiotic microorganisms and diverse macromolecules from the mesohyl matrix (e.g., Maldonado, 2007). As oogenesis progresses, oocytes grow and become round. The initial stages of oocyte growth are previtellogenic, rarely involving yolk production (Fell, 1983; Simpson, 1984). Our observations in several demosponges suggest that massive vitellogenesis starts only after oocytes reach about ¼ of their final size. Yolk bodies are membrane-bound and show a very electron-dense core of striated substructure (suggesting the occurrence of highly condensed proteins) and a less electron-dense, narrow, peripheral band (figure 6*a*). Nevertheless, some species produce yolk bodies in which lipids, multi-membrane structures and the rests of digested microorganisms are packed into atypical heterogeneous structures (e.g., figure 6*b*; Fell, 1983; Gaino *et al*., 1986; Gaino and Sarà, 1994; Lepore *et al*., 1995; Riesgo and Maldonado, 2009). As in other animals, yolk production in Porifera can take place via autosynthesis, heterosynthesis or both processes simultaneously (Riesgo and Maldonado, 2009). Autosynthesis is carried out by the oocytes themselves using basic precursors acquired through the oocyte membrane by endo-, pino-, or phagocytosis and followed by intense synthetic activity (Fell,

1983). Sponges that appear to use strict autosynthesis are *Suberites massa* (Diaz *et al*., 1975), *Aplysina cavernicola* (Gallissian and Vacelet, 1976), *Tetilla serica* (Watanabe, 1978), *Stelleta grubii* (Sciscioli *et al*., 1991), and *Raspaciona aculeata* (figure 6*c*; Riesgo and Maldonado, 2009). Others use a semi-autosynthetic process, in which the precursors are not basic material but endocytosed microorganisms and large incorporated compounds, such as in *Erylus discophorus* (Sciscioli *et al*., 1989), *Tethya aurantium* (Sciscioli *et al*., 2002), *Corticium candelabrum* (Riesgo *et al*., 2007*a*), and *Axinella damicornis* (figure 6*d*; Riesgo and Maldonado, 2009).

Yolk heterosynthesis involves participation of different types of somatic cells (e.g., archaeocytes, spherulose cells, microgranu-

