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Sexual reproduction in demosponges: ecological and evolutive implications

Reproducción sexual en demosponjas: implicaciones ecológicas y evolutivas

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General introduction:

- **General body organization:**

The phylum Porifera is commonly referred to as sponges. The phylum, that comprises more than 6,000 species, is divided into three classes: Calcarea, Hexactinellida and Demospongiae. The latter class contains more than 85% of the living species. They are predominantly marine, with the notable exception of the family Spongillidae, an extant group of freshwater demosponges whose fossil record begins in the Cretaceous. Sponges are ubiquitous benthic creatures, found at all latitudes beneath the world's oceans, and from the intertidal to the deep-sea.

Sponges are considered as the most basal phylum of metazoans, since most of their features appear to be primitive, and it is widely accepted that multicellular animals consist of a monophyletic group (Zrzavy et al. 1998).

Poriferans appear to be diploblastic (Leys 2004; Maldonado 2004), although the two cellular sheets are difficult to homologise with those of the rest of metazoans. They are sessile animals, though it has been shown that some are able to move slowly (up to 4 mm per day) within aquaria (e.g., Bond and Harris 1988; Maldonado and Uriz 1999). They lack organs, possessing cells that develop great number of functions. The sponge body is lined by a pseudoepithelial layer of flat cells (exopinacocytes). Anatomically and physiologically, tissues of most sponges (but carnivorous sponges) are organized around an aquiferous system of excurrent and incurrent canals (Rupert and Barnes 1995). These canals are lined by a pseudoepithelial layer of flat cells (endopinacocytes).Water flows into the sponge body through multiple apertures (ostia)

to the incurrent canals which end in the choanocyte chambers (Fig. 1). Chambers, scattered in the sponge body creating the choanosome, contain flagellated cells (choanocytes) whose beating drive the incurrent water through the canal system (endopinacoderm) in order to capture particles and bacteria to feed the sponge. Exhalant current leaves the body through the oscula. Between the exopinacoderm and the endopinacoderm, there is a mesenchymal region (the mesohyl) with considerable variability in organization, being often modified in response to multiple factors. It consists of an extracellular matrix of fibers (collagen and/or spongin), skeletal elements (siliceous or calcareous spicules), and a relatively abundant population of amoeboid cells with diverse functions (Harrison and De Vos 1991).

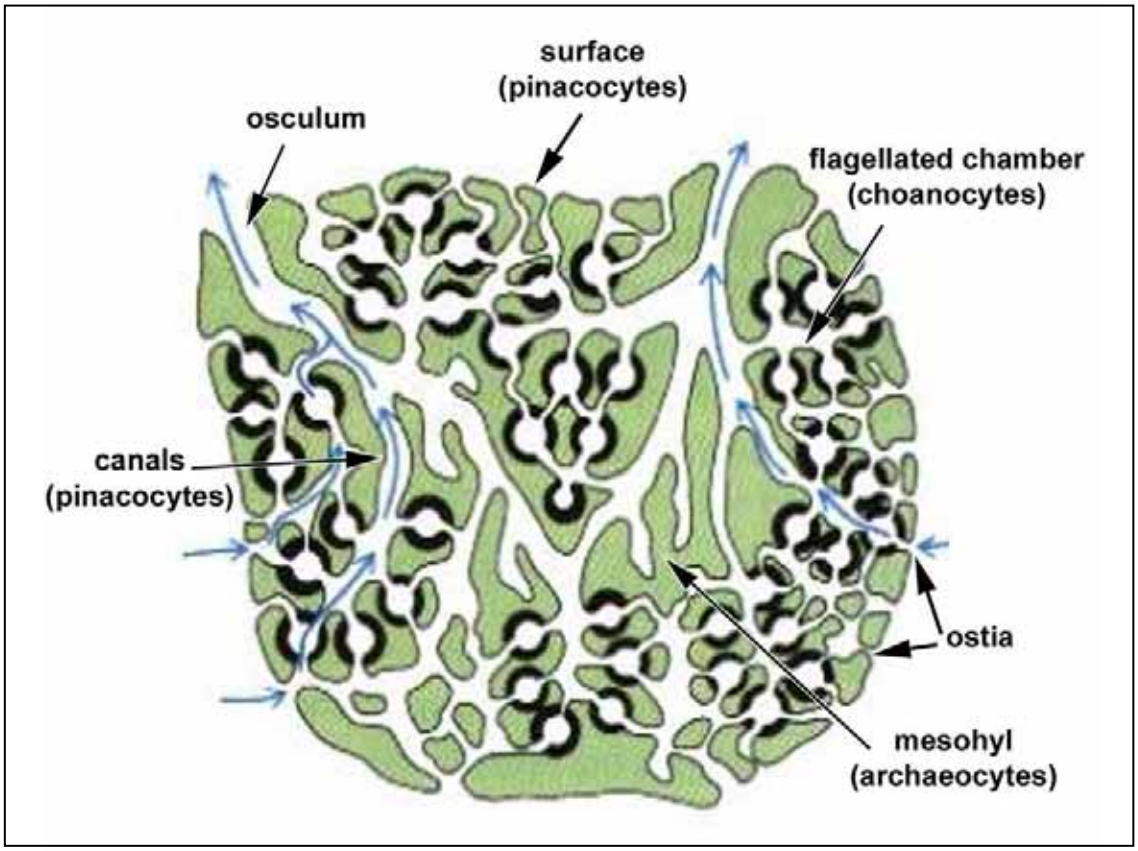

Figure 1. General organization of the sponge body. Blue arrows indicate the direction of the water flow.

