Siliceous Spicules and Skeleton Frameworks in Sponges: Origin, Diversity, Ultrastructural Patterns, and Biological Functions

MARÍA-J. URIZ,[1]* XAVIER TURON,[2] MIKEL A. BECERRO,[1] AND GEMMA AGELL[1]
[1]*Center for Advanced Studies (CSIC), Girona, Spain*
[2]*Department of Animal Biology (Invertebrates), University of Barcelona, Barcelona, Spain*

KEY WORDS silification; spicules; ultrastructure; sponges

ABSTRACT Silica deposition is a fundamental process in sponges. Most sponges in the Classes Demospongiae and Hexactinellida secrete siliceous elements, which can subsequently fuse, interlock with each other, or form three-dimensional structures connected by spongin. The resulting skeletal frameworks allow sponges to grow upwards and facilitate water exchange with minimal metabolic cost. Several studies on sponge skeletogenesis have been published. We are beginning to understand the mechanisms of spicule secretion and the role of spicules and skeletal frameworks in the biology, ecology, and evolution of sponges. Molecular techniques and ecological experiments have demonstrated the genetic control of the process and the contribution of environmental factors to the expression of a sponge spicule, respectively. However, other classic topics such as the role of membranes in silicon transport or whether spicules are formed in situ or secreted anywhere in the sponge mesohyl and then transported to the skeletal framework require further investigation. We review the process of silica deposition in sponges at the molecular and cellular levels, as well as the biological and ecological functions of spicules and skeletons. The genetic control of spicule shapes makes them useful in the reconstruction of sponge phylogeny, although recent experiments have demonstrated the influence of environmental factors in modulating spicule size, shape, and the presence or absence of one or more spicule types. The implications of such variations in sponge taxonomy may be important. Besides supporting sponge cells, spicules can help larvae stay buoyant while in the plankton or reach the bottom at settlement, enhance reproduction success, or catch prey. Conversely, the role of spicules and skeletons in deterring predation has not been demonstrated. Knowledge of several aspects is still based on a single or a few species and extrapolations should be made only with caution. With the advent of new molecular techniques, new lines of research are presently open and active in this field. *Microsc. Res. Tech. 62:279–299, 2003.*

INTRODUCTION

Sponges, regardless of the Class to which they belong (i.e., Calcarea Bowerbank, Demospongiae Sollas, or Hexactinellida Schmidt), secrete mineral or proteinaceous structures that give them a variety of three-dimensional shapes, which minimizes the metabolic cost of water exchange (Vogel, 1974; Larsen and Riisgard, 1994; Riisgard and Larsen, 1995).

Most Demospongiae and Hexactinellida produce silica-made skeletons consisting of individualized elements (spicules) of lengths ranging from micrometers to centimeters, which can subsequently fuse or interlock with each other. The two classes differ from a skeletal point of view in the number of symmetry axes of their megascleres, which are monaxons and tetraxons in demosponges and monaxons and triaxons in hexactinellids (Fig. 1).

The high diversity of spicule shapes and sizes (Fig. 2) in both fossil and living sponges has been repeatedly reported (e.g., Hinde, 1887–1893; Hartman, 1981; Simpson, 1984) and has received particular attention in taxonomic and cladistic studies (e.g., Chombard et al., 1998; Hooper, 1990; Rosell and Uriz, 1997; Uriz and Carballo, 2001). However, the mechanisms that determined such diversity remained elusive until recently. Harrison and Simpson (1976) and later authors (e.g., Garrone et al., 1981) attributed to both the proteinaceous spicule core (the axial filament) and the surrounding membrane (silicalemma) a role in shaping the spicules. Most of those early contributions were reviewed ~20 years ago (e.g., Volcani, 1981; Simpson, 1984, 1989), and here we focus on progress since then. Recent molecular studies (Shimizu et al., 1998; Cha et al., 1999, 2000; Krasko et al., 2000) cast light on the genetic control of spicule deposition. In contrast, the role of membranes in modulating spicule ornamentation (spines and swellings) or the terminal formations

Contract grant sponsor: CICYT; Contract grant number: MAR98-1004-C02; Contract grant sponsor: Generalitat of Catalonia; Contract grant numbers: 1999SGR00184 and REN2001-2312-CO3/MAR and INTERREG-III-2002 (to MJU and XT).

*Correspondence to: María-J. Uriz, Center for Advanced Studies (CSIC), Accés a la Cala St. Francesc, 14 17300 Blanes, Girona, Spain. E-mail: Iosune@ceab.csic.es

Received 7 August 2002; accepted in revised form 20 April 2003
DOI 10.1002/jemt.10395
Published online in Wiley InterScience (www.interscience.wiley.com).

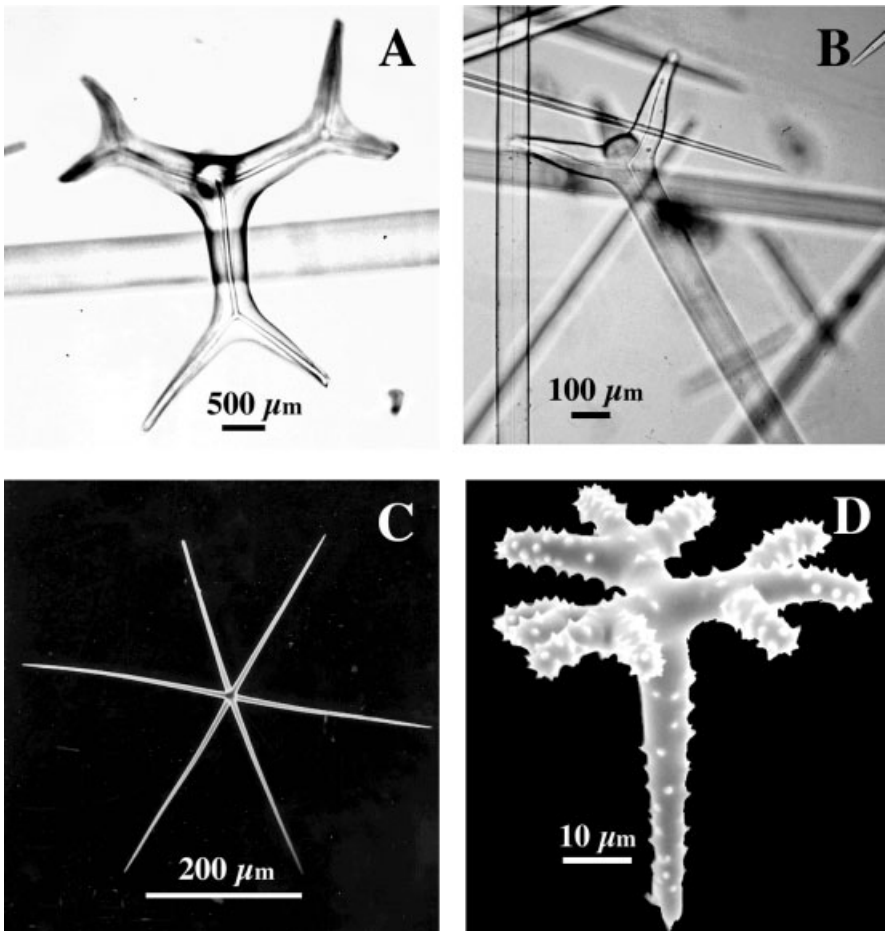


Fig. 1. Typical megascleres of demosponges and hexactinellids. **A,B:** Light microscope image of tetraxons (triaenes) of several families of astrophorid Demospongiae. **C:** SEM photograph of a triaxon (hexactine). **D:** SEM acanthotriaene of *Thrombus* (**C**, modified from Uriz, 1988; **D**, modified from Uriz, 2002).

of desma arms (which interlock to form rigid skeletons) are only implicitly accepted (e.g., Garrone et al., 1981).

SPICULE DIVERSITY

Siliceous sponge spicules have traditionally been separated into two categories termed, according to their size, megascleres and microscleres (e.g., Lévi, 1973). However, size alone does not suffice to separate the two categories in all cases. Some microscleres (e.g., toxas of *Microciona*, sigmas of *Mycale* or onychaetes of *Tedania*) can be larger than some megascleres (e.g., oxeas in *Haliclona*). Moreover, there are species with spicules of intermediate size (e.g., family Plakinidae), which are called mesoscleres only in hexactinellid sponges (Reiswig, 2002). On the other hand, several spicule shapes are exclusively present among either megascleres or microscleres, but there are also a number of exceptions (e.g., microxeas and microstrogyles are similar in shape to, but smaller than, oxeas and strongyles).

A more "functional" diagnostic character used to separate megascleres from microscleres is their role in skeleton organization. Megascleres usually form the main skeletal framework. In contrast, microscleres are widespread in the sponge body and only rarely are they embedded in collagenous material (e.g., when they are concentrated in a peripheral layer—the cortex—as in Geodiidae, Ancorinidae, or Tethyidae). An accessory role of microscleres in the sponge skeleton can be assumed after observing sponge species that grow and reproduce normally but lack one or several types of microsclere. In adverse environmental conditions, such as limited silicic acid availability (Yourassowsky and Rasmont, 1983; Maldonado et al., 1999), sponges may not produce microscleres, which has no detectable effect on the main skeleton framework, species shape, or thickness or ecological success. For instance, *Crambe crambe* is one of the few sponge species that competes successfully for the substrate with seaweeds in the western and central Mediterranean sublittoral (e.g., Uriz et al., 1992), although silica concentration in these waters does not allow the species to produce its characteristic microscleres (Uriz and Maldonado, 1995).

Siliceous spicules are highly diverse in sponges and the selection pressures responsible are difficult to envisage. There are over 12 basic types of megasclere and 25 types of microsclere reported in Demospongiae, 20 basic types of megasclere, and 24 types of microsclere in Hexactinellida, besides a long list of variations of the basic types (Fig. 3) (Boury-Esnault and Rützler, 1997; Tabachnick and Reiswig, 2002).

In several orders of demosponges, some megascleres or microscleres (desmas) become hypersilified and interlock to form a compact ("lithistid") skeleton (Fig. 4)

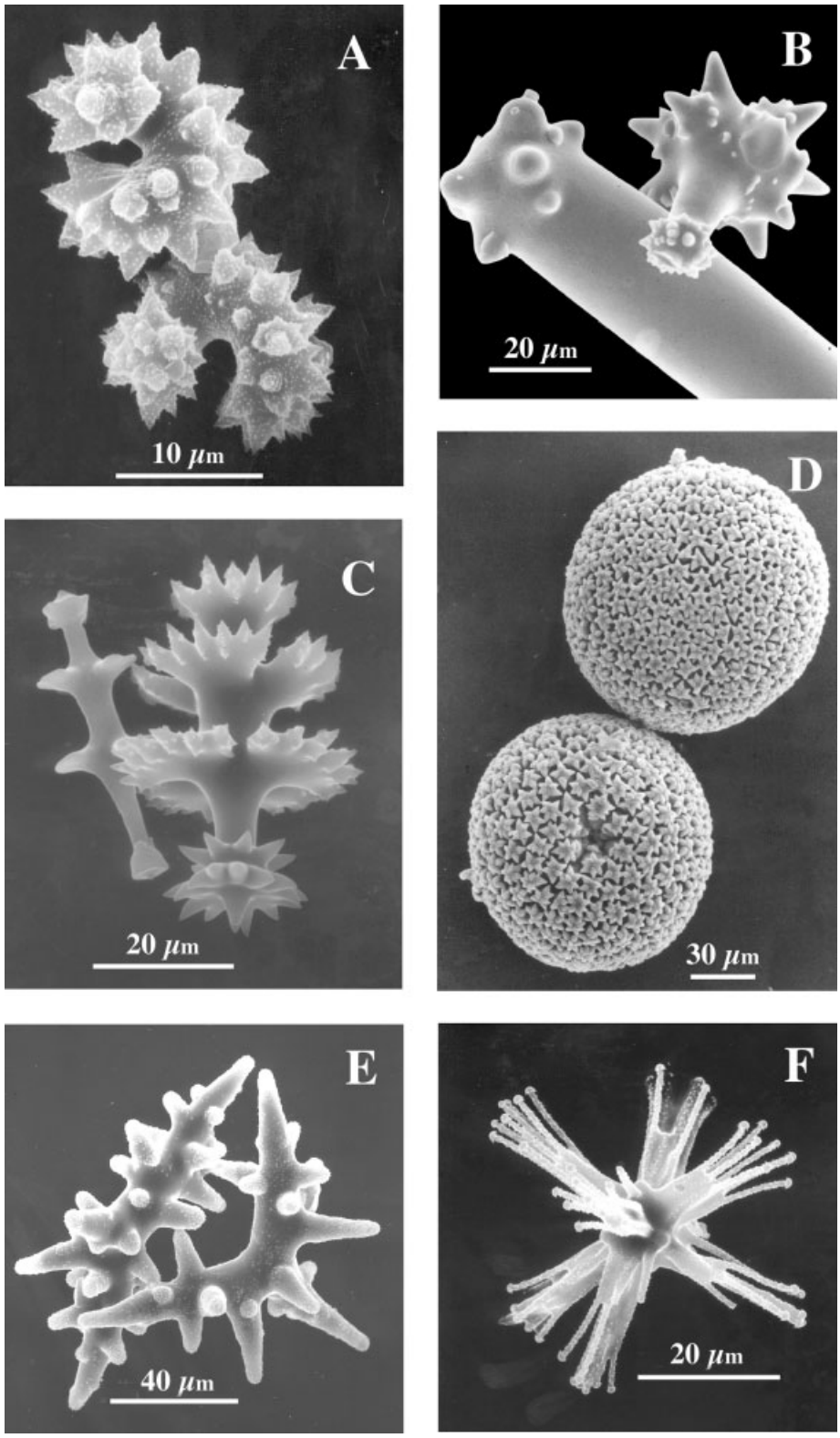


Fig. 2. SEM images of microscleres from different orders of Demospongiae and Hexactinellida. **A:** spirasters of *Spirastrella*. **B:** asterose microacanthostyle of *Discorhabdella*. **C:** discorhabds of *Latrunculia*. **D:** sterrasters of *Geodia*. **E:** microscleres of *Paradesmanthus*. **F:** floricome (microhexaster) of *Sympagella* (**B,C,F**, modified from Uriz, 1988; **E**, modified from Boury-Esnault et al., 1994).

that may confer a stony consistency to the sponge. In species that have the genetic potential to produce desmas, the concentration of silicic acid in the environment may determine whether these spicules are expressed (e.g., *C. crambe*, Maldonado et al., 1999). Conversely, in high Si concentrations, no desmoid spicules accumulate additional silica, giving rise to "abnormalities" (Fig. 5). In upwelling regions such as the Namibia

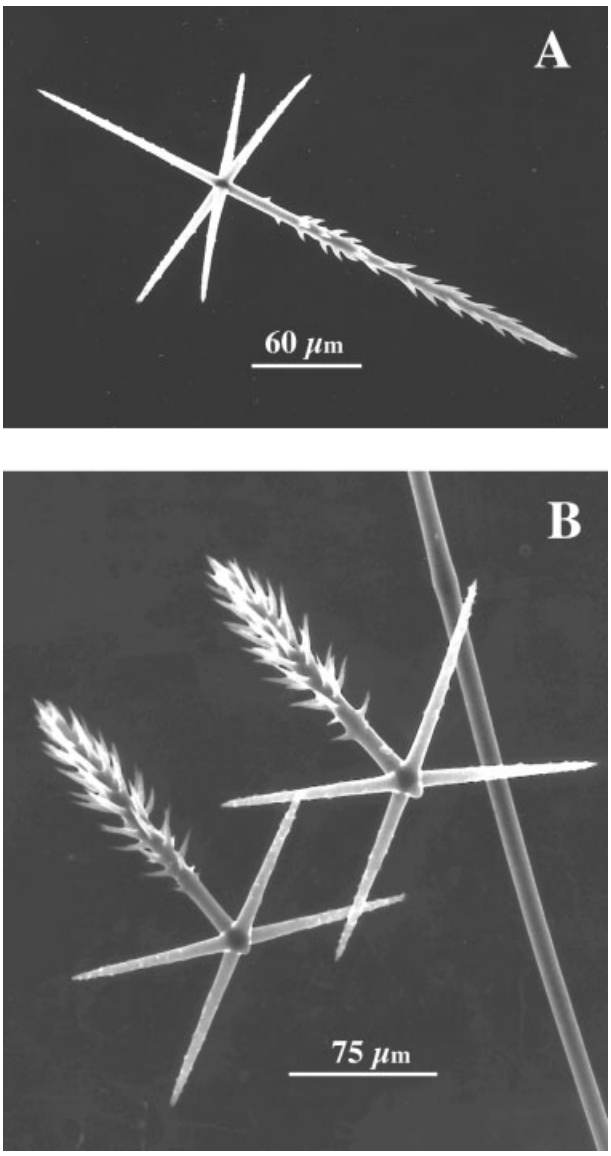


Fig. 3. SEM images of plumules (modified triactines) of hexactinellids. **A:** Hexactine. **B:** Pentactines (Modified from Uriz, 1988).

shelf (Uriz, 1988), the tylostyles of a *Suberites* species have greatly enlarged shafts and rounded points, as a result of which they resemble abnormal tylostrongyles (Fig. 6). In other species, such as *Guitarra flamenca*, silica spheres may accompany normal spicules (Carballo and Uriz, 1998; Fig. 7). Further examples of hypersilification include the proximal tyle of some tylostyles of *Crambe, Discorhabdella* (Uriz and Maldonado, 1995), or *Terpios* (Rützler and Smith, 1993). All these tylostyles have an axial filament, which is polyaxial at the tyloid (proximal) end. Consequently, young spicules have a polyactinate head with short actines, which are seen as pseudospines or pronounced lobes. This proximal zone is further enlarged by silica apposition and become a subspherical tyle similar to those of normal tylostyles.

