

Sponge coordination, tissues, and the evolution of gastrulation

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Abstract: One of the unifying features of animals is that they carry out rapid, coordinated movement. This ability results from the early evolution of tissues that can both conduct signals and contract. The origin of tissues is thus intimately tied to the origin of nerves and muscle, which have long been considered the major innovations of cnidarians (anemones and jellyfish). However, the hypothesis that muscle may have conferred important selective advantages in preying and escape in the cnidarian ancestor suggests that this ancestor also had nerves, or at least the ability to coordinate contractions of its muscle. These ideas are supported by a considerable body of work showing that the genes which in triploblasts are involved in muscle specification and differentiation are expressed during the development of medusa and polyp in three cnidarian model species. Recent data suggest that sponges are paraphyletic, implying that cnidarians and sponges share a common ancestor that had a sponge-like body plan. Here I re-examine evidence for a coordinated contraction system in modern cellular sponges as evidence for functional tissues. I suggest that coordination of the animal is evidence that sponges do possess tissues which arise by gastrulation-like processes during embryogenesis as is the case in other metazoans. The sponge peristaltic contractile system may represent the foundations of coordinating tissues and have set the stage for the innovation of nerves and muscle in later animals.

Keywords: evolution of nerves, coordination, evolution of tissues, Porifera

Introduction:

Although the Porifera have long been regarded as an evolutionary side-branch of metazoans (Parazoans), recent molecular phylogenies propose that sponges are paraphyletic – that calcareous sponges (Calcispongia) and possibly Homoscleromorphs (Peterson, personal communication) are more closely related to cnidarians and other metazoans than they are to other sponges (Borchiellini *et al.* 2001, Medina *et al.* 2001). A paraphyletic Porifera suggests that the ancestral metazoan was a sponge-like organism, a suggestion that may not be as revolutionary as it first appears. The sponge body plan is only difficult to grasp in the light of work which suggests that sponges are colonies of a few types of cells organized around a system of water canals (Simpson 1984), animals that lack true epithelia (Mackie 1984, Tyler 2003), and the ability to coordinate activity at the level of the whole organism (Mackie 1979). In contrast, recent work shows that sponge epithelia are sealed units (Gonobobleva and Ereskovsky 2004) with tight junction proteins (Adell *et al.* 2004) and basement membranes (Boute *et al.* 1996, Boury-Esnault *et al.* 2003, Maldonado 2004). Sponges lack neurons (Pavans de Ceccatty 1989) but syncytial sponge tissues are nonetheless excitable and propagate electrical signals that control the feeding current (Leys and Mackie 1997). In cellular sponges external stimuli (such as contact by amphipods) trigger waves of contraction (Nickel 2004), and similar contractile waves are known to be widespread among

all cellular sponges. Though slow, the contractions in cellular sponges illustrate the components of peristaltic waves seen in animals with a nervous system. Peristalsis is an efficient mechanism for controlling fluid movement through a tube, and typically consists of a series of motor patterns that control relaxation in front of and contraction behind the object being moved by the fluid. The same pulsating movement occurs in the hearts of the invertebrate chordate *Amphioxus* (Holland *et al.* 2003) and ascidians, as well as in the gut and circulatory systems of insects and molluscs. In the sea pansy *Renilla*, a member of the most basal cnidarian group the Pennatulacea, peristaltic contractions of the gastrovascular cavity (GVC) – a tube-like gut that pervades the entire animal – control the movement of fluid for feeding and respiration as well as gamete release (Mechawar and Anctil 1997).

I suggest that the sponge body represents the first elaboration of a peristaltic contractile system in Metazoa, a system that was later adapted for locomotion, digestive and circulatory activity, and which gave rise to the hydrostatic skeleton. It is likely that elements of signaling used in these activities in higher animals may be found in extant sponges. I further suggest that coordinated contractions in sponges is evidence that these animals do possess tissues, and that these must have arisen during embryogenesis via gastrulation-like processes as in other animals. It is our challenge to understand what elements of tissues known in higher animals are used in sponges.