Not all the sponges follow the histological structure detailed above. Sponges of the family Cladorhizidae (class Demospongiae) are especially unusual in their feeding

habit. They completely lack an aquiferous system or have it extremely reduced, so that they typically feed by capturing and digesting whole animals. That means they are carnivorous. They capture small crustaceans with their spicules which act like Velcro when they come in contact with the crustacean exoskeletons. Cells then migrate around the helpless prey and digestion takes place extracellularly.

- **Cytology of demosponges and the problem of germ cells:**

Sponge tissues often hold a consistent uniformity in their composition across the phylum. When compared with the cells of other animals the sponge cells appear to have great functional independence, which make them similar to a protozoan colony (Rupert and Barnes 1995). There are more than 10 types of cells described in sponges so far, summarized in Figure 2 and their location sketched in Figure 3. All of them appear in demosponges (Simpson 1984).

Type of cell	Function	Location
Pinacocytes	pseudoepithelial cells	epithelia: internal and external
Choanocytes	feeding	epithelia: internal
Myocytes	contraction	epithelia (around oscules)
Archaeocytes	totipotential amoeboid cells (=similar to macrophages)	mesohyl
Collencytes	secretion of collagen	Mesohyl
Lophocytes	secretion of collagen	mesohyl
Spongocytes	secretion of spongin	mesohyl
Megasclerocytes	secretion of megasclere spicules	mesohyl
Microsclerocytes	secretion of microsclere spicules	mesohyl and epithelia
Special cells (with inclusions)	with highly differentiated inclusions	mesohyl

Figure 2. Major categories of sponge cells, their function and their location within the sponge body (modified from Simpson 1984).

Apart from these cellular types, female and male gametes can be found in the sponge body, since sponges can reproduce both sexually and asexually. The origin of gametes varies depending on the species, because a predetermined germ cell line during embryogenesis does not occur (or at least remain undiscovered to date). Instead of that, somatic cells (in most cases archaeocytes and choanocytes) become germ cells (oogonia and spermatogonia).

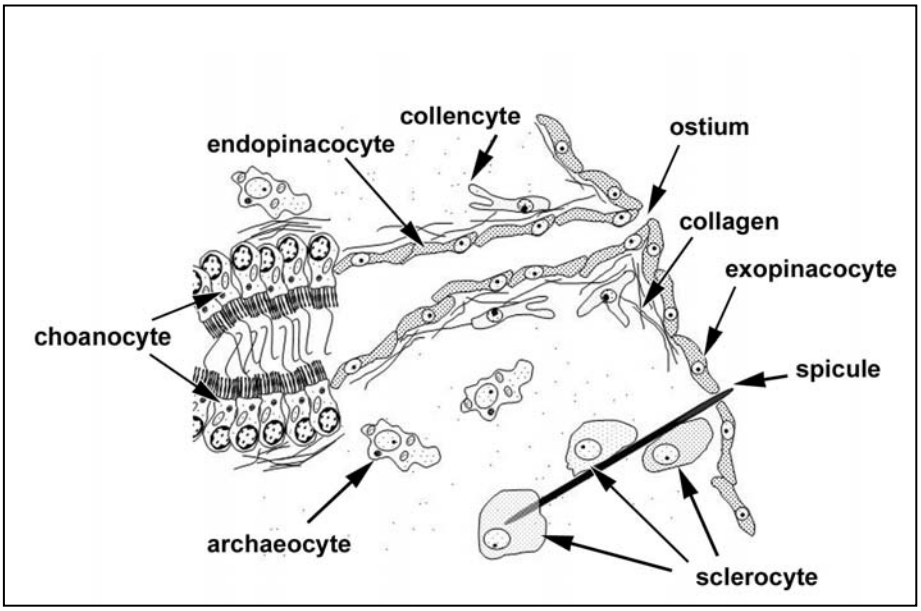

Figure 3. Simplified scheme of the location of the most frequent cells of sponges.

A predetermined germ cell lineage is also absent in placozoans and cnidarians, appearing by the first time in ctenophors (Extavour and Akam 2003). However, there are other animals (turbelarians, entoprocts, phoronids, some chordates and hemichordates) in which the induction of the germ cells occur also in a population of totipotential somatic cells (similar to the archaeocytes of poriferans and the cells I of cnidarians) (Buss 1983; Extavour and Akam 2003). Therefore, such absence in poriferans and cnidarians (the most basal phyla of metazoans, together with placozoans) implied that this ability was acquired later in the evolution, although in some other cases it was lost secondarily. The recent discovery of genes (vasa and nanos) involved in the germ lineage determination in cnidarians (Torras et al. 2004; Extavour et al. 2005) leaves sponges and placozoans as the most basal groups lacking such predetermination

in the germ cell line (although there are higher metazoans that also lack it), but opens the possibility of similar markers in sponges.

- **Reproductive biology of sponges and its relationship to environmental factors:**

The class Demospongiae exhibits an apparent structural and histological uniformity which contrasts with the diversity displayed in the reproductive models: gonochorism, successive hermaphroditism and contemporaneous hermaphroditism. While gonochoristic sponges are oviparous (and predominantly externally-fertilised), hermaphroditic sponges are mostly viviparous (internally-fertilised) (see Fell 1974 and Simpson 1984 for reviews). To our knowledge, only *Aplysina (Verongia) aerophoba* (Scalera-Liaci et al. 1971) and *Tetilla* sp. (Scalera-Liaci et al. 1976) are oviparous and hermaphroditic species, both of them demosponges. The rest of oviparous species are consistently gonochoristic (Fig. 4) (Simpson 1984). Both indirect and direct development appear in the Class Demospongiae (Figure 4), although the latter one is quite uncommon (Maldonado and Bergquist 2002).