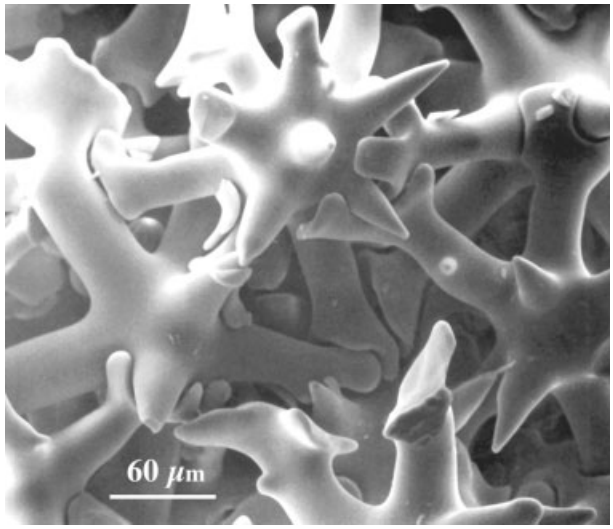


Fig. 4. SEM image of interlocked desmas of *Crambe acuata* (Modified from Uriz, 1988).

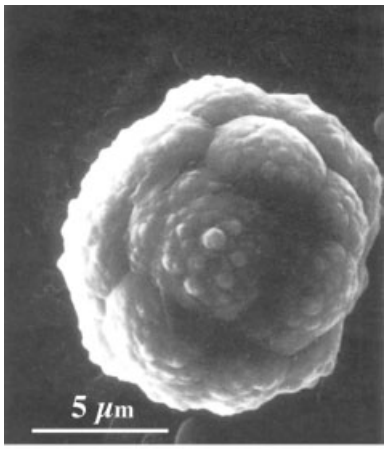
Spicules up to several cm long (e.g., Lévi, 1989) and strongly hypersilified skeletons are typical of many hexactinellid sponges.

SKELETAL FRAMEWORKS

Spicules and most particularly megascleres can be distributed throughout the sponge mesohyl but they generally frame two- or three-dimensional structures joined by spongin (most demosponges), interlock with each other (lithistids), or are cemented by additional silica (Hexactinellids).

In contrast to the high diversity of spicules, there are relatively few basic types of skeletal framework in Demosponges. Six elemental types of skeletons with intermediate forms can be differentiated: hymedesmoid, plumose, axial, radiate, reticulate, and arranged in strength confusion (Boury-Esnault and Rützler, 1997). Similar arrangements may be found in unrelated taxonomic groups as a result of convergent evolution (e.g., radial arrangement in the hadromerid Tethydae and the spirophorid Tetillidae).

The sponge growth habit usually reflects the underlying skeletal arrangement in demosponges. With few exceptions, thinly encrusting sponges have a hymedesmoid skeleton (megascleres arranged singly with their points directed upwards (e.g., *Eurypon, Hymedesmia*, early stages of *Microciona*). Thickly encrusting forms may hide a plumose skeleton (e.g., *Dictyonella, Phorbas*), while solid, cylindrical, and branching forms generally have axial skeletons (e.g., *Raspailia, Axos*). Subspherical or globular forms usually have a radiate spicule arrangement (*Tethya, Geodia, Polymastia, Aaptos*). Massive, irregular forms may have either disarranged (*Topsentia, Epipolasis, Halichondria*) or reticulated skeletons (e.g., *Petrosia, Haliclona*). Finally, tubular sponges are often made of reticulated skeleton walls (Fig. 8) (e.g., *Haliclona*). Other more sophisticated skeletons occur as a result of combinations of the basic types (e.g., in papilla or fistule-bearing species, Fig. 9).



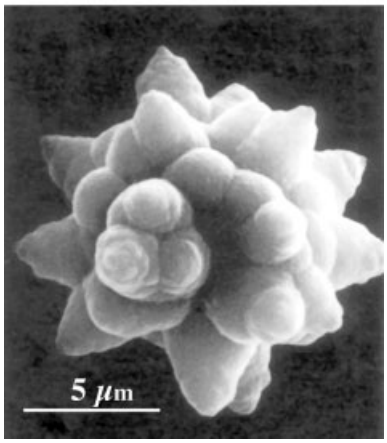


Fig. 5. SEM images of abnormal (hypersilicified) asters of *Chondrilla nucula* (Modified from Bavestrello et al., 1993).

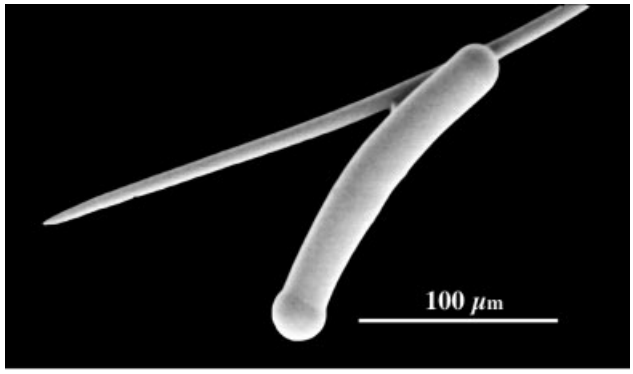


Fig. 6. SEM image of an hypersilicified tylostyle of *Suberites tylobtusa*.

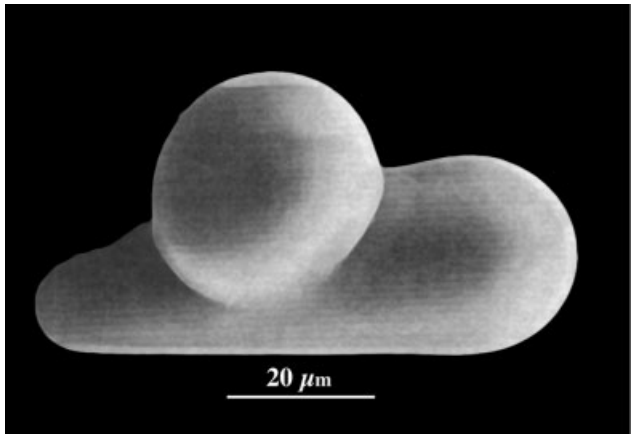


Fig. 7. SEM image of an abnormal hypersilicified spicule in *Guitarra* sp. (Modified from Carballo and Uriz, 1998).

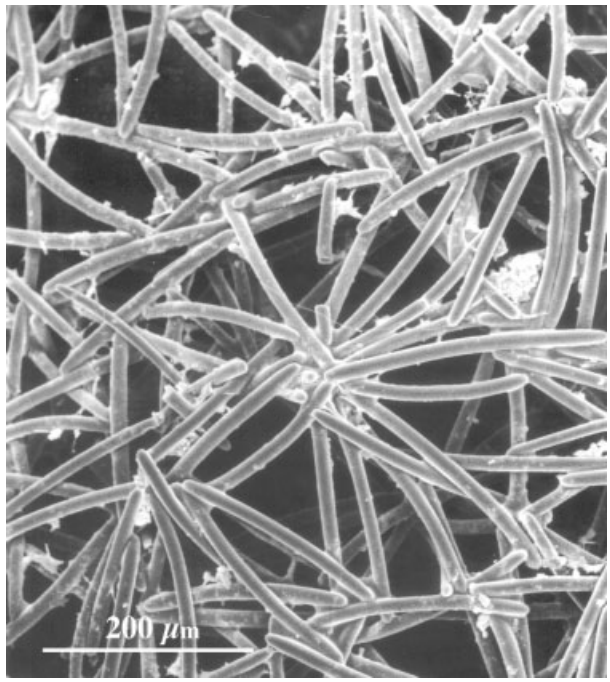


Fig. 8. SEM image of a monactines-made reticulated skeleton of an haplosclerid demosponge.

Conversely, the relationship between body shape and skeletal frameworks is less evident for hexactinellids. They are usually massive sponges with a clearly defined shape: erect, pedunculated, or sessile, either unbranched or branched, tubular saccular, mushroom, fan, blade, and funnel shapes. Many of these forms (Fig. 10) share a primarily dictyonal framework and the specific body shape relies on the secondary structure of the reticulate framework.

The two structural "layers" that can be distinguished in a demosponge, the ectosome and the choanosome (e.g., Galera et al., 2000), usually have different spicule types and arrangements. The choanosomal skeleton plays the main supportive role in a sponge (Boury-Esnault and Rützler, 1997). But the peripheral (ectosomal) skeletons, which are original in many cases, may contain megascleres, microscleres, or both. Some of the most frequent arrangements of the sponge ectosome are as follows:

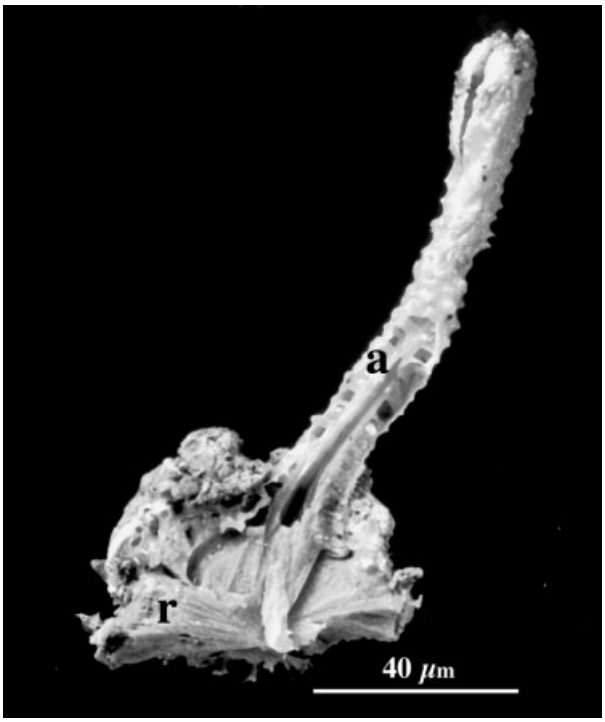


Fig. 9. Longitudinal section of an astrophorid sponge showing a complex skeletal arrangement: radial in the main sponge body and axial in the papilla.

- Dense palisade of upward-pointing megascleres of the same type but shorter than those in the choanosome (e.g., in Suberitidae, Polymastidae).
- Bundles of short megascleres that hispidate the sponge surface (e.g., *Raphidophlus, Willardia*) or surround (echinate) a larger choanosomal megasclere (e.g., styloids in *Raspailia*).
- Tangential, parchment, and paratangential disarranged layer (e.g., acanthoxeas crust in *Crella*, oxeas in *Topsentia*, parchment in *Epipolasis*).
- Particular reticulation of spicules of similar shape as those in the choanosome (e.g., in some *Haliclona*).
- Microscleres concentrated in a crust (e.g., discorhabds in *Latrunculia*, sterrasters in *Geodia*, spirasters in *Spirastrella*).

Furthermore, when an ectosomal skeleton is absent (e.g., in Axinellida), the ectosome is often held up or traversed by choanosomal spicules, which hispidate the sponge surface and allow subectosomal canals to develop.

Siliceous skeletons are particularly well developed in desma-bearing demosponges (Fig. 11) and hexactinellids. Some hexactinellid skeletons (i.e., subclass Amphidiscophora Schulze, 1886) are formed by loose megascleres of different types (hexactins, pentactins, stauractins (tetractines), tauactins (triactines), and diactins), usually at specific sites of the sponge body. Microscleres are always amphidiscs and never microhexactins or derived forms. In contrast, megascleres fuse in most skeletons of Hexasterophora Schulze, 1886, forming a rigid dictyonal network in which the original spicules are obscured by silica (Fig. 12) or are only joined by silica cement at contact points. The frameworks of dictyonal hexactinellids show very different patterns, with meshes being rectangular, rounded, irregular, smooth, or spiny, and formed by fusion of different types of megasclere: pentactines, diactines, and hexactines. Microscleres are microhexasters that can be loose in the sponge body or partially fused to the main skeleton. Fusion of hexactine or hexactine-derived spicules may be simultaneous to spicule formation (Reiswig, 2002). However, the basal long diactines that anchor the sponge to the substrate fuse long after the spicules are secreted (Reiswig, 2002).

Hexactinellid sponges have more silica per biomass unit than demosponges. As a consequence of their silica demands, they are confined to silica-rich environments such as deep bottoms and upwelling zones.

SPICULE FORMATION: ULTRASTRUCTURAL AND MOLECULAR PATTERNS

Ultrastructural Aspects

Whether secretion of spicules in sponges is intra- or extracellular is controversial. Microscleres are mostly secreted intracellularly (e.g., Custódio et al., 2002) with only one known exception (Simpson, 1968). Similarly, several studies support intracellular secretion of megascleres (Garrone, 1969; Simpson and Vaccaro, 1974; Garrone et al., 1981; Hartman, 1981), and only one supports extracellular secretion (megascleres of the demosponge *Crambe crambe*, Uriz et al., 2000). However, all the studies that supported the intracellular secretion of megascleres in demosponges dealt exclusively with small spicules. A single sponge cell cannot completely include a large megasclere, like some of Astrophorid, Spirophorid, Hadromerid, or certain Axinellid sponges, and therefore several sclerocytes appear to be necessary for secreting a single spicule. In contrast, intracellular formation within giant multinucleate sclerocytes has been suggested for the megascleres in Hexactinellida (Mackie and Singla, 1983), although "ad hoc" formation of transient junctions among sclerocytes to secrete some centimeters long hexacts cannot be ruled out (Boury-Esnault and Vacelet, 1994). Consequently, both modes, intra- and extracellular secretion, appear to occur depending on either the spicule type or the taxonomic position of the species considered. The factors determining one or the other type of spicule secretion remain elusive.