Propagated contractions in sponges

Cellular sponges have long been known to contract inhalant and exhalant openings (ostia and oscula), and portions of their canal system (Mackie 1979). The slow rates of propagation ($4\text{--}400\ \mu\text{m s}^{-1}$) and difficulty of observation in large animals have led to the conclusion that events are local and decremental. But at least three species show propagated contractions that are involved in expulsion of sediment (Nickel 2004) or gametes (Reiswig 1970), and rhythmic, diurnal pulses that may assist water flow through the animal (Weissenfels 1990, Nickel 2004). Given the slowness of the events, electrical signaling is not likely to be involved. Although glass sponges can propagate electrical signals, they do this through an uninterrupted giant syncytium (Leys and Mackie 1997). So far there is no convincing evidence of gap junctions (or other communicating junctions) in cellular sponges that would allow equivalent, rapid signalling (see Leys and Meech 2006). The slowness of the events suggests a slower mechanism of signaling via extracellular molecules is possible. Neurotransmitter molecules have been localized in tissues of calcareous sponges (Lentz 1966) and demosponge larvae (Weyrer *et al.* 1999), and many of these chemicals have been shown to affect the contraction of ostia and oscula (Parker 1910, Prosser *et al.* 1962, Emson 1966, Prosser 1967). Fascinating videos showing rhythmic contractions of the asconoid calcareous sponge *Leucosolenia* (C. Bond, personal communication) and time-lapse photographs of contractions in leuconoid calcareous sponges (Gaino *et al.* 1991) suggest the habit is widespread among cellular sponges.

Mechanism(s) of coordination of contractions

Video microscopy and image analysis of contractile behaviour show that both marine and freshwater sponges (de Vos and van de Vyver 1981, Weissenfels 1990, Nickel 2004) control the movement of water through a single aquiferous system (one osculum) in a manner similar to peristaltic contractions in the pennatulacean anthozoan *Renilla*. However, whereas most adult sponges are opaque to microscopy, juvenile demosponges are usually transparent so that individual cells crawling and cells forming epithelial linings to canals can be observed *in vivo*. Freshwater sponges have the added advantage that they can be readily grown *in vitro* from gemmules (overwintering cysts), thereby providing a relatively easy preparation for analysis of the mechanism of signaling. Experiments can be carried out on 7 day-old juvenile sponges hatched at room temperature using well-established protocols (de Vos 1971, de Vos and van de Vyver 1981, Francis and Poirrier 1986, Elliott 2004). Contractions begin after mechanical or chemical stimuli, and kinetics of contractions can be determined by computer assisted image analysis (1 image/10s) (Fig. 1, Elliott and Leys 2007). After each treatment the sponge carries out a stereotypical behaviour involving dilation and contraction, effectively expelling water (and any particulates) from the canal system.

How are contractions propagated? The problem has been considered in depth by Jones (1962) who suggested the following possibilities: local changes in pressure that induce contractions some distance away; stretch receptors acting

sequentially in adjacent cells; release of an aqueous hormone into the water or of a chemical messenger into the mesohyl; and local (non-propagating) action potentials that function to excite adjacent cells. Although recent work has focused on the secreted hormone hypothesis (Ellwanger *et al.* 2004, Ellwanger and Nickel 2006), it is most likely that several mechanisms interact. For example, a local change in pressure could activate stretch receptors, which in turn could trigger the release of a locally acting messenger. In *Ephydatia muelleri* responses to mechanical stimuli involve a peristaltic-like wave of dilation and contraction (Leys and Meech 2006), yet spasms also occur simultaneously on either side of a single sponge (Ellwanger *et al.* 2004, Elliott and Leys 2007) – how are these triggered? Perhaps minute changes in pressure stretch cell membranes at a distant location and trigger an apparently simultaneous contraction. Waves of contraction can also be seen to travel down (*along*) a canal and at the same time *across* canals. While a pressure wave could precede the contraction in both directions, evidence that amoeboid cells in the mesohyl cease crawling as the contraction passes (Fig. 2) point to a secreted messenger.

Many molecules have been shown to trigger oscular and ostia closure, and to initiate contractions (reviewed by Jones 1962, Lawn 1982, Leys and Meech 2006). Widespread evidence for glutamate in signaling in plants (Demidchik *et al.* 2004), *Paramecium* (Yang *et al.* 1997) and astrocytes (Nedergaard *et al.* 2002), and evidence for metabotropic Glu/GABA receptors in sponges (Perovic *et al.* 1999), makes this molecule an especially good candidate for a signaling molecule. Indeed, recent experiments in both *Tethya* (Ellwanger and Nickel 2006) and *Ephydatia* (Elliott and Leys 2007) suggest that contractions can be triggered by application of glutamate in a concentration dependent manner. The working hypothesis is that contractions propagate via calcium waves as in mammalian astrocytes (Nedergaard 1994) and mast cells (Osipchuk and Cahalan 1992), either via the direct action of stretch receptors or by the release of locally acting chemicals, much as outlined by Jones (1962): a stimulus (pressure/mechanical) causes stretch receptors to trigger a rise in intracellular calcium, causing contraction of the cell, tension on adjacent cells, and stimulating release of a secreted messenger (such as glutamate), which in turn stimulates contraction of nearby cells, and so on.