Sponge reproduction also exhibits a great variability in the duration of the reproductive period. Some sponges undergo gametogenesis during 5 to 12 months (Lévi 1956; Fell 1974, 1976a; Scalera-Liaci and Sciscioli 1967; Ayling 1980; Reiswig 1983; Corriero et al. 1998), while others complete the process in less than 3 months (Scalera-Liaci et al. 1973a; Wapstra and van Soest 1987; Fromont 1994, 1999; Usher et al. 2004), this latter mode being less frequent. Differences in the duration of the reproductive cycles are mainly produced by the time required by each sponge to complete the gamete growth and maturation, which in the particular case of oogenesis is tightly related to the different types of vitellogenesis displayed by each sponge.

Seasonally changing sea temperatures may influence the reproductive activities of marine animals (Kinne 1970; Giese and Pearse 1974; Fell 1976b). Because of the high heat capacity of water and the large volume of the ocean, sea temperatures often vary slowly and rhythmically through the year. Changes in temperature therefore, may provide reliable clues to marine animals that may serve to synchronize their reproduction. Many studies emphasize this relationship (Gunter 1957; Kinne 1970; Sastry 1970; Goss and Bunting 1983). In 1946, Thorson proposed the relationship

between sea temperature and reproduction be termed “Orton’s rule”, distinguishing between the effects of gradual changes on gametogenesis from the effect of sharp temperature changes, which induce spawning. However, the fact that many species are known to have discrete breeding seasons in areas where temperature fluctuations are slight (polar, tropical, and deep-sea) does not fit well with Orton’s rule. But as temperature could vary seasonally from only half a degree centigrade to several degrees in such areas, Orton’s rule might not be completely discarded for these particular situations.

Among poriferans temperature is the most studied environmental factor that potentially control and/or regulate reproduction. Gametogenesis and larval release are often triggered/accelerated by maximum temperatures (Hartman 1958; Storr 1964; Fell 1974, 1976b; Scalera-Liaci and Sciscioli 1975; Johnson 1978; Tanaka-Ichihara and Watanabe 1990; Kaye and Reiswig 1991; Fromont 1994, 1999; Fromont and Bergquist 1994; Witte et al. 1994; Ereskovsky 2000; Mercurio et al. 2007), even though minimum values can operate in the same way (Fromont and Bergquist 1994; Corriero et al 1998; Ereskovsky 2000).

It is known that, most marine invertebrates, not only poriferans, have their reproduction regulated or moderated by environmental factors. Therefore, if a certain temperature value serves as the threshold that initiates gametogenesis, modifications of such values because of the climate change can result in the animal initiating a physiological response at the wrong time in relation to calendar date (Lawrence and Soame 2004). However, in some cases sponge gametogenesis appear to be unrelated to temperature and subjected to other stimuli instead (Elvin 1976; Witte 1996; Corriero et al. 1996). Such diverse a range in responses to environmental stimuli, and the possible impact of climate change in sponge reproductive behaviour, reveals the necessity of thorough studies of the reproductive timing of sponges and its potential relationship with this and other environmental factors.

- **Gametogenesis:**

Ultrastructural features of gametogenesis have been well studied during the 70s, 80s, and 90s. But, the approaching to the problem of the origin of gametes is in most cases speculative (Tuzet 1964, 1970; Gaino et al. 1984; Paulus 1989), since the

ultrastructural studies are based upon fixed material. However, archaeocytes and choanocytes are the cells that originate gametes in most sponges (see Fell 1974 and Simpson 1984 for reviews).

Taxon	*Reproductive mode*	*Development mode*
Class Hexactinellida		
Subclass Amphidiscophora		
Order Amphidiscosida	?	?
Subclass Hexasterophora		
Order Hexactinosida	?	viviparous
Order Lychniscosida	?	?
Order Lyssacinosida	hermaphroditic	viviparous
Subphylum Cellularia		
Class Calcarea		
Subclass Calcinea		
Order Clathrinida	hermaphroditic	viviparous
Order Murrayonida	hermaphroditic	viviparous
Subclass Calcaronea		
Order Leucosoleniida	hermaphroditic	viviparous
Order Lithonida	hermaphroditic	viviparous
Class Demospongiae		
Subclass Homoscleromorpha		
Order Homosclerophorida	hermaphroditic	viviparous
Subclass Tetractinomorpha		
Order Astrophorida	gonochoristic	oviparous
Order Spirophorida	hermaphroditic and gonochoristic	oviparous
Order Chondrosida	gonochoristic	oviparous
Order Hadromerida	gonochoristic	oviparous and viviparous
Order Axinellida	gonochoristic	oviparous
Order Agelasida	gonochoristic	oviparous
Subclass Ceractinomorpha		
Order Verticillitida	hermaphroditic	viviparous
Order Halichondrida	hermaphroditic and gonochoristic	viviparous
Order Poecilosclerida	hermaphroditic	viviparous
Order Haplosclerida	gonochoristic	oviparous and viviparous
Order Dendroceratida	hermaphroditic	viviparous
Order Dictyoceratida	hermaphroditic and gonochoristic	viviparous
Order Verongida	hermaphroditic and gonochoristic	oviparous
Order Halisarcida	hermaphroditic and gonochoristic	viviparous

Figure 4. Reproductive and development modes in the major taxonomic categories of the phylum Porifera. Modified from Simpson (1984) and Maldonado (2006).

Oogenesis is one of the best documented processes of the sponge reproductive biology. It has been studied in detail both in demosponges (Fell 1974; Simpson 1984; Barthel 1986; Kaye 1991; Witte and Barthel 1994; Lepore et al. 1995; Corriero et al. 1996; Usher et al. 2004; Mercurio et al. 2007) and in calcareans (Fell 1974; Simpson 1984; Gallissian 1988; Gallissian and Vacelet 1992; Anakina and Drozdov 2001). In hexactinellids, however, only the oogenesis of *Oopsacas minuta* has been described (Boury-Esnault et al. 1999).