The cells that secrete the spicules (sclerocytes) are well typified in demosponges but less so in hexactinellids. They are characterized by abundant small clear vesicles and mitochondria (Fig. 13), which are an indication of their high metabolic activity, and a Golgi apparatus, and may occur both isolated in the sponge mesohyl and forming clusters (Uriz et al., 2000; Wilkinson and Garrone, 1980). Megasclerocytes are larger than microsclerocytes and have a nucleolated nucleus (e.g., Custódio et al., 2002; Garrone et al., 1981; Simpson, 1968; Wilkinson and Garrone, 1980). Moreover, in experimental cultures of *Spongilla lacustris*, micro- and megasclerocytes also respond differently to changes in silicic acid concentration (Jørgensen, 1944).

Although no differences have been found at the ultrastructural level, sclerocytes seem to be specific in

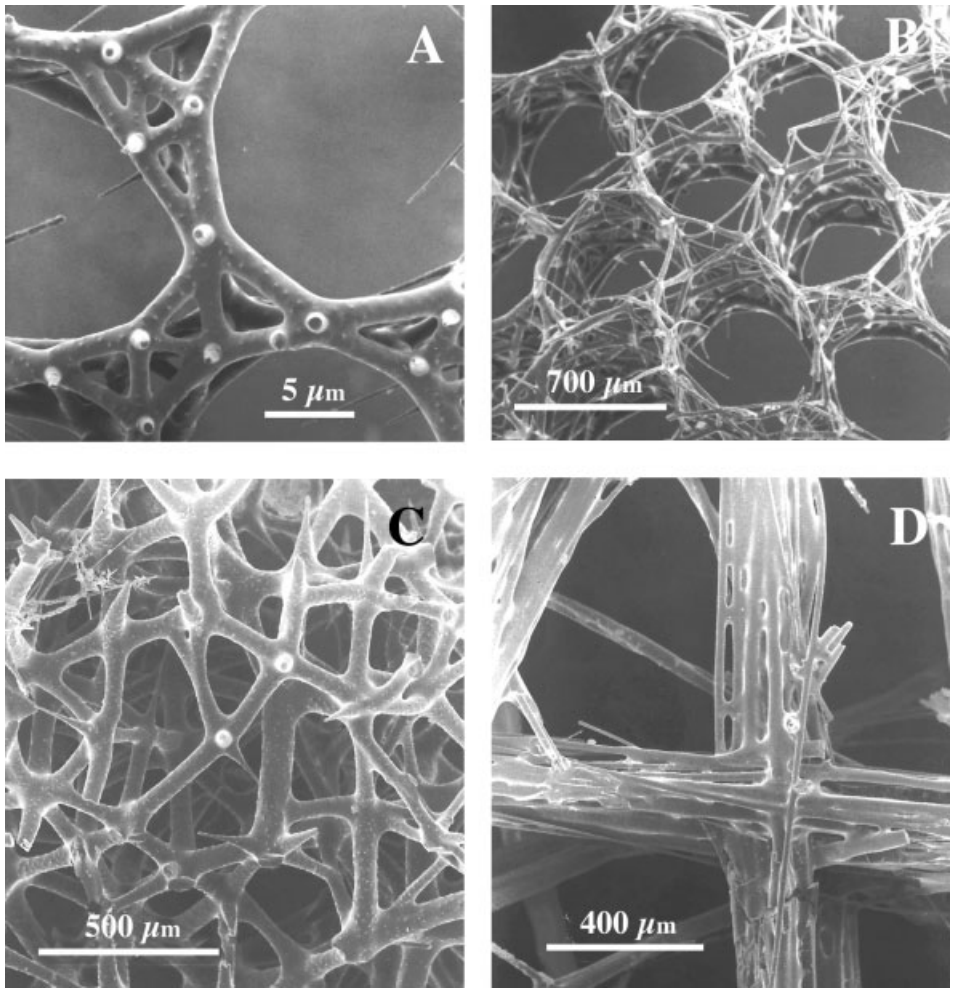


Fig. 10. SEM images of dictyonal frameworks in Hexactinellida. **A,B:** *Aprhocallistes*. **C:** *Tretodictyum*. **D:** *Regadrella* (Modified from Uriz, 1988).

the type of spicule they secrete. The differences rely on the genetic control of the axial filament that chiefly determines spicule shape. Each type of sclerocyte appears to require a particular silicon concentration, below which it does not secrete the corresponding spicule. In the demosponge *C. crambe*, this threshold appears to be higher for microsclerocytes than for megasclerocytes, for microsclerocytes that secrete desma than for those producing isochelae, and for megasclerocytes secreting large styles than those secreting small ones. Consequently, small styles are the only spicule type produced in *C. crambe* living in the silicon-poor western Mediterranean waters (Maldonado et al., 1999).

A unit-type membrane surrounds most growing spicules. This membrane, called silicalemma (Fig. 14), was thought to be something different from the plasmalemma or cell membrane (Garrone and Lethias, 1990). However, a connection between both membranes was first speculated (Simpson, 1984) and later shown in TEM images (Uriz et al., 2000). The secretion of spicules without a surrounding membrane has been reported only once: the microxeas of *Neofibularia irata*, which are secreted all together in a vacuole (Wilkinson and Garrone, 1980). The existence of spicules without an individual membrane questions the role of the silicalemma in shaping the final spicule by contributing to the formation of swellings and spines (Simpson, 1984). However, desmas usually form articulate frameworks, and the presence of intermediate membranes may be the only structure preventing fusion among neighboring desmas (Fig. 15).

Axial Filament: Role in Silica Polymerization and Spicule Shaping

All the siliceous spicules have a central core filled by a proteinaceous material with silica bound to the organic matrix (Fig. 16), called the axial filament. Silica forms part of the axial filament in *C. crambe* (Uriz et al., 2000) and strongly impregnates the axial filaments of spicules in *Corticium candelabrum* and *Phorbas fictitius* (this article). In the former, silica nanospheres (Shimizu et al., 1998) are seen at the external zone of the filament (Fig. 17), while in *P. fictitius*, transversal sections of the filament show similar fractures to those of the spicule walls (Fig. 18A), which is an indication of the filament hardness due to silica.

Desmoid (hypersilicified) spicules also have a proteinaceous axial core but differ from "normal" spicules in that the filament is shorter than the spicule arms (Fig. 19) and their width may vary. The question remains as to how silica deposition takes place in the

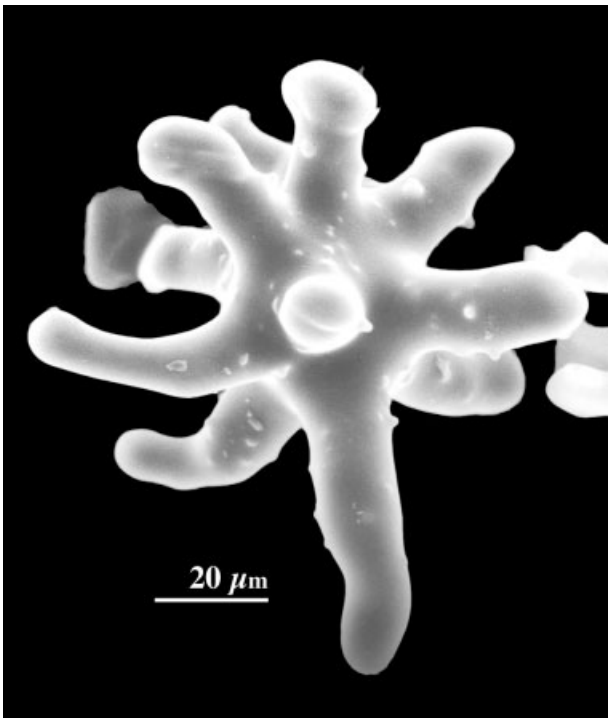


Fig. 11. SEM image of an asteroid desma of *Crambe acuata* (Modified from Uriz, 1988).

terminal arms of desmas, which are deprived of axial filaments. Pisera (pages 312–326 in this issue) suggests the presence of organic molecules that would induce silica polymerization without forming discrete filaments.

The cross-section of the axial filament in the several megascleres and microscleres examined has generated a great deal of discussion, as it was thought that the filament shape might explain spicule morphology (Reiswig, 1971). There is agreement in that the cross-section of the axial filament in hexactinellid species is quadrangular (e.g., Sandford, pages 336–355 in this issue), but whether it is triangular or hexagonal in demosponges is still controversial. However, according to SEM images both shapes are present in Demosponges. Astrophorid and Spirophorid (Bütschli, 1901; Reiswig, 1971; Simpson et al., 1985), Hadromerid (Bütschli, 1901), and Poecilosclerid (Uriz et al., 2000) filaments are triangular in cross-section. Moreover, "true" hexagonal shapes have been found in the axial filaments of Haplosclerid spicules, which are also thinner and clearly show a paracrystalline structure (Garrone, 1969; Donadey et al., 1990; Wilkinson and Garrone, 1980). A distinct paracrystalline structure is also seen in triangular filaments of the Poecilosclerid *P. fictitius* and *P. tenacior*. Three crystallization planes, parallel to the three sides of a triangle, overlap to produce hexagonal cells, 3 nm in diameter (Fig. 18B). This hexagonal nanostructure matches recent findings on the fine structure of the axial filaments of oxeas (diffraction studies), which show a 2D hexagonal lattice with a repeating distance of ~6 nm (Croce et al., pages 378–381 in this issue).

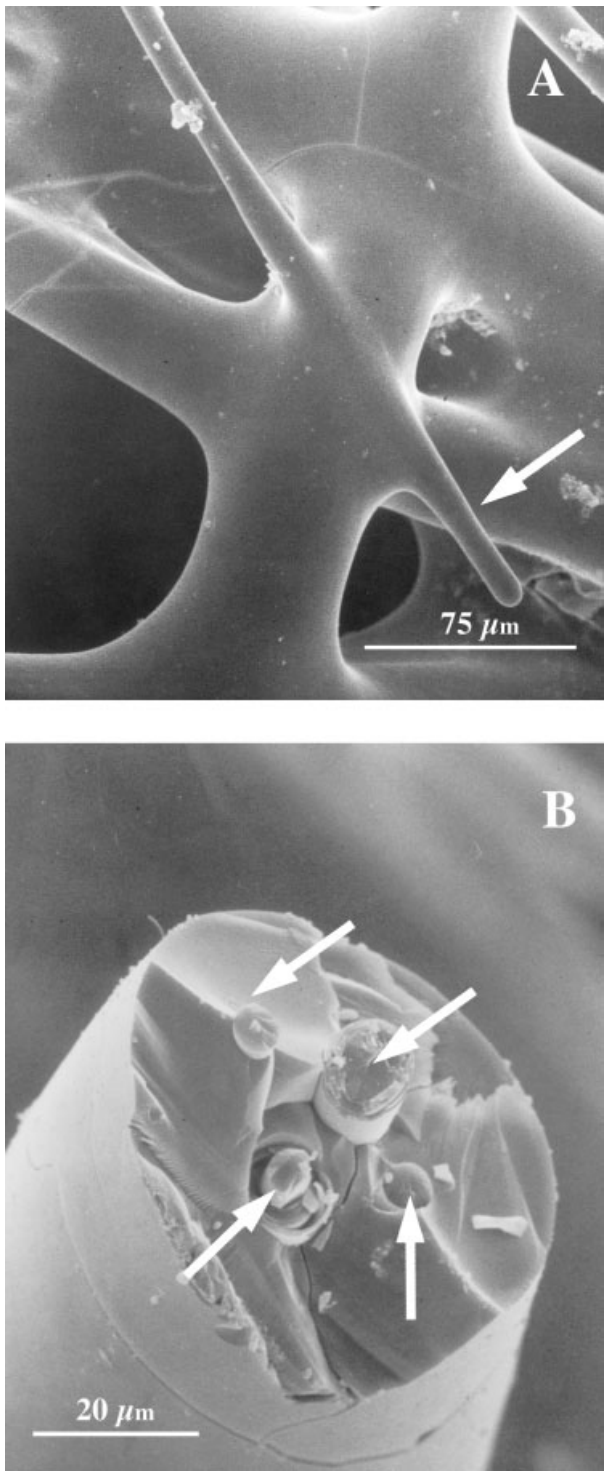


Fig. 12. SEM photograph of hexactinellid spicules embedded in silica. **A:** Partially embedded spicule (arrow). **B:** transversal section showing spicules (arrows) surrounded by large amounts of additional silica.

On the basis of the single species studied to date, the order Homosclerophorida appears to show clear differences with the other Demospongiae in the shape of the axial filament. The spicules of *C. candelabrum* have

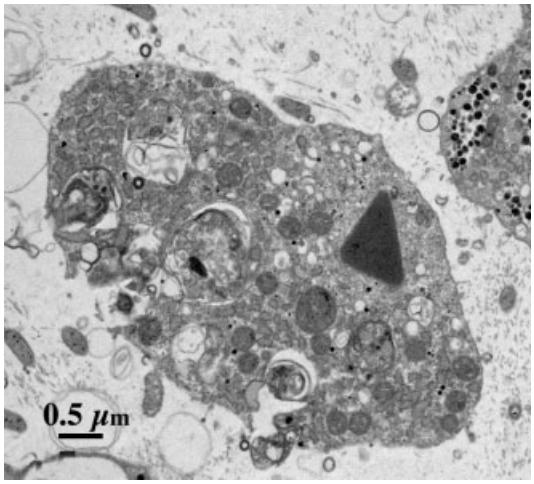


Fig. 13. TEM photograph of a sclerocyte containing an axial filament, abundant mitochondria, and clear vesicles.

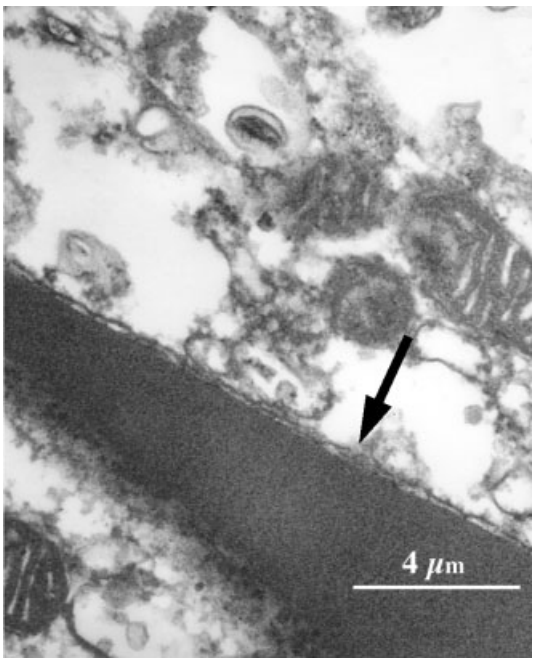


Fig. 14. TEM detail of a sclerocyte containing an axial filament. See the membrane (arrow) surrounding the filament (silicalemma).

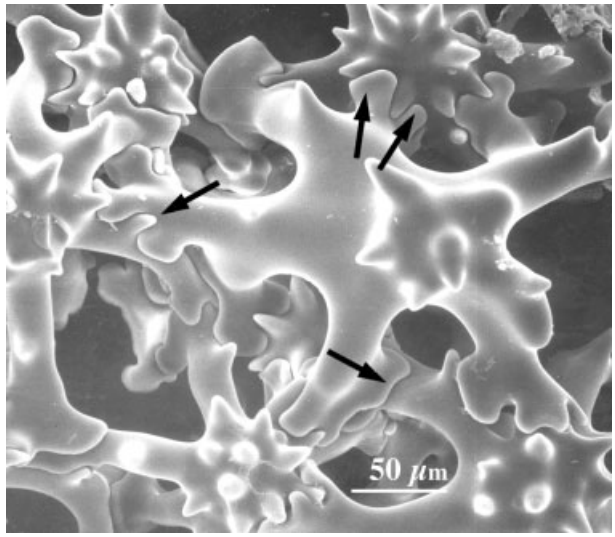


Fig. 15. SEM image of interlocked desmas (arrows) (Modified from Uriz and Maldonado, 1995).