The slow rates of contraction – around $20\ \mu\text{m s}^{-1}$ – lend support to this hypothesis. However, some contractions, like that which travels up the osculum of *E. muelleri* at up to $375\ \mu\text{m s}^{-1}$ (McNair, 1923), are much faster. Since gap junction coupling is enhanced in the presence of glutamate (Enkvist and McCarthy 1994), it is possible that cells may be coupled by ‘almost gap junctions’, which connect cells as glutamate levels rise. During peristalsis in cnidarians and higher animals, nitric oxide signaling allows relaxation prior to contraction (Moroz *et al.* 2004, Anctil *et al.* 2005). Preliminary results show that nitric oxide (NO) synthase staining in *E. muelleri* tissues fixed for NADPH-diaphorase (Elliott and Leys 2007), and experiments by Ellwanger and Nickel (2006) suggest that NO modulates contractions in *Tethya*. Thus waves of contraction along the aquiferous canals in sponges may be modulated much in the same way they are in cnidarians

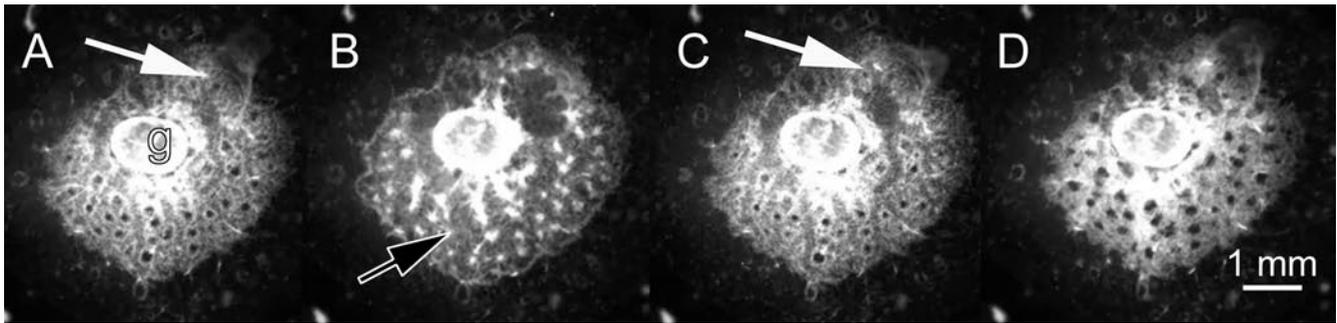


Fig. 1: Dilation and contraction of the aquiferous system of a 7-day old juvenile *Ephydatia muelleri*. White arrows point to the osculum, which increases in diameter by frame C as the canals dilate in A and B, and contract in C and D. The white regions between the dilated exhalant canals in (B) are the compressed inhalant canals (Elliott and Leys 2007).

