

# Sponge embryology: the past, the present and the future

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**Abstract:** Developmental biology of sponges has a 140-year-old history. It made important contributions to spongiology in general: the creation of the subkingdom Enantiozoa, the separation of the Calcinea and the Calcaronea in the Calcarea, and the separation of the Tetractinomorpha and the Ceractinomorpha within the Demospongiae. Nevertheless, embryonic development has been studied in only 93 sponge species. This review must be restricted primarily to embryonic development and metamorphosis of sponges, because full modern information on its normal development is limited to only a few studies. Mechanisms of morphogenetic movements emerged in the course of evolution prior to the separation of the covering cell layer as ectoderm and the internal digestive cell mass as endoderm. Therefore, it is incorrect to apply the term “gastrulation” to sponge development. It is difficult to use comparative embryological data on sponges for phylogenetic interpretations because their development is highly polymorphic. The same cleavage pattern and blastula type may be characteristic of different larval types. On the other hand, the same larval types may develop from different cleavage patterns and types of morphogenesis. However, embryological data do indicate groups or ‘types’ of sponge development. The observed variety of developmental patterns indicates that no linear ways of developmental evolution are common for all Porifera. It testifies to an early divergence of sponge macrogroups or, probably, paraphyly and their long parallel evolution. The basal phylogenetic position of the Porifera among the Metazoa and its suggested paraphyly make new investigations on embryology and larvae especially important. Relatively few homologues of developmental genes are known in the Porifera. Comparison of transcription factors that regulate genes expression during sponge morphogenesis will provide an evolutionary perspective to relationships among basal metazoan phyla.

**Keywords:** development, evolution, morphogenesis, phylogeny, sponges

## The past

Developmental studies of sponges have a 140-year-old history. Ever since the work of Ernst Haeckel (1866) (Fig. 1), they have inspired, enriched and modified evolutionary thought. Altogether, about 540 articles concerning sponge embryology have been published. They involve approximately 36 species of Calcarea, 140 species of Demospongiae, and as little as 3 Hexactinellida species.

The **first** period of sponge development studies falls on the last third of the 19<sup>th</sup> century. It was the “Golden Age” of sponge embryology. About 110 articles on this topic were published (Fig. 2). Uncontested leadership in this research field belonged to German zoologists: Schulze, Maas, Schmidt, Keller (Fig. 3) and others.

The basis of sponge comparative embryology was laid at that time. Haeckel (1874) admitted that embryological studies of calcareous sponges (Haeckel 1872) were the starting point for his ideas about the origin of Metazoa, later formalized as the Gastraea theory of ontogeny recapitulating phylogeny, in which the gastrula is viewed as the recapitulation of a gastracan ancestor that evolved via selection on a simple,

planktonic hollow ball of cells to develop the capacity to feed (Haeckel 1874). On the basis of comparative embryological data of some demosponges, Delage (1892, 1899) (Fig. 4) discovered that during a metamorphosis of parenchymella larvae external flagellated cells migrate inward to form the choanoderm of the adult sponge. These observations have allowed Delage to propose a hypothesis of “inversion of the germ layers”. Being based on this hypothesis, he separated sponges from Metazoa into a special group, Enantiozoa that signified “inside out animals”. Bidder (1898), following Minchin (1896), subdivided Calcarea into two subclasses, Calcinea and Calcaronea, distinguished deep embryological differences (e.g., coeloblastula in Calcinea, amphiblastula in Calcaronea), and the position of the nucleus in the choanocytes (with nucleus basal in choanocyte independent of flagellum in Calcinea and with nucleus apical in choanocyte linked to the flagellum in Calcaronea).

The **second** period falls on the first half of the 20<sup>th</sup> century (1900-1960), when interest in sponge development declined (Fig. 2). Almost the only active researches were made in Belgium by Brien (Fig. 5), Meewis, Leveaux, and in France by Tuzet, Duboscq and Lévi (Fig. 6). Claude Lévi



**Fig. 1:** Ernst Haeckel (1834-1919) and Nicolas Miklucho-Maclay (1846-1888) during the expedition to the Red Sea in 1866 (From: I.I. Kanaev, 1966).

in his famous work (1956) was the first to use embryological characters of sponges in systematics.