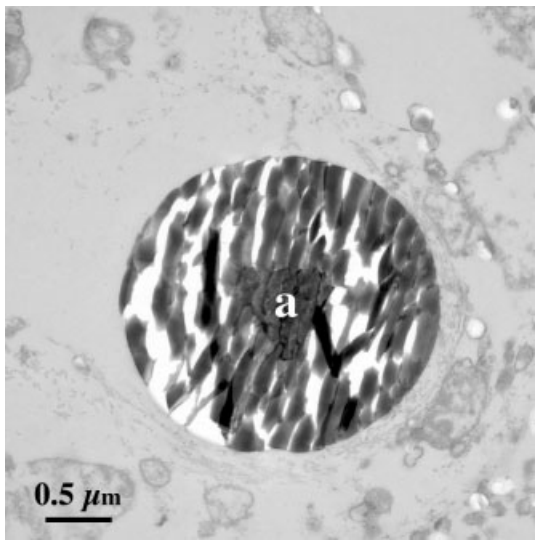


Fig. 16. TEM transversal section of a spicule of *Phorbas fictitius* showing a triangular axial filament (a), impregnated with silica.

irregularly shaped filaments (Fig. 20), closely intermingled with silica micro- or nanospheres (Fig. 17).

Axial filaments vary in width across spicule types. Spicules with thicker walls could be expected to have thicker axial filaments, since these would control the amount of silica polymerized. However, filament and spicule diameters do not appear to be positively correlated. The ratio axial filament/total spicule width is 0.1–0.3 for *C. crambe* spicules (Uriz et al., 2000), 0.2–0.4 for the thin (0.1 μm in diameter) raphides of *A. polypoides* (Donadey et al., 1990), ~0.3–0.4 in *P. fictitius*, and between 0.2–0.7 for the tetraxonid spicules of *C. candelabrum*. Conversely, this ratio only reaches 0.03 for the asters and thick triaenes of *S. grubei* (Simpson et al., 1985).

Reiswig (1971) attributed a phylogenetic value to the cross-section shape of the axial filament. He found triangular filaments in Astrophorids, Spirophorids, Hadromerids, Axinellids, and some Poecilosclerids, and hypothesized that both the triangular morphology and a polyactinal spicule shape would be plesiomorphic within demosponges and would be potentially present in either of the two subclasses established by Lévi (1973) (i.e., Tetractinomorpha and Ceractinomorpha) independently of whether the polyactinal shape is visible. Reiswig's hypothesis is currently gaining strength after TEM and SEM examination of more species belonging to several orders of Demospongiae. Further-

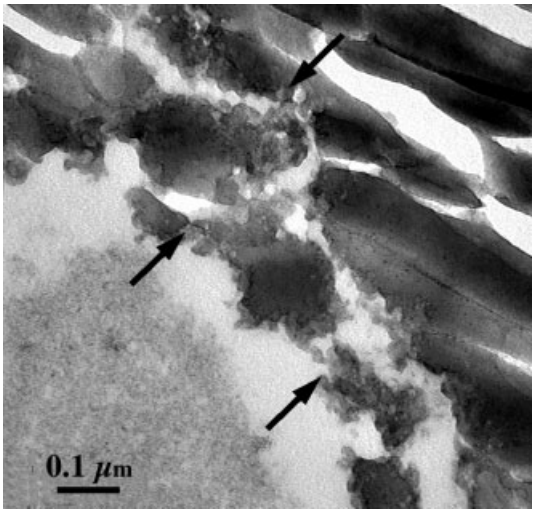


Fig. 17. TEM transversal section of a *C. candelabrum* spicule. Note the silica nanospheres in the zone between the axial filament and the spicule wall (arrows).

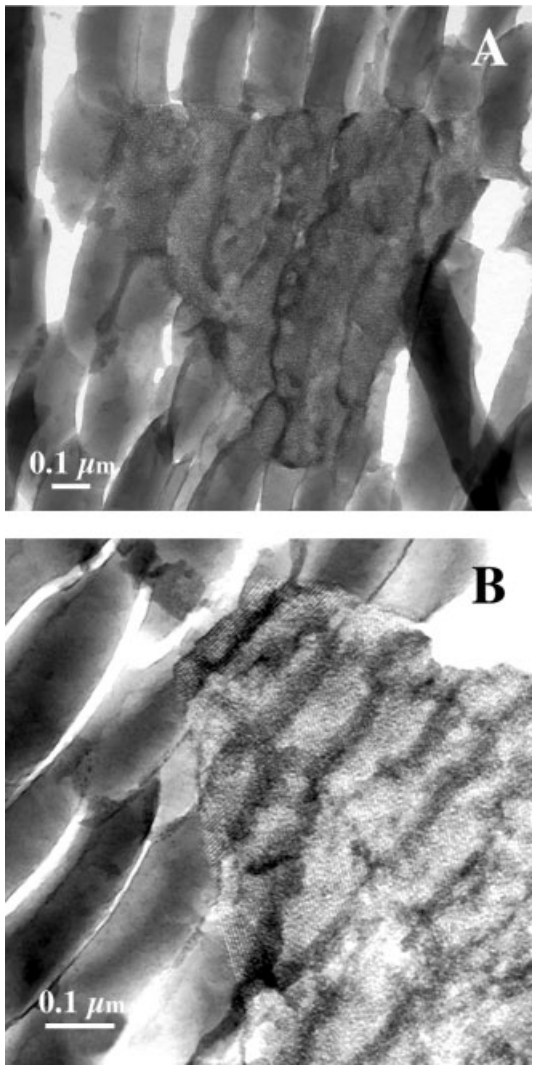


Fig. 18. TEM transversal sections of *Phorbas fictitius* spicules. **A:** The axial filament shows similar fracture to that of the spicule wall. **B:** The axial filament shows a paracrystalline structure with three different crystallization planes.

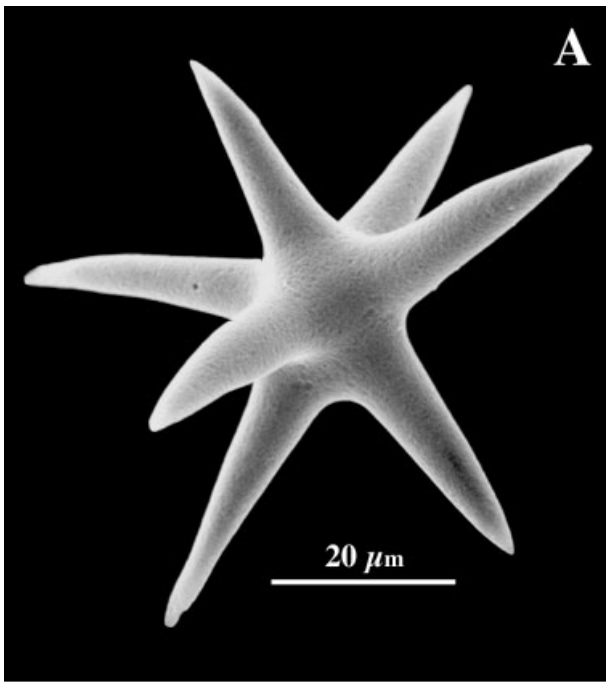
more, the triangular axial filaments reported in some monaxonid spicules of poecilosclerids (Uriz and Maldonado, 1995) and hadromerids (Rützler and Smith, 1993) have a polyaxial origin, since their axial filament branches at one end. In contrast, all the haplosclerid sponges (either with monactinal or diactinal spicules) examined so far showed axial filaments hexagonal in cross-section (Garrone, 1969; Simpson and Vaccaro, 1974; Wilkinson and Garrone, 1980; Weissenfels and Langenbruch, 1985). Although filaments with hexagonal cross-section have also been attributed to the spicules of the Poecilosclerid *Neofibularia irata* (Wilkinson and Garrone, 1980) and the raphides of *Axinella polypoides* (Donadey et al., 1990), other profiles can be found: in the TEM figures shown by these authors: triangular, triangular with cut angles (i.e., "faux hexagonal"), rounded, and irregular shapes. The spicules of the order Homosclerophorida may be exceptional since in the only species examined so far (*C. candelabrum*), the filaments are irregular in cross-section.

The triangular or hexagonal filament sections correlate with the two models of secretion postulated by Simpson (1990). A triangular section is associated with monactines and hexagonal with haplosclerid diactines. Oxeas of fresh-water sponges with hexagonal filaments, when cultured either in a silica-rich medium (Simpson, 1981) or in the presence of germanium (Simpson, 1990), produced central swellings, which have been interpreted as centers from which silica extends in opposite directions towards both spicule pointed ends. In contrast, the growth of monactine spicules with triangular filaments would be unidirectional from a proximal silification center (Simpson, 1990). These alternative hypotheses of spicule growth are supported by the observation of young diactines or monactines that show a swelling at a central or proximal position, respectively (Corriero et al., 1996; Uriz, 1983).

Chemical and Molecular Aspects

The chemical composition of demosponge and hexactinellid spicules varies slightly depending on the species and the water composition in the habitat, but it is mostly silica (SiO_2) and water (e.g., Jørgensen, 1944), with some trace elements (see Sandford, pages 336–355 in this issue). Other mineral ions, which do not take part of the spicule composition, such as Fe(++), appear to be decisive in activating the enzymes that catalyze silica polymerization (Le Pennec et al., 2002; Müller et al., pages 368–377 in this issue).

Sponges take up silicon in the form of soluble silicic acid. Si uptake by sponges has been measured in laboratory experiments (Frohlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 1999) and may vary according to Si concentration in the water, temperature, and other environmental factors that affect sponge physiology and metabolism. Positive corre-



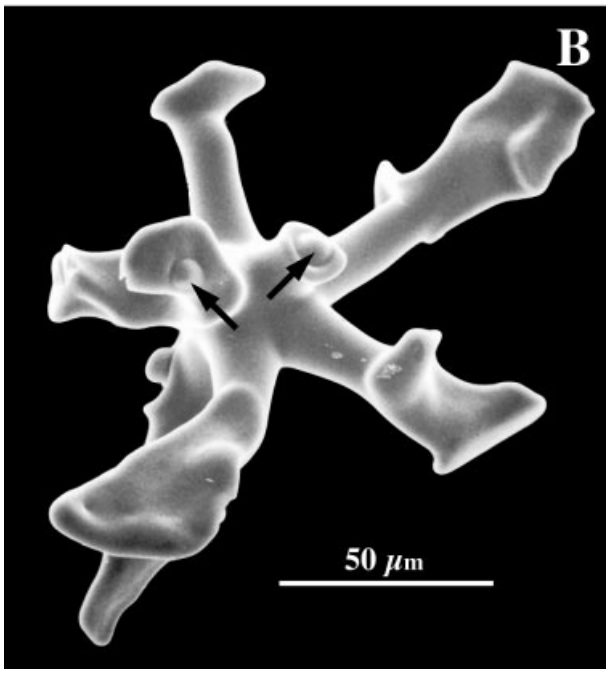


Fig. 19. SEM photographs of different phases in the formation of an asterose desma. **A:** First stage: silica is deposited along the axial filament given rise to an apparently "normal" aster. **B:** Later stages of silicification show additional silica deposited around the initial asterose spicule (note the ends of the initial aster protruding from the desma arms (arrows)).

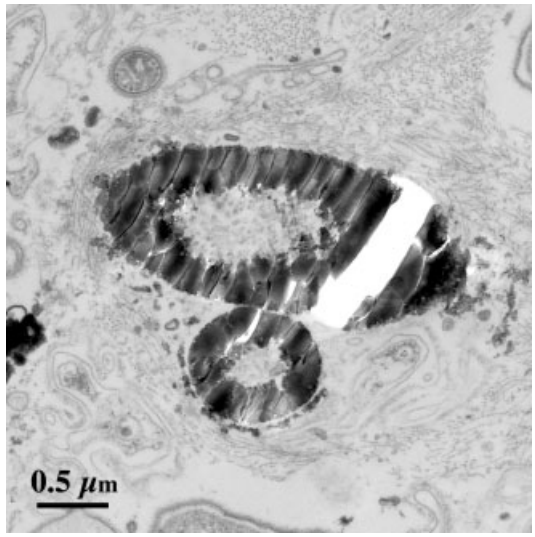


Fig. 20. TEM transversal sections of *C. candelabrum* spicules. The axial filament has an irregularly rounded shape.

lations between the concentration of silica, the nutritional condition of the sponge, and its silica uptake have been reported for *Halichondria panicea* (Frohlich and Barthel, 1997), while temperature did not affect uptake rates. In contrast, Si concentration above a threshold may even reduce silica uptake (Maldonado et al., 1999). It has been speculated that some sponges may compete for silica with diatoms in summer (Frohlich and Barthel, 1997). However, this competition seems unlikely according to Reinke and Barthel (1997), given the faster uptake and lower saturation point of diatoms and differences in their spatial distribution (i.e., water column and sea bottom, respectively).

There has been speculation as to whether silicic acid is transferred from the water to the inside of the unit-type membrane that wraps the growing spicule by means of vacuoles, or directly through the cytoplasm of the sclerocyte. X-ray analysis coupled to TEM images has shown that the silicic acid may enter the sponge mesohyl directly through transient spaces between the epithelial cells, since it was present in the cytosol but not contained in particular vacuoles (Uriz et al., 2000). X-ray analysis provides accurate comparisons between cell compartments in the same sample, provided an internal control is used. By using this method, Uriz et al. (2000) found relatively larger amounts of Si in the axial filament of growing spicules (50–70% of Si relative to the spicule wall) than in mature spicules (30–40%), which indicates the intimate relationship between protein and silica during the first steps of silica deposition. No Si was detected in the perispicular collagen, in archeocytes, choanocytes, or in the mesohyl far from the sclerocyte–spicule complex. When sclerocytes secreting spicules were analyzed, mitochondria and vesicles, whether electron-dense or electron-clear, did not contain significant amounts of silica. In contrast, silica was concentrated in the cytoplasm of the pseudopodia that surrounded the growing spicule (50% of Si relative to that contained in the spicule-wall) and in the extracellular space between the spicule and the sclerocyte (50–65%).

The mechanism by which silica is concentrated and polymerized around the axial filament to form spicules was poorly known until recently (Shimizu et al., 1998;

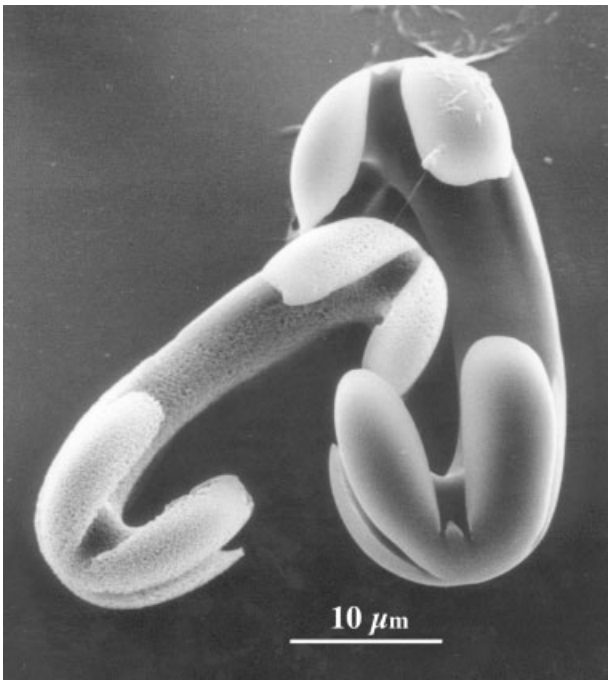


Fig. 21. SEM photographs of two isochelae in different phases of silicification, showing an uneven and even surface, respectively.

Cha et al., 1999; Krasko et al., 2000). Shimizu et al. (1998) identified the main proteins that constitute the axial filament (i.e., silicateins ∝, β, and γ) and proved that they are enzymes highly similar to members of the cathepsin-L and papaine family of proteases. These enzymes catalyze the hydrolysis and polycondensation of silicon alkoxides (Cha et al., 1999) but this activity was abolished after thermal denaturation, suggesting that the enzymatic activity relies on the tertiary structure of the protein. The physical structure of the protein has been elucidated (Croce et al., pages 378–381 in this issue) and, according to these authors, silicatein units would first act as a template for the formation of a highly ordered structure similar to that in mesopore materials, upon which secondary deposition of amorphous silica would proceed. The proposed arrangement of this structure is supported by cross-sections of *P. fictitius* filaments observed at high magnification (Fig. 18B).