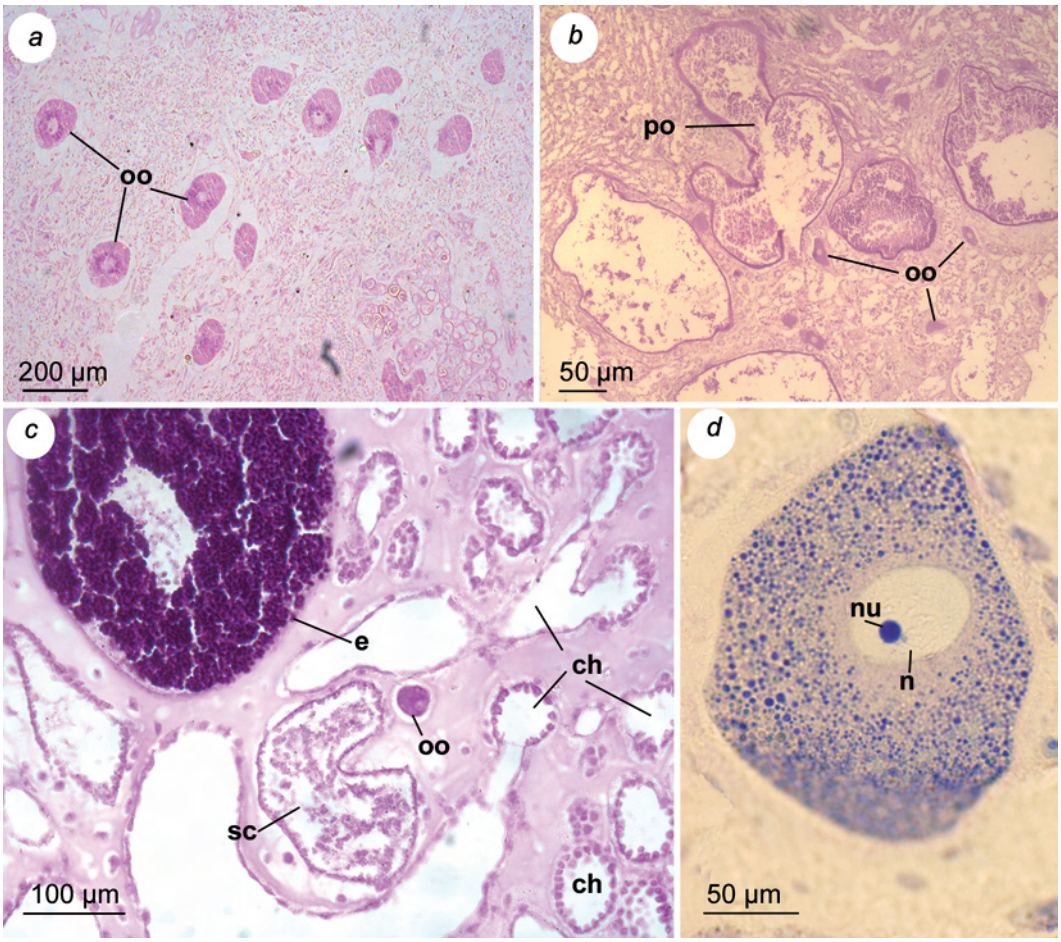


FIGURE 5. *a*) Late-stage oocytes (oo) of the oviparous poecilosclerid *Raspaciona aculeata* maturing synchronously in the mesohyl and *b*) early-stage oocytes (oo) of the viviparous poecilosclerid *C. crambe*. Note section of endobiotic polychaete (po) in the sponge tissue. *c*) An early-stage oocyte (oo) developing adjacent to a gastrulating embryo (e) in the mesohyl of the viviparous *C. candelabrum*. Note also the occurrence of a spermatic cyst (sc), clearly distinguishable from the choanocyte chambers (ch). *d*) A mid-stage oocyte of *R. aculeata* with distinctive amoeboid shape and patent nucleolate (nu) nucleus (n).

lar cells, follicle cells, grey cells, amebocytes, choanocytes, bacteriocytes, etc), which become transiently compromised in oocyte nourishing and are generally referred to as "nurse cells". These cells either elaborate yolk or its precursors in their cytoplasm and also incorporate extracellular microorganisms into vesicles, transporting and transferring all these materials to the growing oocytes (figure 6*e-f*). Reserve materials and microorganisms stored in nurse cells can be either directly transferred to oocytes by fusion of the cell membranes or exocytosed to the perioocytic space to be subsequently incorporated by the oocytes, as in the demosponges *Hippospongia communis* (Tuzet and Pavans de Ceccatty, 1958), *Suberites massa* (Diaz *et al*., 1975), *Halisarca dujardini* (Aisenstadt and Korotkova, 1976) and *Axinella damicornis* (Riesgo and Maldonado, 2009). In some instances, oocytes appear to phagocytose entire nurse cells, as in the calcareous *Ascandra minchini* (Borojevic, 1969) and *Clathrina coriacea* (Jonhson, 1979), and demosponges of the genera *Haliclona* (Fell, 1974), *Halichondria* (Witte and Barthel, 1994) and *Chondrilla* (Maldonado *et al*., 2005). In addition to yolk, the cytoplasm of oocytes contains plenty of other energetic inclusions in the form of glycogen and lipid droplets.