Oogenesis is a process relatively uniform across the phylum, being the origin of gametes, the mechanism of vitellogenesis, and the duration of the process, the most relevant differences between the species. In calcareans and demosponges oocytes can derive from choanocytes (Diaz et al. 1975; Gaino et al. 1986a; Gaino et al. 1987; Sarà 1974) or archaeocytes (Leveaux 1941; Lévi 1956; Simpson 1984; Saller and Weissenfels 1985). In hexactinellids oocytes are (and must be) created from archaeocytes, since choanocytes are enucleated cells (Boury-Esnault et al. 1999). During oogenesis oocyte enlarge and complete the vitellogenesis, by auto-synthesis and/or with the help of nurse cells (see Fell 1974 and Simpson 1984 for reviews). Polar bodies, resulting from the completion of two meiotic divisions, have been observed in few species, most of them calcareans (Tuzet 1947; Gallissian 1981, 1988), and in only two demosponge species (Tuzet and Pavans de Ceccatty 1958; Tuzet and Paris 1964), but never by TEM.

Spermatogenesis is less documented in sponges than oogenesis. Male gametes, both in calcareans and demosponges, derive from choanocytes (see Reiswig 1983; Simpson 1984; Boury-Esnault and Jamieson 1999 for reviews), although an archaeocyte origin has been proposed in few studies (Fincher 1940; Lévi 1956). Hexactinellid choanocytes are enucleated; hence the spermatozoans are derived from archaeocytes congeries (Okada 1928; Boury-Esnault et al. 1999).

Sperm is produced within spermatic cysts, and their formation can be synchronous or asynchronous at the population level (see Reiswig 1983 and Boury-Esnault and Jamieson 1999 for reviews). In general, a choanocyte departs from a chamber and experiences several divisions to give rise to a spermatic cyst. In other cases, a whole chamber can transform entirely into a single cyst (Fell 1974). Modifications suffered by spermatogonia to originate mature spermatozoans are remarkably similar to what is found in most animals (Alberts et al. 1994).

Sponge sperm is widely regarded as primitive round sperm lacking acrosome (Baccetti 1984). However, morphology of mature sperm of the studied sponges is diverse. For instance, round sperm is found in *Verongia archeri* (Reiswig 1970), *Aplysilla rosea* (Tuzet et al. 1970) and *Ephydatia fluviatilis* (Paulus 1989), while elongated sperm is found in *Halichondria panicea* (Barthel and Detmer 1990) and *Crambe crambe* (Tripepi et al. 1984). Organelles and other elements found in sperm are consistently mitochondria, glycogen and Golgi apparatus. In some cases acrosomal complexes or proacrosomal vesicles have been observed (Tuzet 1932; Tuzet and Pavans de Ceccatty 1958; Tuzet and Paris 1964; Reiswig 1970; Diaz and Connes 1980), while a true acrosome have only been described by means of ultrastructure in *Oscarella lobularis* (Baccetti et al. 1986). In invertebrates the acrosome is a membrane-bounded organelle containing proteins, and especially bindin as a major constituent (Lopo 1983). Bindin is known to mediate the binding of sperm to egg. The functional meaning of such structure in sponges is still a matter of study, as well as the phylogenetic importance of both the acrosome and the whole sperm morphology.

- **Gamete release and fertilisation in seawater:**

Once gametes are formed, they are released into the water current. Synchronous gamete release in the sea is highly difficult to observe, since it implies an exhaustive monitoring of the reproductive cycles and an intensive sampling effort. It has been documented when occurred spontaneously in the field or in the laboratory but never induced. Viviparous species only release sperm, because oocytes are retained by the mother waiting for the fertilisation. Oocyte release has been observed only in few oviparous sponges (Watanabe 1978; Hoppe and Reichert 1987). However, early embryos are easier to observe, and have been documented in a number of species (Borojevic 1967; Lévi 1951, 1956; Sidri et al. 2005). Sperm release is also elusive, documented only in few studies underwent in tropical waters (Reiswig 1970, 1976; Hoppe and Reichert 1987; Ritson-Williams et al. 2004).

Fertilisation modes in marine invertebrates are highly diverse. Summarizing, external and internal fertilisation are distributed equally within marine invertebrates. External fertilisation (which involves broad casters, e.g. individuals that shed both oocytes and sperm to the water) occurs in cnidarians (Fautin et al. 1989), nemerteans

(Cantell 1989), priapulids (Nørrevang and van der Land 1989), sipunculids (Rice 1989), some molluscs (Brahmachary 1989), echiurids (Davis 1989), some annelids (Schroeder 1989), brachiopods (Chuang 1990) and echinoderms (Spinelli and Albanese 1990). Internal fertilisation, however, gathers far more types of performance. Copulation is carried out by gnathostomulids (Mainitz 1989), nematods (Bird and Sommerville 1989) or acanthocephalans (Crompton 1989). The use of spermatophores occurs in rotiferans (Gilbert 1989), gastrotrichs (Hummon and Hummon 1989), some molluscs (Brahmachary 1989), some annelids (Schroeder 1989), pogonophorans (Bakke 1990), phoronids (Emig 1982, 1990) and some brachiopods (Chuang 1990). There is, however, another type of internal fertilisation less frequent, in which sperm casters (individuals that shed sperm while oocytes are retained by mothers) are involved. Such fertilisation model occurs both in poriferans, together with external fertilisation, (Fell 1974, 1989; Simpson 1984) and ascidians (Cloney 1990; Pemberton et al. 2003).