Si polymerization, either in abiotic conditions or by biomineralization, gives rise to a network of micro- or nanospheres. Nanosphere networks have been obtained experimentally in the laboratory by using proteins from diatoms (Krögel et al., 1999) and sponges (Shimizu et al., 1998; Cha et al., 1999). They can also be observed in growing spicules of members of the family Clionidae (Schönberg, 2000; Rosell and Uriz, 2002) and Poecilosclerida living in Si-poor environments (Uriz and Maldonado, 1995). During spicule formation, these nanospheres fuse to each other to form larger spheres. Amorphous silica is added at later stages, giving rise to an even spicule surface (Fig. 21). Various nanosphere structures are visible in growing desmas of several Lithistida (see Pisera, pages 312–326 in this issue). Silica nanospheres can be recognized in the

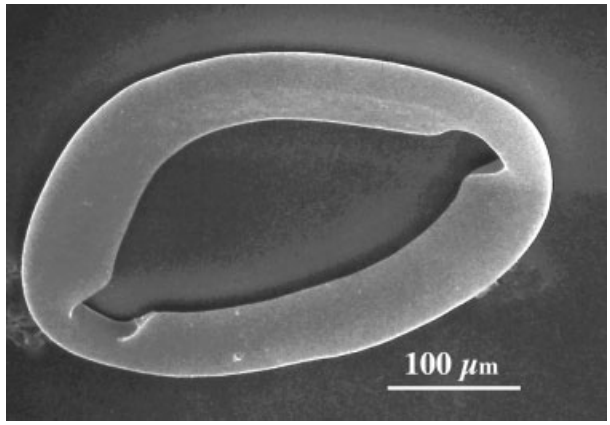


Fig. 22. SEM image of a clavidisc from a *Merlia* species. This spicule may be missing from specimens living in silica-poor environments.

100–120 nm long, dense (silica-rich) zones that surround the axial filament of *C. crambe* (Uriz et al., 2000).

GENOTYPIC VS. ENVIRONMENTAL COMPONENTS IN THE PHENOTYPIC EXPRESSION OF SPICULES

It has been experimentally demonstrated that sponge sclerocytes produce the axial filament (protein) at very low concentrations of silicon (Yourassowsky and Rastmont, 1983). However, several spicule types, which are absent from natural populations living in low concentrations of silicon, can be produced in the laboratory when the concentration of silicic acid is increased artificially (Maldonado et al., 1999). Hence, the potential number of spicule types in a sponge species appears to be genetically fixed, but the environmental conditions, specifically, the availability of silicon, may determine whether a genetically determined spicule type is finally expressed. Several instances illustrate the absence of certain spicule types in Mediterranean specimens due to chronic silicon limitation. The Mediterranean *Axinella polypoides* differs from the sibling Atlantic species *A. dissimilis* in the absence of raphides. However, an ultrastructural study (Donadey et al., 1990) demonstrated that Mediterranean specimens of *A. polypoides* have microsclerocytes containing 20 μm long, 0.1 μm wide raphides, which are hard to detect in spicule preparations under light microscopy due to their extremely small diameter. Furthermore, *Merlia lipoclavidisca*, which lives in silica-poor water of the Balearic Islands, differs from *M. normani* living in the Mediterranean silica-rich waters of the Medes islands by the absence of clavisdiscs (Fig. 22) (Vacelet and Uriz, 1991).

SPICULES ARRANGEMENT: HOW THEY GET THE RIGHT PLACE AND ORIENTATION

The above-mentioned skeletal arrangements are mostly built by several spicule types, which are placed in precise sites within the skeletal framework as the sponge develops and grows. How each spicule type reaches the precise location within those complicated skeletons is difficult to understand without considering

the role of the developmental genes, which involve expression of distinct proteins at several sponge zones during development. There is a clear polarity in the skeletal organization from the choanosome to the ectosome in most sponge species, and a clear radial symmetry can be seen in the skeleton of subspherical sponges (Uriz, 2002). Homeoboxes have been identified in Demosponges (Coutinho et al., 1994; Kruse et al., 1994; Seimyia et al., 1994; Degnan et al., 1995; Richelle-Maurer et al., 1998, 1999) and calcareous sponges (Manuel and Le Parco, 2000) and their temporal and spatial expression has been shown both in adults (Seimyia et al., 1997; Richelle-Maurer et al., 1999; Larroux and Degnan, 1999) and in embryos (Leys et al., 1999). These homeobox genes may be responsible for the establishment of polarity during the sponges' life cycle. Calcareous sponges show a particularly complex skeletal organization in which each distinct spicule type occupies a precise location. Jones (1998) highlighted the importance of mechanical forces caused by the initial contiguity of the founder cells on morphogenesis of *Leuconia fistulosa* (Johnston). Although Simpson (1984) stated that the exact localization of the different spicule types in demosponge skeletons is rarer than in Calcarea, there are also many instances of complex frameworks in demosponges with specific sites for any of the several spicule types. The axial skeleton of the genus *Raspailia* illustrates this point. It consists of a central core formed by long styles joined by spongin in an irregularly reticulated pattern. Long, perpendicular styles arise from this axial core, protruding through (hispidating) the sponge surface. Small acantho-tylostyles are placed perpendicular to the long peripheral styles, with their bases on the style and their points upwards. Furthermore, bundles of thin styloids obliquely surround the hispidating styles at the ectosome level.

Simpson (1984) posed the question as to whether clusters of sclerocytes are developed simultaneously in a particular zone of the sponge and thus spicules are formed in their definite place or, alternatively, spicules are secreted anywhere in the sponge mesohyl and then transported to the appropriated site to form the skeletal framework. Crawling mesohyl cells apparently exert forces that may enable them to move spicules. Spicules transport by cells across the sponge mesohyl to distances up to several hundred micrometers has been recorded in both marine (Bond and Harris, 1988; Bond, 1992) and freshwater (Elvin, 1971; TokyoCinema, 1996; Custódio et al., 2002) sponges. In *Ephydatia muelleri*, sclerocytes containing or surrounding growing spicules were observed wandering quickly through the sponge mesohyl (Elvin, 1971). TEM images of sclerocytes surrounding young spicules by pseudopodia have been reported in *C. crambe* (Uriz et al., 2000), *C. candelabrum* (Fig. 23), and *P. fictitius*, and they may be an indication of spicule transport while completing silica deposition. The long directional pseudopodia of spicule-secreting sclerocytes in *Scopalina lophyropoda* larvae shown through TEM (Fig. 24) also suggest active movement (Uriz et al., 2002). Transport by sclerocytes has also been reported for microscleres (Custódio et al., 2002). Sclerocytes move across the sponge mesohyl following the general cell flow (Elvin, 1971; Bond, 1992; TokyoCinema, 1996) and they appear to move while

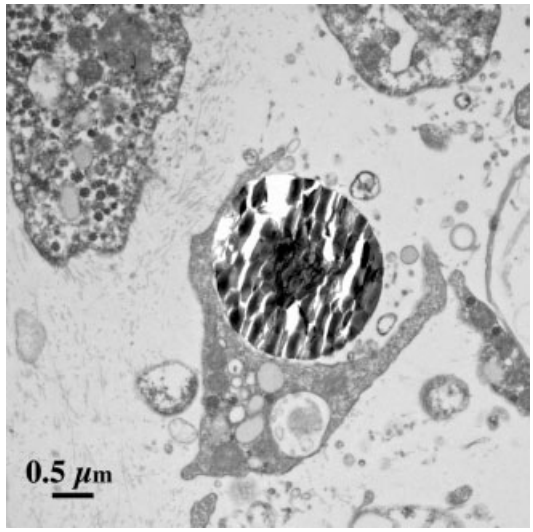


Fig. 23. TEM image of a sclerocyte surrounding (transporting?) a spicule of *Phorbas fictitius*.

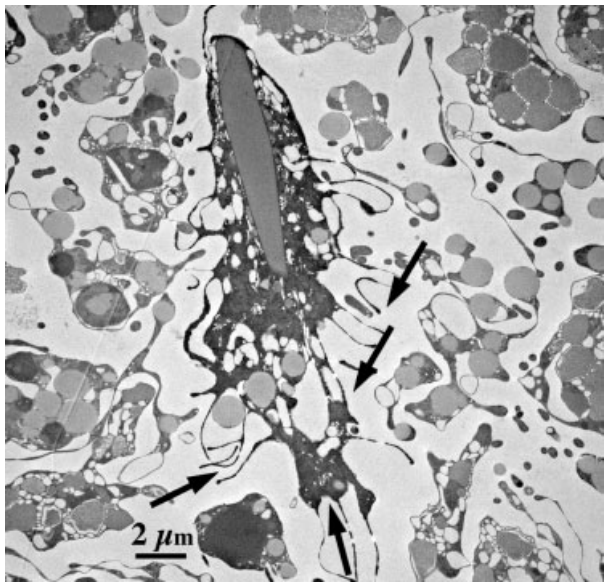


Fig. 24. TEM image of a larval sclerocyte of *Scopalina lophyropoda*, which contains an axial filament. See the long thin pseudopodia at one end of the cell (arrows), which indicates directional active moving.

secreting both the spicules and the axial filament (i.e., before silica secretion starts).

Once silification has finished, spongocytes secrete spongin fibrils around the spicules. Mature spicules might be "identified" as foreign material by the spongocytes, in the same way as basopinacocytes secrete spongin to isolate the sponge cells from the substrate (Fig. 25). After being entrapped and fixed to the basal layer or to other spicules by spongin, spicules may constitute a "barrier" to other transporting spicule cells that move across the mesohyl following the general cell flow, thereby integrating the transported spicules in

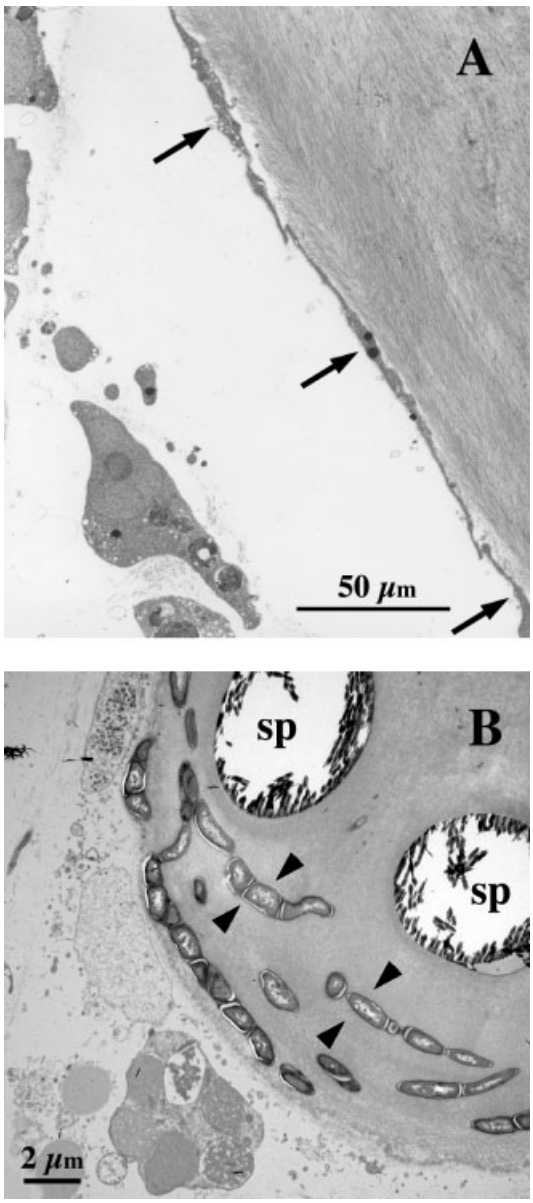


Fig. 25. TEM image of the spongin that surrounds mature spicules. **A:** Spongocytes producing spongin fibrils (arrows) in close contact to the spongin mass. **B:** Two spicules (sp) surrounded by spongin, which is invaded by archeobacteria (arrow heads).

the growing spicule bundle. The iteration of the process would lead to the formation of an elemental skeleton structure: the primary spicule tracks (Bond, 1992). Some of these entrapped spicules are abandoned during the continuous morphogenetic processes of the sponge (Borojevic, 1971; Bond and Harris, 1988; Maldonado and Uriz, 1998) since they do not follow the general cell flow.

In contrast, spicule fusion appears to occur in parallel to spicule secretion in dictyonal hexactinellids (Reiswig, 2002). In these sponges, spicules should be produced at their definite site at fixed distances from each other to conform to the resulting regularly reticulated framework.

The role of tension forces in sponge morphogenetic processes has been discussed previously (e.g., Borojevic, 1971). Teragawa (1990) concluded that the mechanical forces of tension in the dermal membrane of *Dysidea avara* could influence skeletogenesis in this keratose species by affecting both primary fiber growth and the transport of sand particles to the primary fibers. Membrane tension could also influence sponge morphogenesis, since the growth of primary fibers at the conules determines the external shape of the sponge. Many siliceous sponges, particularly those with plumose or ascending reticulate skeletons, show a conulose surface and the mechanical forces acting in the configuration of plumose principal spicule tracts are expected to be similar to those determining the organization of horny skeletons. According to Teragawa (1990), tensile regulation of skeletogenesis could be achieved through a dynamic balance between tensile stresses in the dermal membrane (ectosome) and compressive stresses in the primary spicule tracts.

In many cases bundles of microscleres are secreted in a single cell and are therefore associated in origin. Multiple-spicule secretion by a single cell occurs for microxeas or raphides in *Neofibularia irata* (Wilkinson and Garrone, 1980), raphides in *Axinella polypoides* (Donadey et al., 1990) and *Mycale angulosa* (Custódio et al., 2002), or toxas in *M. angulosa* (Custódio et al., 2002). Conversely, in other microsclere bundles, such as the anisochelae rosettes in the genus *Mycale*, the spicules are secreted individually and appear to be later aggregated by microsclerocyte migration and aggregation while secreting the spicules, in a similar way to that described for megascleres (see above). During anisochelae secretion, the spicule foot seems to be finished first (Custódio et al., 2002) and is then extruded to the mesohyl. Once extruded, the isochelae feet would be fixed by spongin to each other, since a dense collagen-like matrix has been observed surrounding the rosettes (Custódio et al., 2002).

Consequently, both "in situ" secretion and transport of spicules may combine during the frameworks formation.

SKELETAL FUNCTIONS

Intuitively, most spicule shapes appear to be useful in micro-construction. For instance, monaxons (Fig. 26) can be combined in several ways by following the same principles of physics used in human building. Proximal swellings (tyles) of monaxons may provide anchoring points for spicules in hymedesmoid, plumose, and reticulate skeletons. The spongin usually enrobes the spicule base and a terminal swelling (Fig. 27) increases the spicule surface to be immersed in the spongin. The cladome of tetraxons (Fig. 28) anchored in an armored layer of microscleres (the cortex) gives cohesion to the whole structure by interlacing cortex and choanosome and allows subglobular shapes through tension forces. The question arises as to why evolution has allowed the maintenance of multiple spicule shapes and frameworks that may play analogous skeletal functions in sponges. For instance, why are there several monaxonid types with either one or two ends blunt or both ends sharply pointed (e.g., styles, tornotes oxeas, strongyloxeas)?