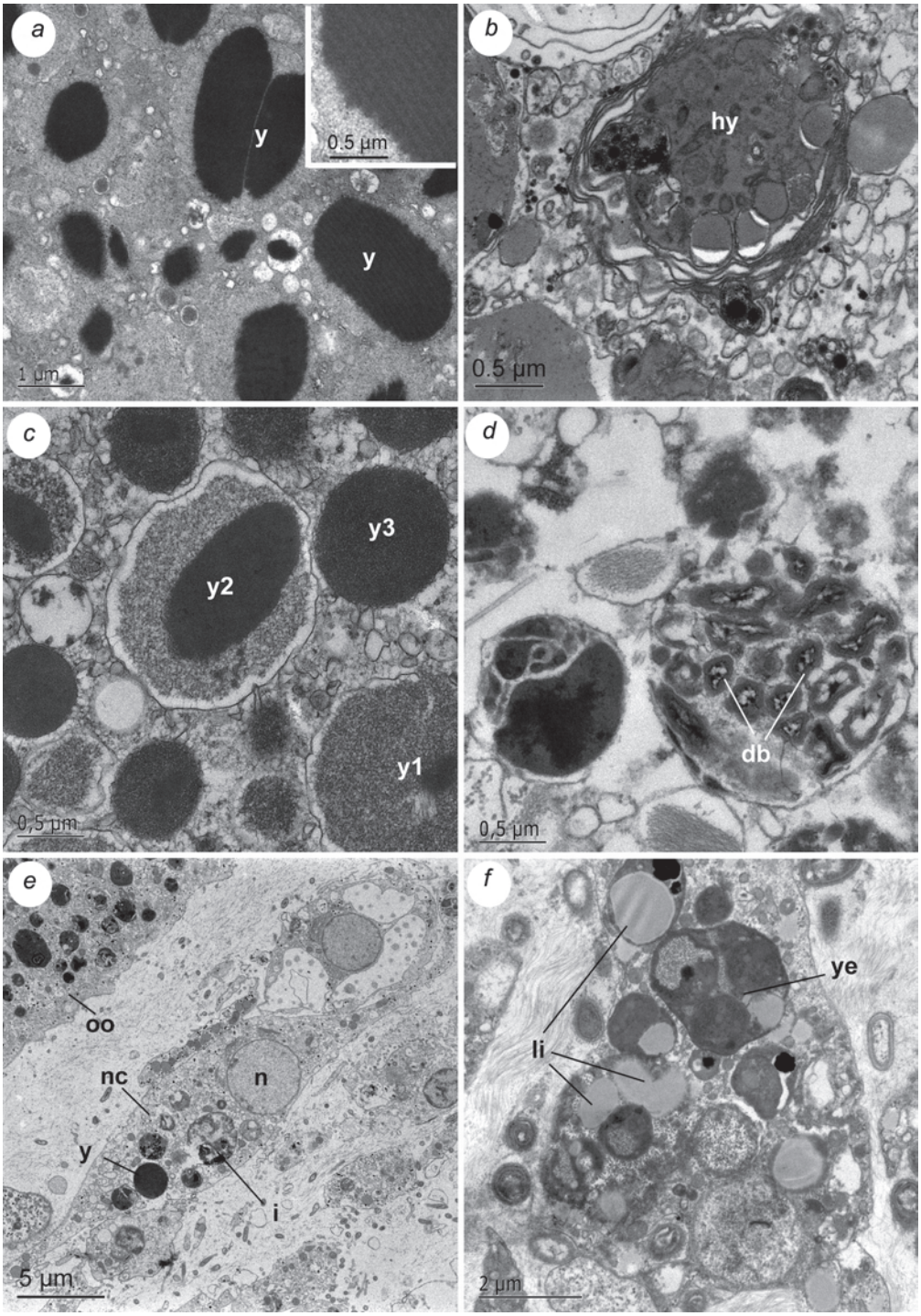
It is assumed that most oocyte growth takes place while arrested in some step of the first meiotic division, since polar bodies have rarely been seen before oocyte maturation is reached. Polar body emission has been observed in very few sponges (e.g., Tuzet, 1947; Lévi, 1951; Tuzet and Pavans de Ceccatty, 1958; Tuzet and Paris, 1964) and documented by light microscopy (figure 7*a*). There is a single TEM report in which putative polar-bodies (but predominantly containing yolk) have been described in a homosclerophorid demosponge (Riesgo *et al*., 2007*a*).