Fertilisation data in poriferans are scarce. Fertilisaton rates have been estimated uniquely for *Xestospongia bergquistia* (Fromont and Bergquist 1994). Indeed, cytological data about fertilisation belongs only to internally-fertilising species (sperm casters). The mechanisms involved in internal fertilisation are described in a bunch of calcareans (Tuzet 1947, 1964; Gaino et al. 1987; Nakamura et al. 1998; Gallisian 1989; Anakina and Drozdov 2001) and in only three demosponges (Tuzet 1930a; Tuzet and Pavans de Ceccaty 1958; Tuzet and Paris 1964). However, fertilisation has been studied ultrastructurally uniquely in calcareous sponges (Gaino et al. 1987; Gallisian 1989; Nakamura et al. 1998). In all of them, fertilisation is mediated by choanocytes that operate as transferring cells (carrier cell) (Figure 5). The choanocytes capture the spermatozoans that have entered the incurrent canals. The spermatozoan disassembles once within the choanocyte, remaining perceivable at this stage only the nucleus, the flagellum and the mitochondria. The choanocyte departs from the chamber and transports the spermatozoan (currently the spermiocyst) to the oocyte, penetrating both into the oocyte until the fertilisation is completed (see Fell 1974 and Simpson 1984 for reviews). Such fertilisation mechanism was extended to the entire phylum, although an unequivocal description of it was provided only for calcareans (Simpson 1989).

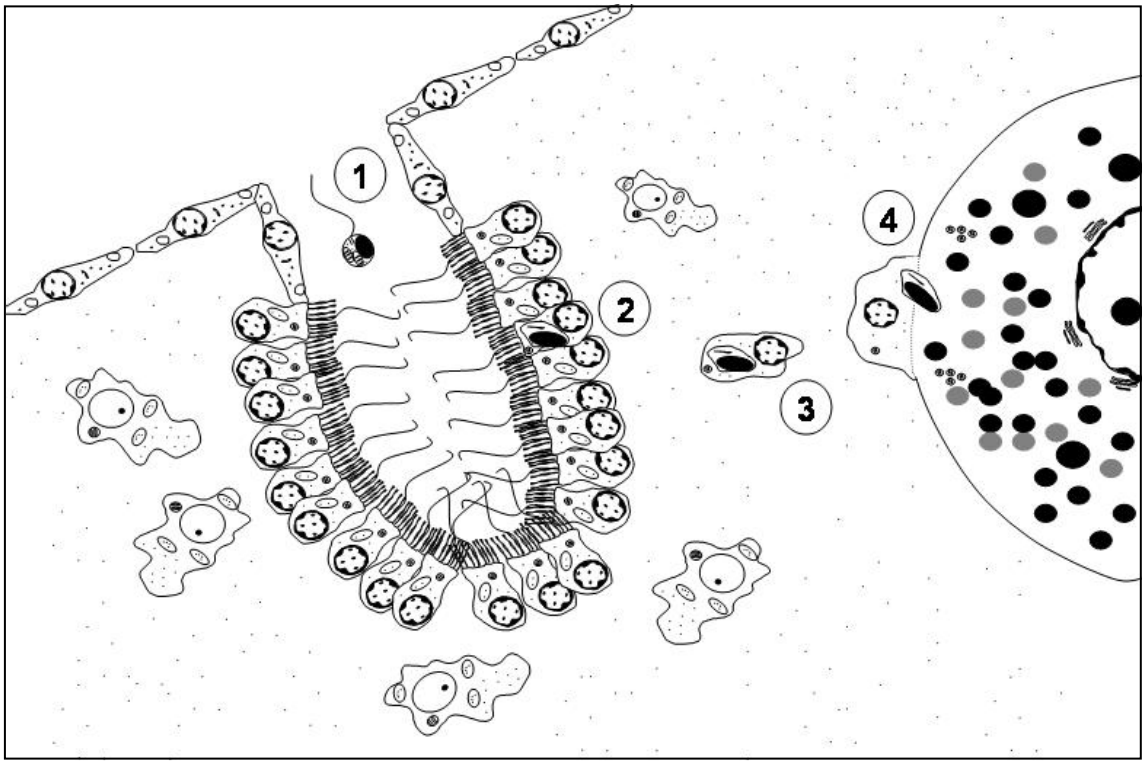

Figure 5. Fertilisation mechanism in sponges. **1)** The sperm enters the canals with the water current. **2)** The spermatozoon is engulfed by a choanocyte in a vacuole (spermiocyst), and inside it the spermatozoon disassembles, remaining only the nucleus, and rests of the mitochondria and the flagellum perceivable. **3)** The choanocyte carrying the spermiocyst (carrier cell) departs from the chamber and loses the flagellum and the collar, transporting the spermiocyst to the oocyte. **4)** The carrier cell penetrates the oocyte transferring the pronucleus of the spermatozoon to the oocyte.

- **Species studied:**

Selection of seven species for study was based on the lack of information available of their reproductive activity and the availability in the studied area. They were: *Chondrosia reniformis*, *Axinella damicornis*, *Crambe crambe*, *Asbestopluma occidentalis*, *Raspaciona aculeata*, *Corticium candelabrum*, and *Petrosia ficiformis*. All selected species are included within the class Demospongiae; six of them are Mediterranean species and one is from the Pacific northwestern coast of Canada. Each species belongs to a different demosponge order (except *Crambe crambe, Asbestopluma oocidentalis*, and *Raspaciona aculeata*, which belong to the order

Poecilosclerida) in order to cover as much variability as possible of reproductive strategies within the class.