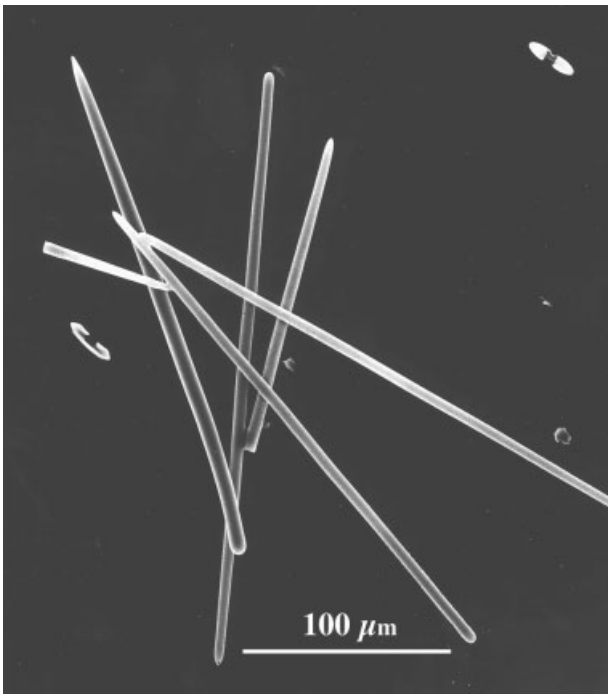


Fig. 26. SEM image of monaxons (styles and strongyles) of demosponges.

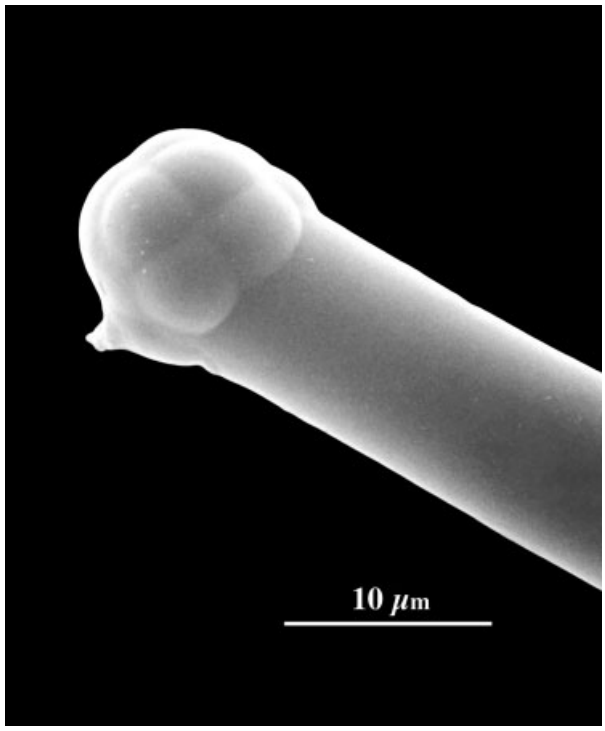


Fig. 27. SEM image of a terminal tyle in a monaxon (tylostyle) spicule.

As for the demosponge microscleres, many of them also have apparently useful forms for framing skeletons. Sigmas, isochelae, anisochelae, diancisters, forceps, plachochelae (Fig. 29), may help hold together some megascleres, provided they have the appropriate size. Asters (poly-actinate microscleres), if in sufficient density and accompanied by spongin, may produce consistent but flexible external layers and may ease the organization of complicated inhalant structures, as in many Astrophorids (Uriz, 2002). However, the precise structural function (if any) of some clusters of microscleres, such as the rosettes of anisochelae in *Mycale* or the trichodragmas (raphides clusters) in *Epipolasis*, remains enigmatic.

Supporting Cells and Improving Sponge Strength

The most obvious function of skeletons in sponges, as in any other living organism, is to allow programmed cell arrangement in a functional body plan (supporting function). Spicules fixed to the basal substrate by spongin can play a decisive role in sponge morphogenesis and posterior rearrangement processes (e.g., Bond, 1992), by acting as anchoring points against moving cells they can exert forces (Borojevic, 1971).

Furthermore, skeletons confer the necessary strength to marine animals to endure hydrodynamic forces: eddies, currents, and waves (e.g., McDonald et al., 2002; Palumbi, 1896). A flexible main skeleton is necessary for erect branching forms to counteract strong currents. Erect branching forms such as the Mediterranean demosponge *Axinella polypoides* arrange their branches in a plane perpendicular to the predominant currents, thus taking advantage of the food transported in the water. Only thanks to a firm but flexible skeleton can the sponge survive high-speed currents. A thick ectosomal crust combined with a radial main skeleton (e.g., *Geodia, Tethya, Aaptos*), a high density of spicules (e.g., *Petrosia*) or desma-made skeletons (Lithistida) also confer resistance to massive sponges. Encrusting sponges, which are particularly abundant in environments exposed to strong water

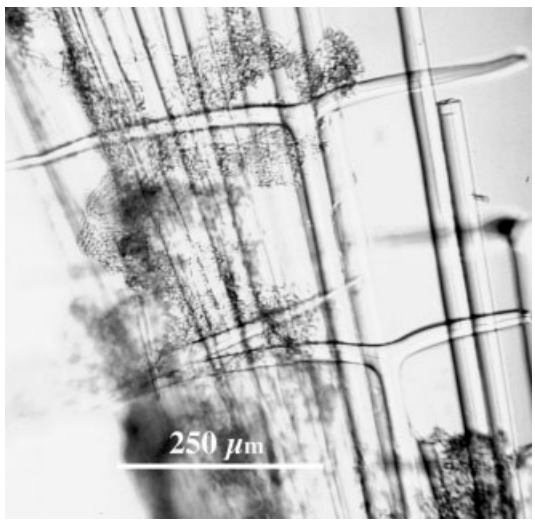


Fig. 28. Light microscope image of triaenes with their cladome arranged to form the skeleton cortex (Modified from Uriz, 2002).

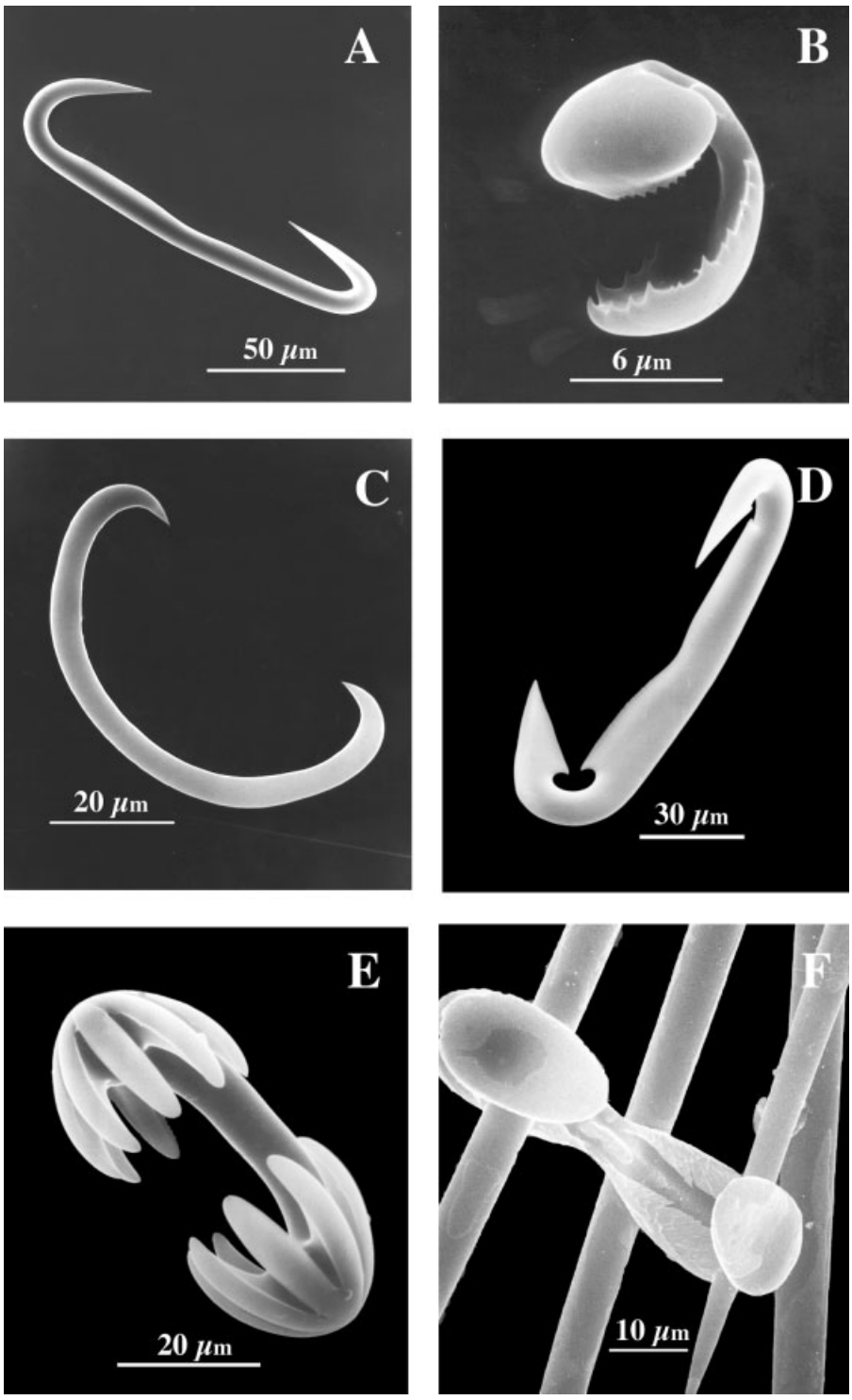


Fig. 29. SEM images of several hooked microscleres of Demospongiae, which may join megascleres in skeletal frameworks. **A,D:** Diancisters of *Hamacantha*. **B:** Bipocillo of *Iophon*. **C:** Sigma of *Gellius*. **E:** Anchorate chela of *Ectyonancora*. **F:** Placochelae of *Guitarra*. (**A–E** modified from Uriz, 1988).

motion, counteract the hydrodynamic forces by adhering firmly to the substratum through their whole area.

Spicules may also play a supporting role for cells during the process of formation of external propagula in species such as *Tethya aurantium, Haliclona loosanoffi*, or *Mycale contarenii*, to cite some examples.

Reaching the Water Column

Another role of the skeletal frameworks is to transport the sponge cells toward the water column (i.e., a few cm above the substrate) by allowing the sponge to grow upwards. The benefits of 3D growth for filter-feeding organisms have often been reported (Jackson,

1979). This ability may help the sponge to elude lateral overgrowth or competition for space with close neighbors as well as the clogging of inhalant orifices by sediment (Jackson, 1979).

Furthermore, thanks to their paradigmatic plasticity (e.g., Becerro et al., 1994; Sarà and Vacelet, 1973), sponges may undergo morphological and skeletal changes along their life, depending on the environmental conditions (McDonald et al., 2002), adapting their shape to perform the physiological functions with a minimal metabolic cost (Riisgard et al., 1993; Riisgard and Larsen, 1995). Sponges that have a genetically fixed capacity for building 3D skeletons can maintain their thin encrusting shape during their whole life or can become thick encrusting or massive, depending on factors such as sediment rates, food availability, and hydrodynamic forces. For instance, thinly encrusting individuals of the genus *Clathria* or *Phorbas* became thick encrusting or massive, and their skeletons change from hymedesmoid to plumose, when water motion is low or the trophic conditions are favorable.

Defense

Spicules seem to represent harmful elements in the food, particularly if we consider that they may represent up to 75% of the sponge biomass (Rützler and Macintyre, 1978; Desqueyroux-Faundez, 1990) and that they are often arranged in the skeleton with their sharp end towards or protruding the sponge surface. As a consequence, the siliceous skeleton of sponges has often been interpreted as an effective mechanism for deterring predation (Randall and Hartman, 1968; Sarà and Vacelet, 1973). Spicule concentration appears to be a plastic trait that can be induced by damage in some morphotypes of *Antosigmella varians* Duchanssaing and Michelotti (Hill and Hill, 2002). These authors found that sponges unprotected from predators increased spicule yields, and suggest that the large spicule-rich cortex of these morphotypes is an inducible structural defense.

However, studies specifically designed to test the deterrent capabilities of sponge structural defenses (spicules) have produced contrasting results, depending on the energy content of the artificial food assayed (Chanas and Pawlick, 1995, 1996; Uriz et al., 1996). Chanas and Pawlick (1995) found that spicules deterred predators when incorporated into prepared food of a nutritional quality lower than that of the sponge tissue. A combination of poorly digestible spongin and indigestible silica may result in tissue of low nutritional quality that may be more effective in deterring predators than a harmful effect of spicules per se (e.g., Waddell and Pawlik, 2000).

The spicule arrangement and concentration at the sponge surface might also exert some influence in avoiding predation. The food offered to fish or echinoderms in the above-mentioned experiments (Chanas and Pawlick, 1995, 1996; Uriz et al., 1996) did not mimic the spicule orientation or concentration at the sponge periphery (e.g., protruding the sponge surface or densely packed). However, large predators of sponges such fish (Wulff, 1994) and turtles (Meylan, 1988, 1990) may elude the troublesome spicules thanks to a different size scale of the predator's mouth (cm) and the spicules (μm), respectively. Fish and turtles usually predate on several sponge species, which may be the result of coevolution since partial predation by fish is not fatal for sponges (Wulff, 1997). Moreover, some specialized invertebrates can also circumvent the defenses by crushing the spicules thanks to special tools for grazing hard materials (e.g., mollusks' radula, sea urchins' lantern, polychaetes' mandibles) (e.g., Cimino and Sodano, 1994; Martin and Britayev, 1998; Uriz et al., 1996), or obtain cellular material by means of thin suction trunks (e.g., Siphonostomatoid copepods, Mariani and Uriz, 2001) or external digestion such as sea-stars that feed exclusively on sponges in the Antarctic (Dayton, 1974, 1979), despite the fact that siliceous skeletons are particularly well developed in this ocean.

However, few vertebrates or macro-invertebrates feed on temperate (e.g., Sala and Ballesteros, 1997) or tropical sponges (e.g., Randall and Hartman, 1968). Production of deterrent and noxious chemicals appears to be more effective than spicules in deterring predators (e.g., Pawlik et al., 1995; Uriz et al., 1996) and sponges seem to have a recurrent selection for chemical defenses as a part of their life strategy (Becerro et al., 2003).

Spicules may be present in sponge larvae. Hexactinose spicules are abundant in the trychymella larva of the hexactinellid *Farrea sollasii* Schulze (Okada, 1928) and their arrangement, covering the whole larval body points to a supporting function. Some authors have proposed that larval spicules would protect sponge larva from predators (e.g., Young and Chia, 1987). However, this does not seem very reliable because larval spicules are rather scarce, and if high amounts of spicules do not protect adult sponges in most cases (see above), it is unlikely that a few spicules would protect larvae. Few experimental studies addressed larval defense of sponges, but, for instance, spicule-bearing larvae of *C. crambe* were palatable, while spicule-free larvae of *Dysidea avara* were unpalatable (Uriz et al., 1996).

Other Functions

Several other functions, sometimes complementary to the main supporting role, can be envisaged for sponge spicules, although most of them appear exclusive of particular species. In extreme environments where an active filter feeder has a low yield, the family Cladorhizidae Dendy has developed particular tools for capturing living prey passively, which consist of long thin tentacles provided with a dense layer of protruding upraised hook-shaped microscleres (Fig. 30A,B). These carnivorous sponges capture small crustaceans (less than 1 mm in size), which are entrapped thanks to the sponge microscleres (Vacelet and Boury-Esnault, 1995).