Many sponge species produce mature oocytes that are surrounded by cells such as pinacocytes or collenocytes that are apparently not involved in the nourishing process (Fell, 1974). In many instances, oocytes are surrounded by packages of striate fibers, as in *Tetilla serica* and *Tetilla japonica* (Watanabe, 1978), by a thin layer of collagen fibrils, such as in *Suberites massa* (Diaz *et al*., 1975) and *Aplysina cavernicola* (Gallissian and Vacelet, 1976), or by a thick collagenous cover (probably some form of spongin) which remains undegraded for a long time after egg hatching, such as in the genera *Agelas* and *Cliona* (figure 7*c-d*).

Fertilization

Upon completion of gametogenesis, spermatozoa are released into the water column,. Male spawning takes places by fusion of the spermatic cysts to the wall of the exhalant aquiferous canals so that the mature spermatozoa are released into the outgoing flow and finally expelled through the oscules in the form of milky clouds. The process is similar for oocyte release in oviparous sponges. Oocytes or eggs are released either individually through the oscules or as chains embedded in mucous threads and abundantly accompanied by nurse cells (e.g., Lévi and Lévi, 1976; Reiswig 1970, 1976; Watanabe, 1978; Hoppe and Reichert, 1987).

Fertilization is suspected to take place externally in many oviparous sponges (e.g., figure 7*a-b*; Fell 1983, 1989; Reiswig, 1970, 1983). However, personal observations in a Caribbean population of *Chondrilla nucula* in 2001 revealed that sperm release took place more than 3 days before female spawning, which consisted of mucous threads charged with both eggs and nurse cells (Maldonado *et al*., 2005). This observation corroborated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that *Chondrilla nucula*, despite being viviparous, experience internal fertilization, a condition also suggested from previous studies in Mediterranean populations (Sidri *et al*., 2005). Experimental external fertilization also performed in the laboratory by mixing male and female gametes of *C. nucula* rendered a high percentage of zygotes that developed until the late blastula stage (Maldonado, personal observations). Therefore, the natural process of internal fertilization in *Chondrilla* does not require oocytes to be nested in the mesohyl and it probably takes place when oocytes have already been discharged into the exhalant aquiferous canals for imminent spawning. External fertilization occurs in a manner similar to that observed in other lower invertebrates. There are even cases in which the egg produces a fertilization-like membrane, such as in the genus *Tetilla* (Watanabe, 1978; 1990). However, the mechanisms by which eggs that are shed within follicle-like envelopes or cases are fertilized remain unclear.

Viviparous sponges are spermcasters, i.e., they release sperm but retain the oocytes in the mesohyl for internal fertilization and subsequent embryo incubation. Interestingly, male sperm spawning in viviparous sponges has never been documented, probably because it is not a massive obvious event at the population level, but rather a subtle asynchronous process unnoticed by most divers. The process of internal fertilization in brooding sponges is only partially understood. Internal fertilization has been well documented in a variety of calcareous sponges. The process is mediated by choanocytes, which phagocytose the spermatozoa entering the chambers in the inhalated water (e.g., Gallisian, 1980, 1989; Gaino *et al*., 1987; Gallisian and Vacelet, 1990; Anakina and Drozdov, 2001; Nakamura *et al*., 1998). These choanocytes which do not digest the phagocytosed spermatozoa, leave the chamber and de-differentiate into amoeboid cells called carriers cells. They migrate through the mesohyl to find an oocyte and transfer the encysted spermatozoon to it. During cell migration, the encysted spermatozoon, now referred to as spermiocyst, loses its flagellum and most other organelles. Spermiocyst transference may occur at early-stage oocytes or just immediately before their maturation, depending on the species. In contrast to the class Calcarea, the above-described fertilization mechanism has not been properly documented in Demospongiae and Hexactinellida. Thus, serious doubts are raised as to the universality of this process among Porifera (Reiswig, 1976). We have found evidence in the homosclerophorid demosponge *Corticium candelabrum* that spermatozoa are able to enter the choanocyte chambers (Riesgo *et al*., 2007*a*). Additionally, a «putative» spermatozoon in a "putative" carrier cell adjacent to choanocyte chamber was documented (Riesgo *et al*., 2007*a*). Both findings suggest that the carrier-cell mechanism appears to operate in at least members of this small demosponge order. An alternative mode of internal fertilization has been