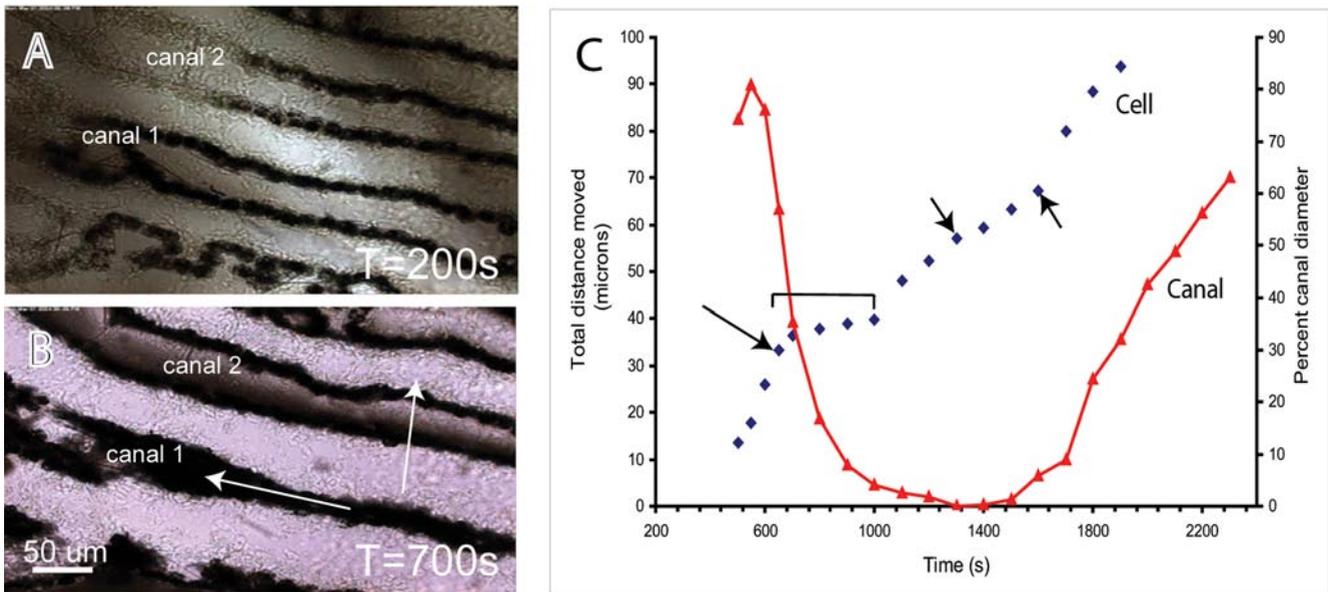


Fig. 2: Cells crawling through the mesohyl stop when a wave of contraction passes by. **A, B.** India ink added to a dish with a sandwich preparation is taken into the chambers (black oblique lines). When all chambers are filled a wave of contraction propagates along the canals (white arrow pointing left) and across canals (white arrow pointing across). There is a slight delay before the contraction is seen moving along the second canal (2). **C.** Plot of the track (diamond) of a single cell crawling in the mesohyl of the first canal during the first contraction. As the canal diameter narrows during a contraction (10 minutes, 600 s after addition of dye), the cell ceases forward movement (long arrow and bar). Crawling commences once the canal is fully contracted (10 minutes later). Forward motion is slowed a second time (small arrows) when the incumbent canal (1) begins to dilate once more (Modified from Elliott and Leys, 2007).

– even though the events occur at a much slower rate than allowed by nerves and true muscle.

Histology and ontogeny of contractile and signaling tissues

Coordination of contractions in cellular sponges remains a difficult problem because the concept of the sponge as an animal (Eumetazoa) is controversial. If sponges have a cellular level of organization (Parazoa) as is traditionally thought, there are no tissues: thus along what structure do contractions propagate and how does the animal maintain integrity among the cells during contractions?

Most modern texts suggest that during the evolution of basal metazoans there was a graded acquisition of structured

tissues (Gilbert 2003), culminating in the invention of mesoderm by primitive bilaterians. Cnidarians are usually regarded as diploblastic animals with only endoderm and ectoderm; however genes whose bilaterian homologs are implicated in mesodermal specification and differentiation are expressed during development of the anthozoan cnidarian *Nematostella* (Martindale *et al.* 2004) as well as in a tissue that gives rise to striated muscle in the hydrozoan jellyfish *Podocoryne* (Spring *et al.* 2002). It has therefore been suggested that mesoderm in triploblasts may have arisen from the endoderm of diploblastic animals, or, alternatively, that cnidarians arose from a triploblastic ancestor and that diploblasty is a secondary simplification (Martindale *et al.* 2004, Siepel and Schmid 2005).

Tissues

Under the hypothesis of sponge paraphyly (see above), a sponge-like animal is presumed to have given rise to early cnidarians. The leap is large. Sponges are filter feeders that lack nerves and muscle. Furthermore, sponges are not generally considered to have tissues, yet to some extent this last point may be a problem of definition. For example, transport strands in the tropical sponge *Aplysina* function as a distinct tissue, carrying phagocytosed material to the tip of the sponge presumably for growth (Leys and Reiswig 1998); regional concentrations of symbiont-containing cells that provide nutrition to sponges may do the same (e.g., Yahel *et al.* 2003). Tissues arise from germ layers during ontogeny and are mesenchymal or epithelial in nature. While current theory holds that epithelia preceded mesenchyme both during evolution and in development (Hay 1968, Pérez-Pomares and Muñoz-Chàpuli 2002, Price and Patel 2004), extant sponges are considered to be largely mesenchymal animals. The idea that their covering layer is not a true epithelium comes from the absence of a well-defined basement membrane (Woollacott and Pinto 1995). Strictly speaking, epithelia are considered to be sheets of cells with apical-basal polarity, cell-cell junctions and an extracellular matrix – ECM, cuticle or equivalent apically, and basement membrane basally – that maintains cell polarity (Tyler 2003, Cereijido *et al.* 2004). Other authors however, consider epithelia as “physical barriers between two different extracellular environments, with or without the presence of a basal lamina” (Pérez-Pomares and Muñoz-Chàpuli 2002). Although homoscleromorph sponges are considered to have a ‘true’ basement membrane, the condensation of ECM below choanocyte chambers is extremely slight (Boury-Esnault *et al.* 2003), and readily can be confused with the mucous coat on the apical surface of cells. An image showing similar condensation of ECM under the epithelium of the larva of the demosponge *Crambe crambe* (Maldonado 2004), coupled with evidence that other sponges have a type of collagen functionally equivalent to Type IV collagen (a typical constituent of basement membranes) (Aouacheria *et al.*, 2006) suggests that a comprehensive morphological study of sponge epithelia with a focus on seeking structures equivalent to a basal lamina is well-warranted.