- **Order Chondrosida**:

Family Chondrillidae

Chondrosia reniformis Nardo, 1833 is a common Mediterranean demosponge which owes its generic name to the cartilage-like consistency of its densely collagenous cortex (ectosome) (Bonasoro et al. 2001). It is a spotted sponge, dark-brown with white spots on the parts exposed to light, and white on parts unexposed to light (Figure 5A). Specimens are lobate and can reach 30 cm in great dimension and 3 cm thick. The consistency in life is cartilaginous, firm and tough. It possesses a great ability of contraction when touched or disturbed, and an astonishing capacity for tissue plasticity and morphological deformation (Bonasoro et al. 2001; Wilkie et al. 2006). The surface is smooth and shiny. The oscula are always visible. Specimens lack spicules but sometimes engulf and contain foreign spicules. It has a differentiated cortex characterised by a net of large fascicles of collagen fibrils and spherulous cells (containing spherules of about 3 μm). Extracellular symbiotic bacteria are present in the mesohyl (Manz et al. 2000). It grows in sublittoral rocky outcrops, usually in vertical walls from 5 to 30 m deep.

- **Order Halichondrida**:

Family Axinellidae

Axinella damicornis (Esper, 1794) is an erect, branching sponge, with short flattened or lamellate branches fused together, that gives the sponge a slight brain-like appearance (Fig. 5B). The color in living animals is yellow. The surface is generally smooth with choanosomal spicules protruding slightly. Superficial canals lead to openings (the oscula) which are not always visible (Hooper and van Soest 2002). The internal structure is organized around large bundles of spongin that contain needle-like megasclere spicules (styles and oxeas). The mesohyl of the sponge contains abundant

microorganisms, some of them identified as Archaea (Margot et al. 2002). It is characteristic component of semi-sciaphilous and sciaphilous communities, growing at rocky outcrops and crevices, between 10 and 40 m depth.

- **Order Poecilosclerida**:

Family Crambeidae

Crambe crambe (Schmidt, 1862) is a thin encrusting bright-red to orange sponge, occasionally lumpy, forming thicker tubercular masses (Fig. 5C). It can reach up to 0.5 m^2 in area and 0.5-1.5 cm in thickness (Turón et al. 1998). The surface is translucent, slightly hispid, with clear veinal channel pattern in life ending in volcano-shaped oscula. It possesses both megasclere and microsclere spicules, but these latter ones are often absent (Uriz et al. 2000). Megascleres are situated in bundles rising up from a spongin basal plate, occurring also tangentially to the sponge surface. Sand and other material are frequently incorporated in the basal parts. It is the most common sponge species in the western Mediterranean (Maldonado et al. 2005).

Asbestopluma occidentalis (Lambe, 1893) is a carnivorous sponge in which the aquiferous system is completely absent. The colour in living specimens is white and slightly transparent (Fig. 5D). Mature adults exhibit a stipitate morphology, consisting of a slender cylindrical shaft up to 6 cm long and 1.5 mm wide, from which long filaments arise. The surface of the sponge is slightly hispid due to protruding microscleres involved in prey capture. Together with microscleres, the sponge possess, in the central shaft and radiating from it, large bundles of collagen that enclose needle-like spicules (predominantly styles). It usually inhabits deep-sea habitats, but can be found not shallower than 30 m in Vancouver Island.