FIGURE 6 (facing page). *a*) Yolk body (y) of *Corticium candelabrum* showing conventional homogeneous structure. Note the inset showing the striated substructure of yolk. *b*) Highly heterogeneous yolk (hy) body of *Petrosia ficiformis* incorporating multimembranes, lipids and diverse granules. *c*) Early (y1), mid (y2) and late (y3) stages of autosynthesis of yolk bodies of *C. candelabrum*. *d*) The early stage of formation of a yolk body in *Axinella damicornis* using mostly phagocytosed, digested bacteria (db). *e*) A nurse cell (nc) of *P. ficiformis* sectioned at the nucleus level (n) carrying homogeneous yolk bodies (y) and diverse inclusions (i) towards the oocyte (oo). *f*) A nurse cell of *Chondrilla nucula* transporting groups of phagocytosed symbiotic yeasts (ye) and lipid droplets (li).

postulated for carnivorous demosponges, such as *Asbestopluma hypogea* (Vacelet, 2007) and *Asbestopluma occidentalis* (Riesgo *et al*., 2007*b*), which lack aquiferous systems and choanocyte chambers. In these sponges, spermatophore-like spermatic cysts are released from the sponges. The wall of these cysts consists of a complex, cellular envelope that also contains a peculiar type of protruding, hairpin-shaped spicules. Such spicules are thought to allow the spermatic cyst to both reach neutral buoyancy in the water column and become trapped by adjacent reproductive sponges. It is suspected that the thick envelope of the spermatic cysts is partially digested by symbiotic bacteria within special sponge cells, thus setting the spermatozoa free in the mesohyl for subsequent fertilization.

Data about zygote formation are scarce. Pronuclei typically swell before fusion. In some sponges, oocyte meiotic divisions proceed only after transference of the male pronucleus. This appears to be the case in *Clathrina coriacea* (Tuzet, 1947), *Oscarella lobularis* (Tuzet and Paris, 1964, as *Octavella galangaui*), and *Hippospongia communis* (Tuzet and Pavans de Ceccatty, 1958). Very little information exists concerning fertilization success. Fromont and Bergquist (1994) estimated that fertilization success rate in the oviparous demosponge *Xestospongia bergquistia*, was about 71%, with most eggs fertilized and starting cleavage within 5 hours. In contrast, *Petrosia ficiformis*, a Mediterranean species in the same suborder, exhibited an «in vitro» fertilization success rate of about 70%, their zygotes needing at least 12 hours to start cleavage (Maldonado, personal observation). Experimental «in vitro» fertilization has also been investigated in the verongid demosponge *Aplysina aerophoba*. In this species, the naturally released oocytes which were fertilized in