One practical tool for studying sponge epithelia is simple labeling of the actin cytoskeleton in epithelia (Pavans de Ceccatty 1986). Using modern fluorescent labels for actin microfilaments (Bodipy-phalloidin fluorescein, Molecular Probes, OR) the surface ‘epithelium’ of 6-day old juvenile freshwater sponges shows remarkably extensive tracts of actin (Fig. 3). Bundles run from cell to cell forming continuous paths from the choanosome to the edge of the animal, a distance of several hundred microns. In fact, continuous tracts can traverse over 1 mm through the apical pinacoderm of these sponges. Where cells adjoin one-another there are dense plaques of actin reminiscent of adhesion plaques in smooth muscle, as shown in freeze fracture and thin section by Pavans de Ceccatty (1986) (Fig. 3C, D). The apical pinacoderm is a tri-layered structure formed by two epithelial sheets that sandwich a very thin collagenous (and

cellular) mesohyl (Elliott and Leys 2007). Like the transport pathways in *Aplysina* (Leys and Reiswig 1998), the apical pinacoderm of the juvenile sponge is designed to function as an epithelium.

Gastrulation

If sponges can be considered to possess functional tissues, how do these tissues arise? The problem of the origin of tissues and germ layers in sponges has been a long-standing debate with growing disagreement as to whether gastrulation-like processes occur (Efremova 1997, Leys 2004, Ereskovsky and Dondua 2006, reviewed in Leys and Ereskovsky 2006). Most studies of sponge development are largely descriptive. Given that the vast majority of sponges are ovoviviparous (brood their young), development cannot be readily followed *in vitro* as it can in many other phyla. Furthermore, mechanisms of development (cleavage patterns and segregation of cells to form layers) are diverse (reviewed in Leys and Ereskovsky 2006), and the lack of uniformity has led to a great disparity in views on comparative development. Experimental data is now needed to test the hypothesis that sponges undergo gastrulation-like processes during ontogeny (Leys and Degnan 2002, Leys 2004, Leys and Eerkes-Medrano 2005). Homologs of genes known to be involved in the formation of mesoderm in other animals have been sequenced from a calcareous sponge (Manuel *et al.* 2004) and from demosponges (Adell *et al.* 2003, Bebenek *et al.* 2004, Hill *et al.* 2004), but expression patterns in early gastrula-like stages are only just being examined (Larroux *et al.* 2006). Of specific interest should be the expression pattern in calcareous sponges, a group in which embryogenesis involves distinctly epithelial movements and invagination of the larva to form a blastopore-like structure (Leys and Eerkes-Medrano 2005).

Speculations and considerations

It is valuable to remember that “all is possible with sponges” (Boury-Esnault 2006). These animals are specialized for suspension feeding on bacteria and ultraplankton (Pile *et al.* 1996, 1997, Ribes *et al.* 1999), but this poses certain problems: the filter may occasionally become clogged, and the flow bringing in food may not be sufficient for gas exchange in certain habitats. The idea that peristaltic-like contractions (condensation contractions) may have arisen to assist the filtering mechanism in sponges is not new (Weissenfels 1990). However, with increasing knowledge of the physiology and development of other basal metazoans, it is now interesting to note how similar the rhythmic contractions in sponges are to those in pennatulaceans such as *Renilla* (Anctil 1989, 1991). Apparently the absence of nerves and true muscle in sponges is no handicap given the power of a hydrostatic skeleton. Seen in this light, the sponge body plan with its aquiferous system could be considered to contain the underpinnings for the evolution of peristaltic contractile systems found in later animals.