However, this period was marked by the emergence of a major branch in developmental biology of sponges. Wilson

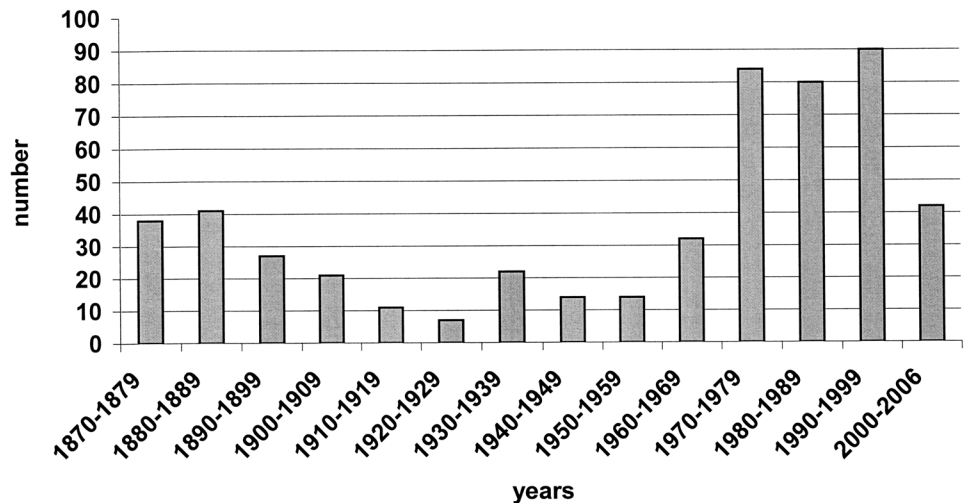
(1907) pioneered the use of sponges as model animals for cell adhesion research. He described species-specific reaggregation of mechanically dissociated sponge cells. His works provided an impulse for studies of behaviour of separate cells and regeneration in sponges.

The **third** period started with the application of electron microscopy and new optical and experimental methods to sponge studies (Fig. 2). Spermatogenesis and oogenesis, fertilization (in Calcaronea) and larval structure (in all poriferan classes) were investigated ultrastructurally. The results of these works were extensively applied to evolutionary and phylogenetic constructions concerning both Porifera and Metazoa in general. At the same time, complete development from egg to juvenile was investigated at the ultrastructural level only in some species, including some Spongillidae (see: Weissenfels 1989), *Halisarca dujardini* Johnston, 1842 (Demospongiae, Halisarcida) (Ereskovsky and Gonobobleva 2000, Ereskovsky 2002, Gonobobleva and Ereskovsky 2004a, 2004b, Ereskovsky *et al.* 2005, 2007a, Mukhina *et al.* 2006), some species of *Oscarella* (Ereskovsky and Boury-Esnault 2002, Boury-Esnault *et al.* 2003, Ereskovsky 2005, Ereskovsky *et al.* 2007b) and *Amphimedon queenslandica* Hooper and van Soest, 2006 (as *Reniera* sp.) (Leys and Degnan 2002, Degnan *et al.* 2005, Larroux *et al.* 2006).

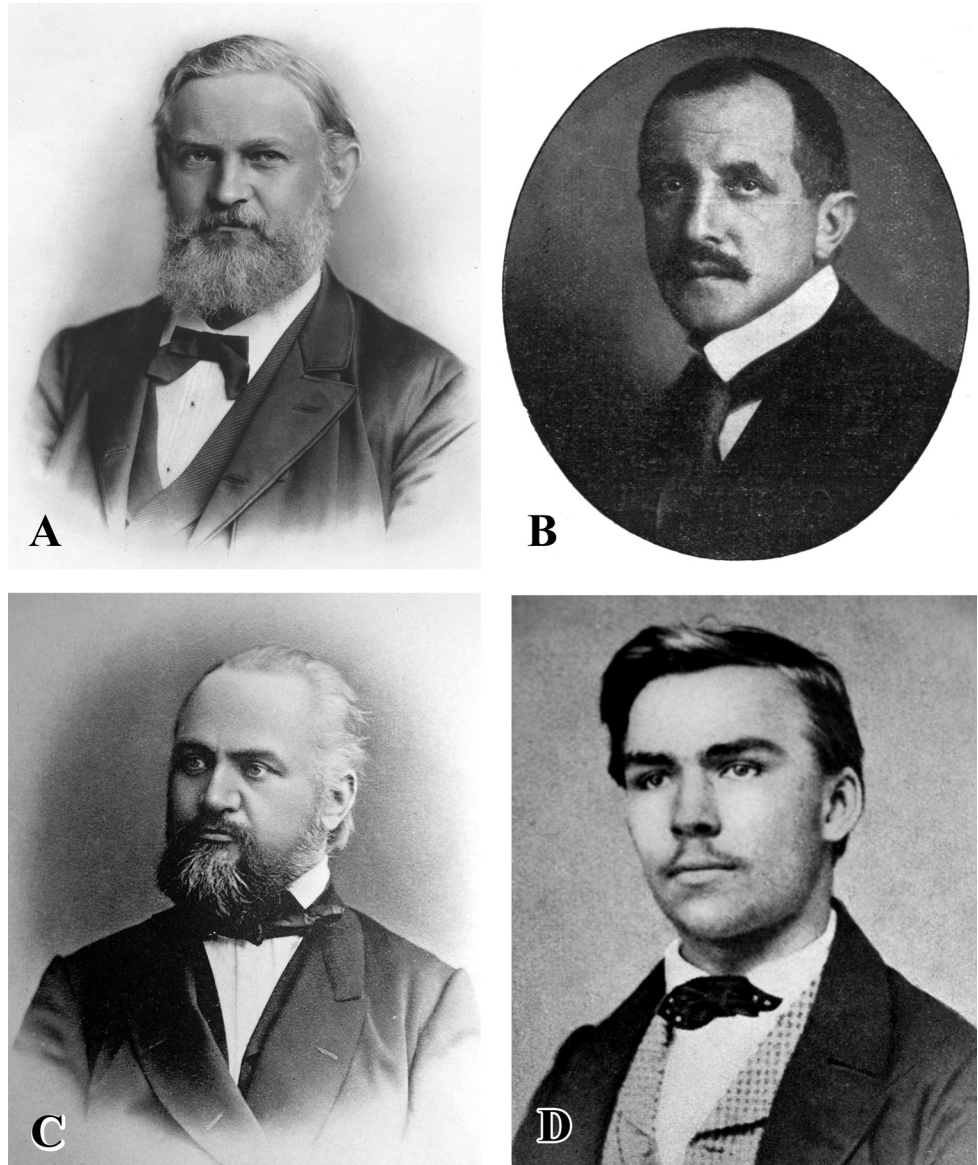
Looking back, we can see that out of the 540 articles on sponge embryology, only 93 are devoted to embryonic development in the strict sense. They deal with 21 species of Calcarea, 2 species of Hexactinellida and about 70 species of Demospongiae (Fig. 7). Strangely enough, there is only two publications (Hill *et al.* 2004, Laroux *et al.* 2006) on the development of sponges during sexual reproduction where molecular-biological methods were used. Researchers only start to decode complex embryonic morphogenesis at the ultrastructural level (Boury-Esnault *et al.* 1999, 2003, Ereskovsky and Gonobobleva 2000, Ereskovsky and Boury-