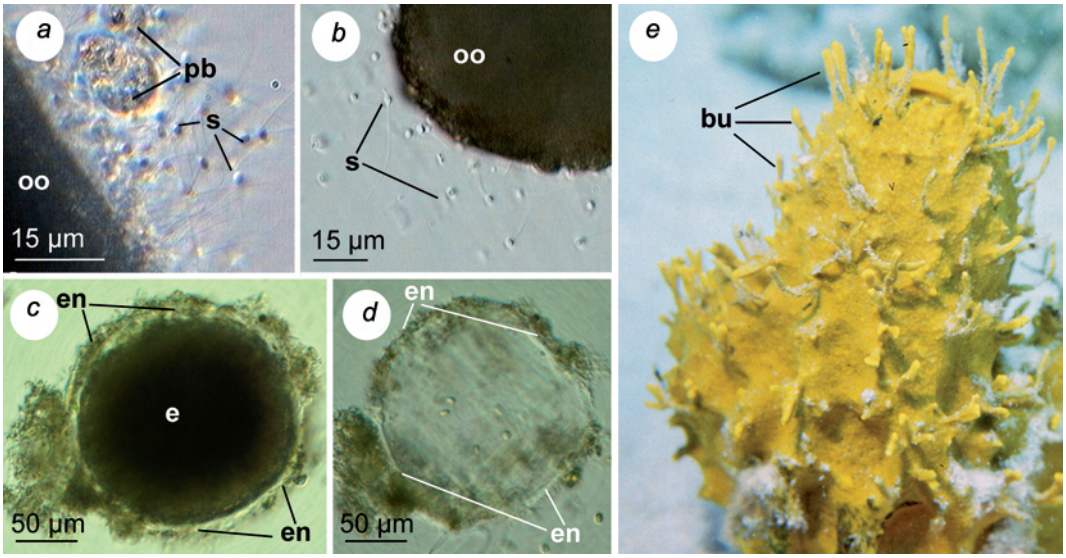

FIGURE 7. *a*) Spermatozoa swimming around recently spawned egg of the haplosclerid demosponge *Petrosia ficiformis*. Note that the 2 polar bodies (pb) are still visible at the egg surface. *b*) Spermatozoa (s) swimming around a recently spawned egg (oo) of the verongid demosponge *Aplysina aerophoba*. *c-d*) The envelope (en) around a developing embryo (e) of the hadromerid demosponge *Cliona viridis*. Note that the envelope resists degradation for weeks after embryo hatching. *e*) Multiple buds (bu) at the body surface of an individual of *Aplysina fistularis*.

more than 99% of cases, started cleavage in less than 4 hours (Maldonado, personal observation). To our knowledge, the success of internal fertilization has only been investigated in the demosponge *C. candelabrum*, in which a comparison between densities of mature oocytes and subsequent brooded embryos revealed a fertilization success rate of about 90% in a reproductive cycle (Riesgo *et al*., 2007*a*).

Embryos and larvae

Cleavage in sponges is usually total and equal, but some calcareous sponges produce blastulae with micromeres and macromeres. Late blastulae experience extensive cellular reorganizations. These embryonic reorganizations have been regarded by some authors as a gastrulation process equivalent to that occurring during the embryogenesis in other animal phyla (e.g., Boury-Esnault *et al*., 1999; Leys and Degnan, 2002; Maldonado and Bergquist, 2002; Maldonado, 2004; Leys, 2004). Some other authors do not accept the occurrence of gastrulation in Porifera (e.g., Ereskovsky and Dondua, 2006), but they propose no satisfactory alternative explanation concerning how cell fates are assigned to form the larval and juvenile stages (see Maldonado and Riesgo, 2008, for discussion). Embryogenesis gives rise to a ciliate or unciliated, swimming or crawling larval stage, except in those very few cases of direct development. Up to eight basic larval types have been described in Porifera (see table 1 for their taxonomic distribution): trichimella, amphiblastula, calciblastula, cinctoblastula or cinctogastrula, dispherula, hoplitomella, clavablastula, and parenchymella. These types are determined according not only to differences in their final morphology but also to a distinct embryogenesis in each case. Because many aspect of the biology and ultrastructure of embryos (Leys 2004; Maldonado 2004; Eerkes-Medrano and Leys, 2006) and larval stages (Maldonado and Bergquist, 2002; Leys and Ereskovsky, 2006; Maldonado 2006) have recently been reviewed by compiling and summarizing most of the findings attained in the past 30 years, readers are re-directed to those papers and the literature cited therein for further information on this issue.