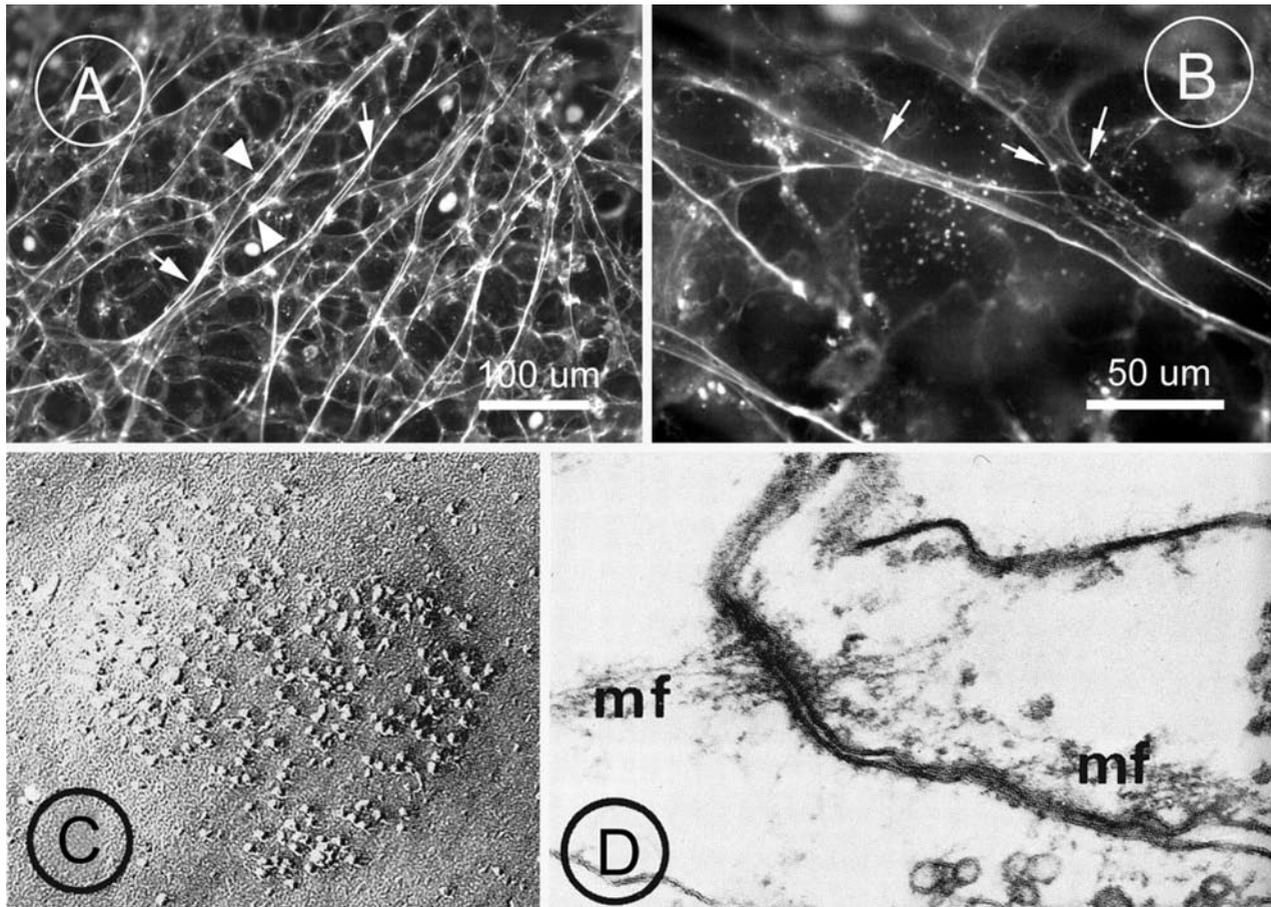


Fig. 3: **A, B.** The actin cytoskeleton in the apical pinacoderm (exopinacoderm) of a 6-day old juvenile sponge (*E. muelleri*). In **A**, arrows indicate actin tracts labeled with Bodipy phalloidin-fluorescein, and arrowheads mark points of contact of individual cells. In **B**, arrows indicate dense plaques of actin at points of contact of cells. **C.** Freeze fracture preparation showing a desmosome-like region on the basal epithelial membrane in *E. muelleri*. **D.** Thin section (TEM) showing actin microfilaments (mf) and a dense plaque at the point of contact between two cells (C and D from Pavans de Ceccatty, 1986).

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