Spicules also appear to play a role in gamete and larvae dispersal of some species. Certain larval spicules such as the discotriaenes that cover the hoplitomella larva of Alectonidae (Vacelet, 1998) are not present in the adults. Some of the larval spicules notably favor larval buoyancy and thus may increase larval dispersal, as reported for the styles that largely protrude through the armored hoplitomella of Alectonidae, which can be found among the oceanic plankton (Trégouboff, 1942). A well-known case of particular

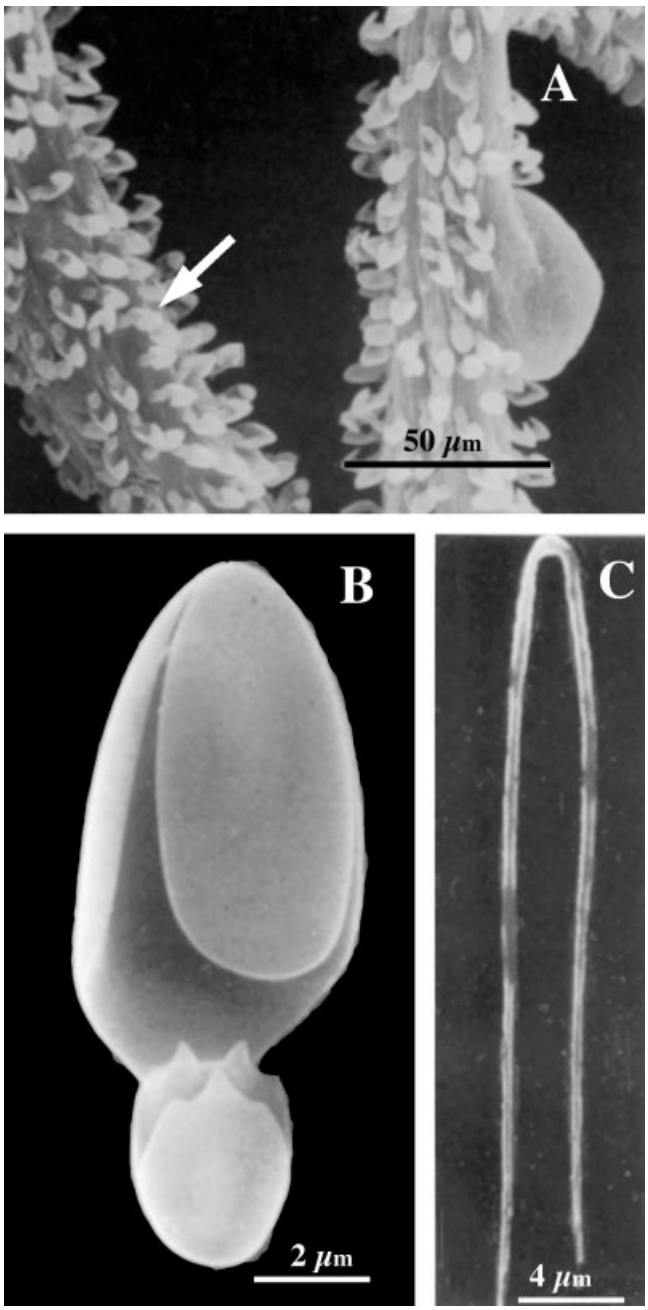


Fig. 30. SEM images of the carnivorous sponge *Abestospluma hypogea*. **A:** SEM photograph of the filaments ("tentacles") provided with protruding hook-shaped microscleres (arrows). **B:** Magnification of a hook-shaped microsclere. **C:** spermatogonia spicule (forceps), which favors spermatogonia buoyancy and dispersal (Modified from Vacelet and Boury-Esnault, 1996).

spicules in sponge propagula is that of gemmoscleres that form a pneumatic layer in the resistance gemmules of the freshwater sponges of the family Spongillidae, which favors gemma buoyancy and dispersal (Hartman, 1981). The carnivorous sponge *Abestopluma hypogea* Vacelet and Boury-Esnault has microscleres (forceps) that accompany the spermatic cysts at release. The shape of these spicules (Fig. 30C) and their protruding position increase spermatocyst buoyancy. They can remain suspended in the water column for a long time, enhancing the possibility of contacting the long filaments of another individual. The shape of the spermatocyst forceps and the hooked anisochelae of the filaments also favors capturing (entrapping and phagocytosis) of the spermatocysts (Boury-Esnault and Vacelet, pers. commun.).

In contrast to the role of spicules in the buoyancy of *Abestopluma* gametes, it has been speculated that internal spicules favor larval sinking by diminishing larval buoyancy, since spicules increase in number as the larvae get older (Meewis, 1939; Woollacott, 1993; Maldonado et al., 1997). According to some of these authors, a buoyancy decrease would enhance the chances of larvae contacting the substratum, thus promoting settlement success. However, larvae may settle before spicule secretion starts (e.g., Uriz et al., 2001) and many larvae do not produce spicules (i.e., keratose sponges) and settle normally.

SPICULES AND SKELETAL FRAMEWORKS: INCONSISTENCIES IN THEIR TRADITIONAL USE IN TAXONOMY

Spicule size, shape, and arrangement are traditionally used as the main diagnostic characters in sponge taxonomy (e.g., Lévi, 1973; Hooper and van Soest, 2002). However, there is no consensus as to the taxonomic value of a given character, and the same character type is used at different taxonomic levels (e.g., Hooper and van Soest, 2002). For instance, the type of skeletal framework is sometimes used to differentiate genera (e.g., *Phorbas* from *Stylostichon* or *Raspailia* from *Eurypon*), families (e.g., Raspailidae from Euryponidae, Lévi, 1973) or orders (e.g., the order Hexactinosida Schrammen from Aulocalycoida Tabachnick and Reiswig in the Hexactinellida (Reiswig, 2002)).

The presence/absence of a type of microsclere separates genera in many cases (e.g., *Phyteas* from *Crella*, with and without isochelae, respectively) also allows us to distinguish between families (e.g., Geodiidae with sterrasters from Stellettidae without sterrasters, and from Pachastrellidae with estreptasters; or Hamacanthidae, with diancisters, from the remaining families of Mycalina). In addition, the presence of a type of microsclere is considered the synapomorphic character for an order (e.g., spinispires for Spirophorida in Demospongiae or amphidiscs Amphidiscosida within the Hexactinellida (Reiswig, 2002)).

Spicule size is often used to distinguish between species of the same genus (e.g., *Haliclona* spp.), although environmental conditions may affect this trait. Individuals of several species living in insular, silicon-poor Mediterranean waters produce shorter and notably thinner spicules than their Atlantic counterparts (Bibiloni, 1990), particularly those in upwelling zones (Uriz, 1888). Moreover, spicules undergo seasonal variation in size in *Halichondria panicea* (Hanna et al., 1998) and *Chondrilla nucula* (Bavestrello et al., 1993). Spicule shape may also change with environmental conditions. For instance, the presence of terminal or subterminal swellings, which transform styloid spicules to tylostyles or subtylostyles (see Boury-Esnault and Rützler, 1997), may depend on the silicon concen-

tration in the water. Moreover, it has been shown experimentally for both freshwater (Yourassowsky and Rasmont, 1983) and marine (Maldonado et al., 1999) sponges that the concentration of silicic acid may affect the phenotypic expression of several spicule types, which are not produced in silicon-poor environments. All these considerations, besides convergent spicule shapes (Fromont and Bergquist, 1990) and skeletal arrangements, may complicate the use of skeleton elements in sponge taxonomy. Although the spicule types are fixed genetically, and may thus be useful in the reconstruction of the sponge's phylogeny, variation in spicule size, shape, and types due to environmental conditions should be taken into account.

CONCLUSION

Several relevant studies on sponge skeletogenesis have recently become available. The diversity of such studies goes beyond taxonomic boundaries to include areas such as spicule chemistry and functional roles, or cellular and molecular control of the process of silica deposition and frameworks building. This ongoing research is improving our understanding of role of spicules and skeletal frameworks in the biology, ecology, and evolution of sponges as well as the mechanisms determining spicules secretion. We are now uncovering the contribution of the axial filament to spicule diversity. As we apply molecular techniques and ecological experiments to sponge research, we are unraveling the role of genetic and environmental factors in spicule formation. Although we have clearly advanced in our understanding of, for example, the genetic control of silica deposition, other subjects such as the role (if any) of the membranes remain unresolved, as they have for the last 20 years, and these warrant further investigation. We now know that basic spicule types are genetically determined and, accordingly, they may be useful in the reconstruction of sponge phylogeny. Yet we have increasing evidence of the role of environmental factors in modulating not only spicule size and shape, but also the presence and absence of spicule types. The implications of such variations in sponge taxonomy loom large and cannot be taken for granted. Contrary to sponge spicules, there is a low diversity of sponge skeletal frameworks with similar arrangements present in unrelated taxonomic groups as a result of convergent evolution. Skeletal frameworks are clearly associated with sponge growth habits in demosponges, but this association is less evident in hexactinellids. These variations in spicule formation and skeletal frameworks as a function of environmental and growth habits may partially respond to the role that these structures have in the biology and ecology of sponges. There is contrasting information on the role of sponge spicules and skeletons in defense against predators, although it seems widely accepted that sponges depend more on chemical defenses than on structural defenses. Besides supporting sponge cells and transporting them toward the water column, spicules may help larvae stay buoyant while in the plankton, reach the bottom at settlement, enhance reproduction success, or catch prey. However, our understanding of numerous processes is still based on a limited number of species. We therefore have to be cautious about the widespread occurrence of many assumptions, trends, and hypotheses currently available in the literature. Still, sponge science remains an open field and in the years to come we will increase our knowledge of the biological functions of siliceous spicules and skeleton frameworks as well as our understanding of the ultrastructural and molecular patterns of silica deposition in sponges.

REFERENCES

Bavestrello G, Bonito M, Sarà M. 1993. Influence of depth on the size of sponge spicules. In: Uriz MJ, Rützler K, editors. Recent advances in ecology and systematics of sponges. Barcelona: Scientia Marina 57(4). p 415–420.

Becerro MA, Uriz MJ, Turon X. 1994. Trends in space occupation by the encrusting sponge *Crambe crambe*: variation in shape as a function of size and environment. Mar Biol 121:301–307.

Becerro MA, Thacker RW, Turon X, Uriz MJ, Paul VJ. 2003. Biogeography of sponge chemical ecology: comparisons of tropical and temperate defenses. Oecologia 135:91–101.

Bibiloni MA. 1990. Fauna de Esponjas de las Islas Baleares. Variación cualitativa y cuantitativa de la población de esponjas en un gradiente batimétrico. comparación Baleares-Costa Catalana. Ph.D. thesis, Universitat de Barcelona.

Bond C. 1992. Continuous cell movements rearrange anatomical structures in intact sponges. J Exp Zool 263:284–302.

Bond C, Harris AK. 1988. Locomotion of sponges and its physical mechanism. J Exp Zool 246:271–284.

Borojevic R. 1971. Le comportament des cellules d'éponge lors de processus morphogénétiques. Ann Biol 10:533–545.

Boury-Esnault N, Rützler K. 1997. Thesaurus of sponge morphology. Smith Contrib Zool 596:1–55.

Boury-Esnault N, Vacelet J. 1994. Preliminary studies on the organization and development of a hexactinellid sponge from a Mediterranean cave, *Oopsacas minuta*. In: van Soest RWM, van Kempen ThMG, Braekman JC, editors. Sponges in time and space. Proc 4th Int Porifera Congr 1993, Amsterdam. Rotterdam: AA Balkema. p 407–415.

Bütschli O. 1901. Einige Beobachtungen über Kiesel-und Kalknadelm von Spongien. Z Wiss Zool 69:235–286.

Carballo JL, Uriz MJ. 1998. *Guitarra flamenca* sp. nov. (Porifera: Poecilosclerida) with a SEM revision of the spiny isochelae and placochelae in the genus. J Mar Biol Assoc UK 78:807–819.

Cha JN, Shimizu K, Zhou Y, Christiansen SC, Chmelka BF, Stucky GD, Morse DE. 1999. Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. Proc Natl Acad Sci USA 96:361–365.

Cha JN, Stucky GD, Morse DE, Deming TJ. 2000. Biomimetic synthesis of ordered silica structures mediated by block polypeptides. Nature 40:289–292.

Chanas B, Pawlik JR. 1995. Defenses of Caribbean sponges against predatory reef fish. II. Spicules, tissue toughness and nutritional quality. Mar Ecol Prog Ser 127:195–211.

Chanas B, Pawlik JR. 1996. Does the skeleton of a sponge provide a defense against predatory reef fish? Oecologia 107:225–231.

Chombard C, Boury-Esnault N, Tillier S. 1998. Reassessment of homology of morphological characters in tetractinellid sponges based on molecular data. Sys Biol 47:351–366.

Cimino G, Sodano G. 1994. Transfer of sponge secondary metabolites to predators. In: van Soest RWM, van Kempen TMG, Braekman JC, editors. Sponges in time and space. Proc 4th Int Porifera Congr 1993, Amsterdam. Rotterdam: AA Balkema. p 459–472.

Corriero G, Liaci L, Pronzato R. 1996. Two new species of *Dendroxea griessinger* (Porifera: Demospongiae) from the Mediterranean Sea. Bull Inst R Sci Nat Belg 66(Suppl):197–203.

Coutinho CC, Vissers S, van den Vyver G. 1994. Evidence of homeobox genes in the fresh-water sponge *Ephydatia fluviatilis*. In: van Soest RWM, van Kemper TMG, Braekman J-C, editors. Sponges in time and space. Proc 4th Int Porifera Congr 1993, Amsterdam. Rotterdam: AA Balkema. p 385–388.

Croce G, Frache A, Milanesio M, Viterbo D, Bavestrello G, Benatti V, Giovine M, Amenitsch H. 2003. Fiber diffraction study of spicules from marine sponges. Microsc Res Tech 62:378–381.

Custódio MR, Hadju E, Muricy G. 2002. In vivo study of microsclere formation in sponges of the genus *Mycale* (Demospongiae, Poecilosclerida). Zoomorphology 121:203–211.

Dayton P. 1979. Observations of growth, dispersal and population dynamics of some sponges in McMurdo Sound, Antarctica. Colloques Internationaux du C.N.R.S. Biology des Spongiaires 291: 271–282.