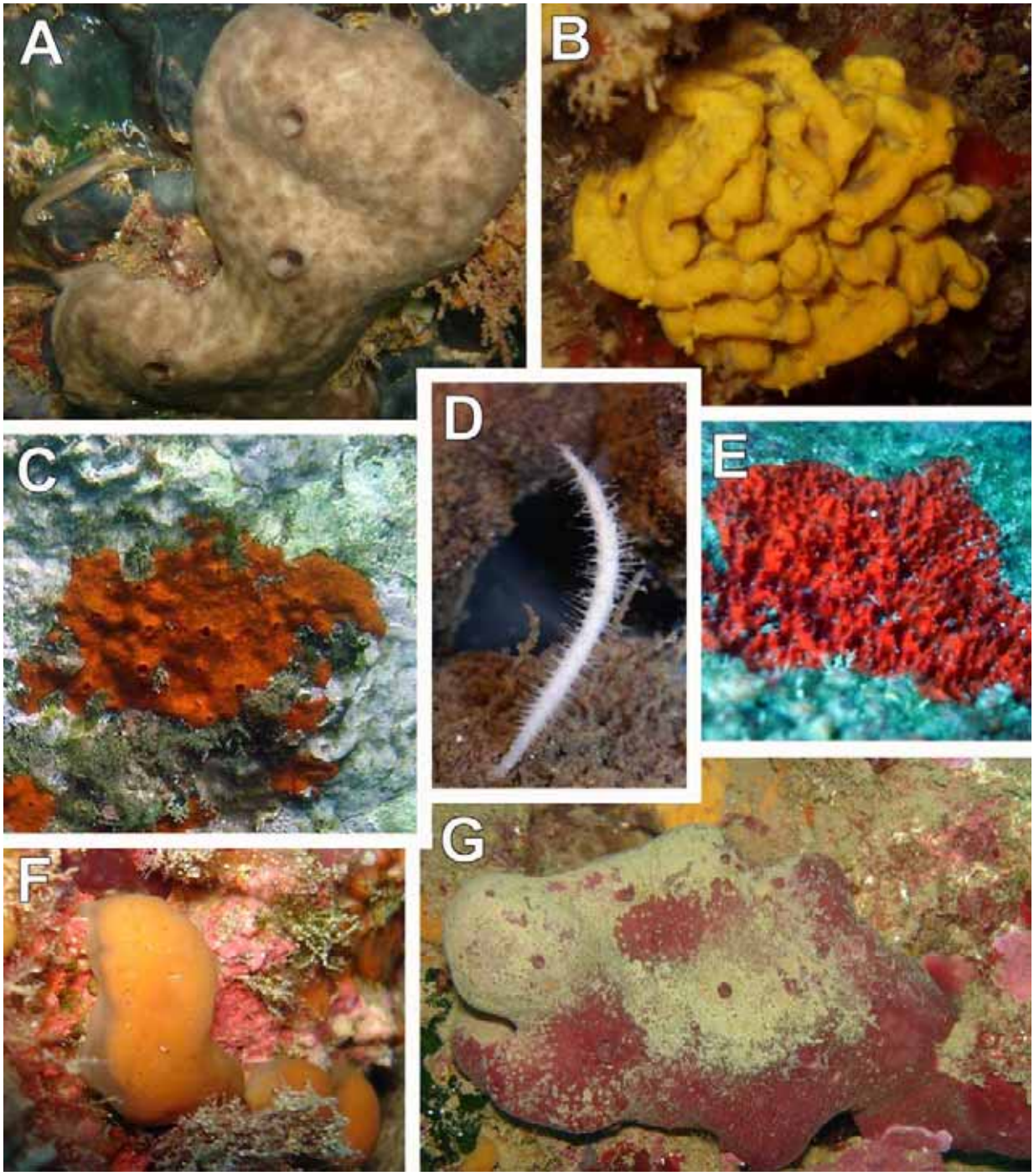

Figure 5. Demosponges studied within this thesis. **(A)** *Chondrosia reniformis,* from the Mediterranean. **(B)** *Axinella damicornis,* from the Mediterranean. **(C)** *Crambe crambe,* from the Mediterranean. **(D)** *Asbestopluma occidentalis,* from the Northwestern coast of Canada. **(E)** *Raspaciona aculeata,* from the Mediterranean. **(F)** *Corticium candelabrum*, from the Mediterranean. **(G)** *Petrosia ficiformis,* from the Mediterranean.

Family Raspailidae

Raspaciona aculeata (Johnston, 1842) is a thickly-encrusting bright-red sponge (Fig. 5E) that grows on pseudohorizontal rocky bottoms, usually covered by sand and sediment. The surface is hispid and clearly conulose. The oscula are not visible. The choanosomal skeleton is ramified in different branches that arise from a central shaft (Hooper and van Soest 2002). The spicules are megasclere needle-like forms (styles of different types). It grows sublittorally on rocky outcrops, stones and shells down to 15 m deep.

- Order Homosclerophorida:

Family Plakinidae

Corticium candelabrum Schmidt, 1862 is a thinly encrusting to cushion-shaped sponge (Fig. 5F), with a lobate structure that can reach 5 cm long and 1.5 cm thick. The body is attached to the substratum by thin filaments. The surface is uneven, slightly rough to touch. Oscula are round, 1-5 mm in diameter, and highly contractile. The colour is light brown to tan. The consistency is firm and cartilaginous. The skeletal spicules are scattered within the choanosome and the pinacoderm (Muricy and Diaz 2002; Maldonado and Riesgo 2007). It is a common sponge, though not abundant, in the sublittoral Mediterranean, inhabiting rocky outcrops, usually growing on vertical walls.

- **Order Haplosclerida**:

Family Petrosiidae

Petrosia ficiformis (Poiret, 1789) is a massive red sponge (Fig. 5G), which colour depends on the presence of autotrophic symbionts (Sarà and Vacelet 1973). It shows a wide range of morphotypes related to different habitats: large, massive specimens live in lighted conditions, while the branched and creeping specimens living in dim light and dark caves have been considered reduced forms because of the decrease in the trophic source represented by associated cyanobacteria (Regoli et al. 2000). The

aquiferous system ends on a terminal deep aquiferous cavity connected with volcano-shaped oscula. The surface is smooth, fine and compact, covered by a hispid ectosomal layer. The texture is firm and hard. The skeleton is comprised of needle-like megasclere spicules (oxeas) contained in large bundles of collagen. It usually grows on sublittoral vertical walls, caves, and crevices, from 10 to 40 m.

Objectives

Sexual reproductive dynamics and features of 7 selected species of demosponges were investigated by light and electron microscopy in order to search for the possible ecological and evolutive implications.

The work is structured from basic descriptions of reproductive biology (dynamics of production of reproductive products and relations of gametogenesis with environmental factors) to special ultrastructural features of gametes which were not studied so far, or were partially unresolved, that may help to understand the biology of the species.

The specific objectives (addressed in 7 different chapters) planned in this thesis were:

1. *Investigate the timing of sexual reproduction in Mediterranean demosponges and the potential effect of temperature.*

Chapter 1. "Revisiting the relationship between temperature and gametogenesis in sublittoral demosponges: a foresight of potential effects of climate change"

The reproductive cycles of sponges have been studied during the past three decades (see Fell 1974 and Simpson 1984 for reviews). However, the role of environmental factors triggering or regulating the gametogenesis are still poorly understood, although it is widely assumed that temperature play a relevant role controlling sexual reproduction in sponges (Reiswig 1983; Simpson 1984). Comprehensive studies comparing the strategies displayed by sponges living in the same habitat and under similar environmental conditions are still scarce, and could be of

much importance to investigate the effect of the global climate change on basic physiological processes of sponges, that determine recruitment, dispersal abilities, and subsequently population dynamics and densities.

The objective of this chapter is to investigate the sexual cycles of four selected common Mediterranean demosponges that live under similar environmental conditions, *Axinella damicornis*, *Corticium candelabrum*, *Raspaciona aculeata* and *Chondrosia reniformis*, and the potential role of the temperature in controlling their reproductive cycles.