ASEXUAL REPRODUCTION

It is thought that while population maintenance of most sponges depends primarily on sexual reproduction, a few sponges largely rely on asexual reproduction or a combination of both processes (e.g., Dayton, 1979; Fell, 1993). Asexual reproduction typically occurs by budding, gemmulation, or fragmentation (Fell, 1993).

Some sponges produce buds (see Fell, 1974, 1993 and Simpson, 1984, for a review) which are cell masses growing at the external surface of the body that subsequently separate from the parental body by constriction of the tissue bridges (figure 7*e*). After being dispersed by currents and waves for days to months, freed buds attach to the bottom and give rise to a small sponge. The cytological composition of buds varies largely between species. The most usual elements in buds are a dense matrix of collagen fibrils, totipotent archaeocytes, and cells charged with large inclusions presumably for energy storage. They may also contain skeletal pieces and choanocytes.

Gemmules are typically produced by sponges adapted to fresh-water and estuarine habitats, although some marine species also generate them (see Fell, 1974, 1993 and Simpson, 1984, for a review). Gemmules are dormant, resistance bodies formed internally,

usually at the base of the sponge. Typically, they are composed of a dense mass of totipotent archaeocytes and storage cells (thesocytes, nurse cells, etc) surrounded by a thick protective envelope. The structure and thickness of the envelope, which may even incorporate skeletal materials and pneumatic cavities, varies largely between species. Unlike buds, gemmules are freed only after extensive tissue damage. They are thought to provide a means to assure timely population restoration after extended severe mortality by transient unfavorable conditions (drainage or freezing of water bodies, extreme temperature events, etc). The relative resistance of gemmules to both desiccation and the digestive process in the tract of large animals makes them suitable for long-range dispersal via winds, birds, etc (Fell, 1993).

Accidental fragmentation of the sponge body as a result of storms, waves, animal or human activity may also function as a mechanism for asexual propagation. This is due to the pluripotent capacity of many sponge cell types and even small fragments of the body that can contain cells enough to regenerate a new complete, small sponge. Before attaching, small fragments can be transported over considerable distances by currents and storms (Wulff, 1985, 1991). Interestingly, fragments of some species have been demonstrated to contain developing embryos of sexual origin. Even small fragments often carry the essentials cells for not only reorganizing as small sponges, but also for nourishing the developing embryos which can successfully complete development and leave fragments as free-swimming larvae (Maldonado and Uriz, 1999). Therefore, fragmentation may interact with sexual reproduction, so that the dispersal capacity of sexually produced propagules is maximized by the additional dispersal of the asexual fragment. The dispersal of embryo-bearing fragments also maximizes the chance that several distinct genotypes will reach a new area, increasing the chance of establishing new populations.

FUTURE DIRECTIONS

Many aspects of sponge reproduction have not been elucidated. For example, the process by which somatic cells become gonial cells has never been investigated using modern techniques. Likewise, the migration mechanisms of mature spermatic cysts and oocytes from the mesohyl to the exhalant aquiferous canals for spawning remain poorly understood. The processes determining and synchronizing spawning at the population level has also been largely neglected by experimental approaches. Many cytological aspects of fertilization need to be clarified, particularly in the case of internal fertilization in Demospongiae and Hexactinellida, where the actual mechanisms for sperm transference to the oocytes have yet to be discovered. There is also a serious scarcity of studies concerning age, size and/or nutritional condition required for an individual sponge to reach sexual maturity. The contribution of sexual versus asexual reproduction for long-term maintenance of natural populations has only been roughly addressed so far and in very few species. Therefore, much investigational effort needs to be devoted to the reproductive biology of Porifera in order to palliate this situation and reach the standard level of knowledge attained in other invertebrate groups.

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