Dayton P, Robilliard GA, Paine RT, Dayton LB. 1974. Biological accommodation in the benthic community at McMurdo Sound, Antarctica. Ecol Monogr 44:105–128.
Degnan BM, Degnan SM, Giusti A, Morse DE. 1995. A hox/hom homeobox in sponges. Gene 155:175–177.
Desqueyroux-Faúndez R. 1990. Silica content of the New Caledonian fauna of Haplosclerida and Petrosiida and its possible taxonomic significance. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 279–283.
Donadey C, Paris J, Vacelet J. 1990. Ocurrence and ultrastructure of microraphides in *Axinella polypoides*. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press, p 259–263.
Elvin D. 1971. Growth rates of the siliceous spicules of the fresh-water sponge *Ephydatia muelleri* (Lieberkühn). Trans Am Microsc Soc 90:219–224.
Frohlich H, Barthel D. 1997. Silica uptake of the marine sponge *Halichondria panicea* in Kiel Bight. Mar Biol 128:115–125.
Fromont JP, Bergquist PR. 1990. Structural characters and their use in sponge taxonomy; when is a sigma not a sigma? In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 273–278.
Galera J, Turon X, Uriz MJ, Becerro MA. 2000. Microstructure variation in sponges sharing growth form: the encrusting sponges *Dysidea avara* (Dictyoceratida) and *Crambe crambe* (Poecilosclerida). Acta Zool 81:93–107.
Garrone R. 1969. Collagène, spongin et squelette minéral chez l'éponge *Haliclona rosea* (O.S.) (Démosponge, Haplosclérides). J Microsc 8:581–598.
Garrone R, Lethias C. 1990. Freeze fracture study of sponge cells. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 121–128.
Garrone R, Simpson TL, Pottu J. 1981. Ultrastructure and deposition of silica in sponges. In: Simpson TL, Volcani BE, editors. Silicon and siliceous structures in biological systems. New York: Springer. p 495–525.
Hanna C, Schönberg L, Barthel D. 1998. Unreliability of Demosponge skeletal characters: the example of *Halichondria*. In: Watanabe Y, Fusetani N, editors. Sponge science. Multidisciplinary perspectives. Tokyo: Springer. p 41–53.
Hartman WD. 1981. Form and distribution of silica in sponges. In: Simpson TL, Volcani BE, editors. Silicon and siliceous structures in biological systems. New York: Springer. p 453–493.
Hildebrand M, Volcani BE, Gassmann W, Schroeder JI. 1997. A gene family silicon transporters. Nature 385:688–689.
Hill MS, Hill AL. 2002. Morphological plasticity in the tropical sponge *Anthosigmella varian*: responses to predators and wave energy. Biol Bull 202:86–95.
Hinde GJ. 1887–1893. A monograph of the British fossil sponges. Palaeontogr Soc Monogr 1–254.
Hooper JNA. 1990. Character stability, systematics and affinities between Microcionidae (Poecilosclerida) and Axinellida. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 284–294.
Jackson JBC. 1979. Morphological strategies of sessile animals. In: Larwood G, Rosen BR, editors. Biology and systematics of colonial organisms 2. London: Academic Press. p 499–555.
Jones WC. 1998. Spicule form and morphogenesis in the calcareous sponge *Leuconia fistulosa* (Johnston). In: Watanabe Y, Fusetani N, editors. Sponge sciences. Multidisciplinary perspectives. Tokyo: Springer. p 41–53.
Jørgensen CB. 1944. On the spicule-formation of *Spongilla lacustris* (L.) 1. The dependence of the spicule-formation on the content of dissolved and solid silicic acid of the milieu. Det Kgl Danske Vidensks Selsk Biol Medd 19:2–45.
Krasko A, Batel R, Schröder HC, Müller IM, Müller WEG. 2000. Expression of silicatein and collagen genes in the marine sponge *Suberites domuncula* is controlled by silicate and myotrophin. Eur J Biochem 267:4878–4887.
Kröger N, Deutzman R, Sumper M. 1999. Polycationic peptides from diatom biosilica that direct silica nanosphere formation. Science 286:1129–1132.
Kruse M, Mikoc A, Cetkovic H, Gamulin V, Rinkevich, Müller IM, Müller WEG. 1994. Molecular evidence for the presence of a developmental gene in the lowest animals: identification of a homeobox-like gene in the marine sponge *Geodia cydonium*. Mech Ageing Dev 77:43–54.
Larroux C, Degnan B. 1999. Homeoboxes genes expressed in the adult and reaggregating sponge. Mem Queensland Museum 44:306.
Larsen PS, Riisgard HU. 1994. The sponge pump. J Theor Biol 168: 53–63.
Le Pennec G, Perovic S, Shokry A, Ammar A, Grebenjuk VA, Steffen R, Brümmer F, Müller WEG. 2003. Cultivation of primmorphs from the marine sponge *Suberites domuncula*, morphogenetic potential of silicon and iron. A review. J Biotechnol 100:93–108.
Lévi C. 1973. Systématique de la classe des Demospongiaria (Démosponges). In: Grassé P, editor. Spongiaires. Traité de Zoologie 3(1). Paris: Masson. p 577–632.
Lévi C. 1989. A remarkably strong natural glassy rod: The anchoring spicule of the *Monorhaphis* sponge. J Mat Sci Lett 8:337–339.
Leys SP, Hinman VF, Degnan BM. 1999. Which way up? Phototaxis and polarity in a sponge. Am Zool 39:38A.
Macki GO, Singla CL.1983. Studies on hexactinellid sponges. I. Histology of *Rhabdocalyptus dawsoni* (Lambe, 1973). Philos Trans R Soc Lond B 301:365–400.
Maldonado M, Uriz MJ. 1998. An experimental approach to the ecological significance of microhabitat-scale movement in an encrusting sponge. Mar Ecol Progr Ser 185:239–255.
Maldonado M, George SB, Young CM, Vaquerizo I. 1997. Depth regulation in parenchymella larvae of a demosponge: relative roles of skeletogenesis, biochemical changes and behavior. Mar Ecol Progr Ser 148:115–124.
Maldonado M, Carmona MC, Uriz MJ, Cruzado A. 1999. Decline in Mesozoic reef-building sponges explained by silicon limitation. Nature 401:785–788.
Manuel M, Le Parco Y. 2000. Homeobox gene diversification in the calcareous sponge *Sycon raphanus*. Mol Phyl Evol 17:97–107.
Mariani S, Uriz MJ. 2001. Copepods of the genus *Asterocheres* (Copepoda: Siphonostomatoida) feeding on sponges: behavioral and ecological traits. Invert Biol 120:269–277.
Martin D, Britayev TA. 1998. Symbiotic polychaetes: review of known species. Oceanogr Mar Biol Annu Rev 36:217–340.
Meewis H. 1939. Contribution à l'étude de l'embryogénèse des Chalinidae: *Halichona limbata* (Mont.). Ann Soc R Zool Belg 70: 201–244.
Meylan A. 1988. Spongivory in hawksbill turtles: a diet of glass. Science 239:393–395.
Meylan A. 1990. Nutritional characteristics of sponges in the diet of the hawksbill turtle, *Eretmochelys imbricata*. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 472–477.
Müller EG, Krasko A, Le Pennec G, Müller CI, Schröder HC. 2003. Biochemistry and cell biology of silica formation in sponges. Microsc Res Tech 62:368–377.
Okada Y. 1928. On the development of a hexactinellid sponge, *Farrea sollasii*. J Fac Sci Imp Univ Tokyo 2:1–27.
Palumbi SR. 1986. How body plans limit acclimation: responses of a demosponge to wave force. Ecology 67:208–214.
Pawlik JR, Chanas B, Toonen RJ, Fenical W. 1995. Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrence. Mar Ecol Prog Ser 127:183–194.
Pisera A. 2003. Some aspects of silica deposition in lithistid demosponge desmas. Microsc Res Tech 62:312–326.
Randall JE, Hartman WD. 1968. Sponge-feeding fishes of the West Indies. Mar Biol 1:216–225.
Reincke T, Barthel D. 1997. Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. Mar Biol 129:591–593
Reiswig HM. 1971. The axial symmetry of sponge spicules and its phylogenetic significance. Cah Biol Mar 12:505–514
Reiswig HM. 2002. Class Hexactinellida Schmidt, 1870. In: Hooper JNA, van Soest RWM, editors. Systema Porifera: a guide to the classification of sponges. New York: Kluwer Academic/Plenum. p 1201–1202.
Richelle-Maurer E, van de Vyver G. 1999. Temporal and spatial expression of EmH-3, a homeobox-containing gene isolated from the freshwater sponge *Ephydatia muelleri*. Mech Ageing Dev 109:203–219.
Richelle-Maurer E, van de Vyver G, Vissers S, Couthinho CC. 1998. Homeobox-containing genes in freshwater sponges: characterization, expression and phylogeny. Prog Mol Subcell Biol 19:1–20.
Riisgard HU, Larsen PS. 1995. Filter-feeding in marine macroinvertebrates: pump characteristics, modelling and energy cost. Biol Rev 70:67–106.
Riisgard HU, Larsen PS, Thomassen S, Jacobsen H, Weeks JM. 1993. Suspension feeding in marine sponges *Halichondria panicea* and

Haliclona urceolus: effects of temperature on filtration rate and energy cost of pumping. Mar Ecol Prog Ser 96:177–188.
Rosell D, Uriz MJ. 1997. Phylogenetic relationships within the excavating Hadromerida (Porifera), with a systematic revision. Cladistics 13:349–366.
Rosell D, Uriz MJ. 2002. Excavating and endolithic sponge species (Porifera) from the Mediterranean: species descriptions and identification key. Org Divers Evol 2:55–86.
Rützler K, Macintyre IG. 1978. Siliceous sponge spicules in coral reef sediments. Mar Biol 49:147–159.
Rützler K, Smith KP. 1993. The genus *Terpios* (Suberitidae) and new species in the "Lobiceps" complex. In: Uriz MJ, Rützler K, editors. Recent advances in ecology and systematics of sponges. Barcelona: Scientia Marina. p 381–393.
Sala E, Ballesteros E. 1997. Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralitoral ecosystem. Mar Ecol Prog Ser 152:273–283.
Sandford F. 2003. Physical and chemical analysis of the siliceous skeletons in six sponges of two sponge groups (Demospongiae and Hexactinellida. Microsc Res Tech 62:336–355.
Sangster AG, Parry DW. 1981. Ultrastructure of silica deposits in higher plants. In: Simpson TL, Volcani BE, editors. Silicon and siliceous structures in biological systems. New York: Springer. p 384–407.
Sarà M, Vacelet J. 1973. Ecologie des Démosponges. In: Grassé P, editor. Spongiaires. Traité de Zoologie 3(1) Paris: Masson. p 462–576.
Schönberg CHL. 2000. Bioeroding sponges common to the Central Australian Great Barrier: description of three new species, two new records, and additions to two previously described species. Senckenberg Marit 30:161–221.
Seimyia M, Ishiguro H, Miura K, Watanabe Y, Kurosawa Y. 1994. Homeobox-containing genes in the most primitive metazoa, the sponges. Eur J Biochem 221:219–225.
Seimyia M, Watanabe Y, Kurosawa Y. 1997. Identification of POU-class homeobox genes in a freshwater sponge and the specific expression of these genes during differentiation. Eur J Biochem 243: 27–31.
Shimizu K, Cha JH, Stucky GD, Morse DE. 1998. Silicatein alpha: cathepsin L-like protein in sponge biosilica. Proc Natl Acad Sci USA 95:6234–6238.
Simpson TL. 1968. The structure and function of sponge cells: new criteria for the taxonomy of Poecilosclerid sponges (Demospongiae). Peabody Mus Nat Hist (Yale Univ) 25:1–141.
Simpson TL. 1981. Effects of germanium on silica deposition in sponges. In: Simpson TL, Volcani VE, editors. Silicon and siliceous structures in biological systems. New York: Springer. p 527–550.
Simpson TL. 1984. The cell biology of sponges. New York: Springer. p 1–660.
Simpson TL. 1989. Silification processes in sponges: *Geodia asters* and the problem of morphogenesis of spicule shape. In: Crick RE, editor. Origin, evolution and modern aspects of biomineralization in plants and animals. New York: Kluwer Academic/Plenum. p 125–136.
Simpson TL. 1990. Recent data on pattern of silicification and the origin of monaxons from tetraxons. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 264–272.
Simpson TL, Vaccaro CA. 1974. An ultrastructural study of silica deposition in the fresh water sponge *Spongilla lacustris*. J Ultrastr Res 47:296–309.
Simpson TL, Langenbruch PF, Scalera-Liaci L. 1985. Silica spicules and axial filaments of the marine sponge *Stelletta grubii* (Porifera, Demospongiae). Zoomorphology 105:375–382.
Tabachnick KR, Reiswig HM. 2002. Dictionary of Hexactinellida. In: Hooper JNA, van Soest RWM, editors. Systema Porifera: a guide to the classification of sponges. New York: Kluwer Academic/Plenum. p 1224–1229.
Teragawa CK. 1990. Mechanical function and regulation of the skeletal network in *Dysidea*. In: Rützler K, editor. New perspectives in sponge biology. 3rd International Conference on the Biology of Sponges, 1985. Washington, DC: Smithsonian Institution Press. p 252–258.
TokyoCinema. 1996. Life of the freshwater sponge. Tokyo: TokyoCinema.
Trégouboff G. 1942. Contribution à la connaissance des larves planctoniques d'éponges. Arch Zool Exp Gén 82:357–399.
Uriz MJ. 1983. Contribución a la fauna de esponjas (Demospongiae) de Cataluña. An Sec Cienc Col Univ Gerona (Spain) 7:1–220.
Uriz MJ. 1988. Deep-water sponges from the continental shelf and slope of Namibia (South-West Africa): Classes Hexactinellida and Demospongia. In: Contributions on the marine fauna of Southern Africa. Monografías de Zoología Marina, vol. 3. Barcelona: Instituto de Ciencias del Mar (C.S.I.C). p 9–157.
Uriz MJ. 2002. Family Geodiidae. In: Hooper JNA, van Soest RWM, editors. Systema Porifera: a guide to the classification of sponges. New York: Kluwer Academic/Plenum. p 134–140.
Uriz MJ, Carballo JL. 2001. Phylogenetic relationship of sponges with placochelae or related spicules (Poecilosclerida, Guitarridae) with a systematic revision. Zool J Linn Soc 132:41–428.
Uriz MJ, Maldonado M. 1995. A reconsideration of the relationship between polyaxonid and monaxonid spicules in Demospongiae: new data from the genera *Crambe* and *Discorhabdella* (Porifera). Biol J Linnean Soc 55:1–15.
Uriz MJ, Rosell D, Martin D. 1992. The sponge population of the Cabrera Archipelago (Balearic Islands): characteristics, distribution, and abundance of the most representative species. PSZN Mar Ecol 13:101–117.
Uriz MJ, Turon X, Becerro MA, Galera J. 1996. Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage. J Exp Mar Biol Ecol 205:187–204.
Uriz MJ, Turon X, Becerro M. 2000. Silica deposition in Demosponges: spiculogenesis in *Crambe crambe*. Cell Tissue Res 301:299–309.
Uriz MJ, Turon X, Becerro MA. 2001. Morphology and ultrastructure of the swimming larvae of *Crambe crambe* (Demospongiae, Poecilosclerida). Invert Biol 120:295–307.
Uriz MJ, Turon X, Becerro M. 2002. Silica deposition in demosponges. In: Müller WEG, editor. Silicon biomineralization. Progress in molecular and subcellular biology. Berlin: Springer.
Vacelet J. 1998. Planktonic armoured propagules of the excavating sponge *Alectona* (Porifera): Demospongiae) are larvae: evidence from *Alectona wallichii* and *A. mesatlantica* sp. nov. Mem Queensl Mus 627–642
Vacelet J, Boury-Esnault N. 1995. Carnivorous sponges. Nature (Lond) 373:333–335.
Vacelet J, Uriz MJ. 1991. Deficient spiculation in a new species of *Merlia* (Merliida, Demospongiae) from the Balearic islands. In: Reitner J, Keupp H, editors. Fossil and recent sponges. Berlin: Springer. p 170–178.
Vogel S. 1974. Current-induced flow through the sponge, *Halichondria*. Biol Bull Mar Biol Lab Woods Hole 147:443–456.
Volcani BE. 1981. Cell wall formation in diatoms: morphogenesis and biochemistry. In: Simpson TL, Volcani BE, editors. Silicon and siliceous structures in biological systems. New York: Springer. p 157–200.
Waddell B, Pawlik JR. 2000. Defenses of Caribbean sponges against invertebrate predators. I. Assays with hermit crabs. Mar Ecol Prog Ser 195:125–132.
Weissenfels N, Langenbruch PF. 1985. Langzeitkulturen von Sübwasserschwämmen (Porifera, Spongillidae) unter Laborbedingungen. Zoomorphology 105:12–15.
Wilkinson CR, Garrone R. 1980. Ultrastructure of siliceous spicules and microsclerocytes in the marine sponge *Neofibularia irata* n. sp. J Morphol 166:51–64.
Wulff JL. 1994. Sponge feeding by Caribbean angelfishes, trunkfishes, and filefishes. In: van Soest RWM, van Kempen ThMG, Braekman JC, editors. Sponges in time and space. Biology, chemistry, paleontology. Rotterdam: AA Balkema. p 265–271.
Wulff JL. 1997. Parrotfish predation on cryptic sponges of Caribbean coral reefs. Mar Biol 129:41–52.
Young CM, Chia F-S. 1987. Abundance and distribution of pelagic larvae as influenced by predation, behavior, and hydrodynamic factors. In: Giese AG, Pearse JS, Pearse VN, editors. Reproduction of marine invertebrates. Palo Alto, CA: Blackwell Scientific and Boxwood Press. p 385–463.
Yourassowsky C, Rasmont R. 1983. The differentiation of sclerocytes in fresh-water sponges grown in a silica-poor medium. Differentiation 25:5–9.