2. Explore the sexual reproduction of common viviparous and oviparous sublittoral demosponges, by means of the study of the dynamics of both gametogenesis and embryogenesis using different techniques of microscopy.

Chapter 2. "Dynamics of gametogenesis, embryogenesis and larval release in a Mediterranean homosclerophorid sponge"

Once the general cycle was understood by means of the previous chapter, we wanted to explore deeply the dynamics of abundance and size of the reproductive elements and embryos found in the tissues, principally because of the detection of continuous production of oocytes, a feature unexpected in a sponge with annual reproduction. We also approached an ultrastructural study of the gametes, cells involved in fertilisation (carrier cells), and early embryos of *Corticium candelabrum*, in order to correlate its cytological features with its fertilisation success and some other ecological issues.

Chapter 3. "Dynamics of gametogenesis and gamete release in *Petrosia ficiformis* (Porifera, Demospongiae): absence of a free-swimming larva"

The dynamics of oogenesis and gamete release of *Petrosia ficiformis* were preliminary approached by Scalera-Liaci et al. (1973) in a population of the sublittoral area of the southern coast of Italy. The ultrastructure of the mature oocyte of *Petrosia ficiformis* was first described by Lepore *et al.* (1995), but the complete process of

oogenesis remains unknown. It is also unknown the developmental mode displayed by this particular species (Simpson 1984).

We wanted to study the sexual cycle of *Petrosia ficiformis* over two years using both light and electron microscopy. For that purpose we tagged several individuals that were monitored over both years of study, in order to fill the gaps in the parts of the process that remain unreported.

3. Examine the sexual reproduction of a peculiar demosponge, a carnivorous species, focusing on the implications of the absence of the aquiferous system for the entire process of reproduction, which, in the rest of the sponges, is completely involved in the development of the whole process.

Chapter 4. "Reproduction of a carnivorous sponge: what are the implications of the absence of an aquiferous system?"

The reproductive cycle of a carnivorous sponge has never been described before, although the presence of gametes and embryos was reported for the Mediterranean *Asbestopluma hypogea* (Vacelet and Boury-Esnault 1996). Sexual reproduction of *Asbestopluma occidentalis* was examined by light and electron microscopy[1] (both TEM and SEM), searching for the special adaptations that make possible sexual reproduction and fertilisation in the absence of both an aquiferous system and choanocytes.

4. Study the development of an atypical sperm cell, which starkly contrasts with the common sperm morphologies known for sponges.

Chapter 5. "Spermatogenesis of the V-shaped sperm of *Crambe crambe*"

Sperm cells in sponges are regarded as primitive, possessing round or conical shapes, a single flagellum, and lacking an acrosome. However, some studies of sperm cells of sponges have reported modified morphologies of sperm (e.g., Barthel and Detmer 1990; Tripepi et al. 1984). The study of Tripepi et al. (1984) briefly described

[1] This work was performed in the Department of Biological Sciences of the University of Alberta (Canada), under the supervision of Dr. Sally Leys

the advanced spermatid of *Crambe crambe*, but the dynamics of spermatogenesis and the ultrastructural features of the entire process remained unknown. Therefore, we investigated the dynamics of the spermatogenesis in a two-year study using both light and electron microscopy. Further, we followed the entire process of the spermatogenesis at the ultrastructural level, describing in detail the morphology of the sperm cells in the different stages of spermatogenesis. We also proposed an alternative mechanism of fertilisation which departs strongly to what assumed for all classes of sponges.

5. Examine the ultrastructural features of the oogenesis of oviparous demosponges that possess similar oocyte morphologies but differ in the duration and timing of their sexual reproductive cycles.

Chapter 6. "Ultrastructure of the oocytes of two oviparous Mediterranean demosponges, *Axinella damicornis* and *Raspaciona aculeata*"

The general features of the oocytes of *Axinella damicornis* and *Raspaciona aculeata* were examined by light microscopy in the first chapter, finding similar morphologies, sizes, and location. However, the duration of oogenesis varied in 3-5 months between both species, and the seasons when both oogenesis underwent were also different. We wanted to investigate ultrastructurally the entire process of oogenesis to show the differences (if any) between both species and their peculiarities.

6. Examine the possible mechanisms of sperm elimination after spawning displayed by demosponges, which are described in many invertebrates and vertebrates.

Chapter 7. "Self-predation of sperm in sponges: the role of motile phagocytic cells in the spermatic cyst of two demosponges, *Raspaciona aculeata* and *Petrosia ficiformis*"

Resorption of unspawned or abnormal gamete cells is reported in corals (Sier and Olive 1994), oysters (Braley 1982; Steele and Mulcahy 1999), polychaetes (Olive 1978) and echinoderms (Franz 1986; Guillou and Lumingas 1998). While oocyte resorption or degeneration is the rule in most invertebrates, the mechanism used by the

individuals to remove the unspawned or abnormal sperm is quite different. Somatic cells (Sertoli-like cells or motile phagocytic cells) usually phagocytose such sperm cells. These cells are thoroughly described in plathelmints (O'Donovan and Abraham 1987), bivalves (Vaschenko et al. 1997), marine gastropods (Buckland-Nicks and Chia 1986), polychaetes (Pacey and Bentley 1992), echinoderms (Chia and Buckland-Nicks 1987; Reunov et al. 2004), ascidians (Jørgensen and Lützen 1997), and cephalochordates (Holland and Holland 1989). We wanted to examine the mechanism used by sponges to remove the unspawned or abnormal sperm, and if any phagocytic cell (Sertoli-like cell) is involved.