**Fig. 2:** The trend in general sponge development publications between 1870 and 2006.

### General number of sponge embryological articles



**Fig. 3:** German spongiologists portraits. **A.** Frans Eilhard Schulze (1840-1921) ZM B IX-608; **B.** Otto Maas (1867-1916); **C.** Oscar Schmidt (1823-1886); **D.** Conrad Keller (1848-1930) ZM B I/1753. (The photos are kindly given by C. Eckert).



Esnault 2002, Leys and Degnan 2002, Gonobobleva and Ereskovsky 2004a, Usher and Ereskovsky 2005, Leys *et al.* 2006) and investigate developmental genes expression during embryonic development (Hill *et al.* 2004, Laroux *et al.* 2006).

### The present

#### *Gastrulation: verbal or real problem in sponges?*

Applicability of the term “gastrulation” to sponge development is one of the sore points in our discussion (see: Efremova 1997, Leys 2004, Ereskovsky and Dondua 2006). There are two principal definitions of this term.

The first is used by most, but not all developmental biologists: Gastrulation is the process in embryonic development in the course of which three primary germ layers are formed and the gut is formed through complex

cell migrations (Technau and Scholz 2003, Stern 2004, Keller 2005, Martindale 2005). The second definition is rare: Gastrulation is the process that results in a multilayered organism during embryonic development (Efremova 1997, Leys and Degnan 2002, Maldonado 2004, Leys 2004). According to these authors, the formation of a multilayered embryo during embryogenesis in sponges should be considered as gastrulation, since mechanisms of cell reorganization in the blastula are similar with those recognized as gastrulation in cnidarians. This contradiction stems from the absence of a generally accepted point of view on the homology of embryonic processes and their derivatives in sponges and other animals.

Despite recent impressive progress in morphogenetic research in general, works on sponge embryonic morphogenesis are very rare. Therefore, investigations of mechanisms of sponge embryonic development are currently



**Fig. 4:** Yves Delage (1854-1920) at the Roscoff Marine laboratory, 1905 (From: Beetschen and Fischer, 2004).

much more important than terminological discussions. *Morphogenesis* is the mechanism responsible for creation of body plan during embryonic development, metamorphosis, asexual reproduction and regeneration. Morphogenetic investigations are a promising branch in developmental biology of sponges.

Formation of multilayer embryos in Metazoa is achieved either by the migration of individual cells, or, by movements of cell sheets (Keller *et al.* 2003, Keller 2005). The former morphogenetic movements are known as mesenchymal morphogenesis or epithelial–mesenchymal transitions. One such example is multipolar ingression (Shook and Keller 2003). The latter is epithelial morphogenesis and invagination is such an example (Keller *et al.* 2003, Gilbert 2003).

Morphogenetic cell movements are determined by complex and specific gene systems. Their origin and evolution resulted in the diversity of metazoan developmental types. Apparently, they are involved in multicellular embryos' formation in all animals, including sponges.

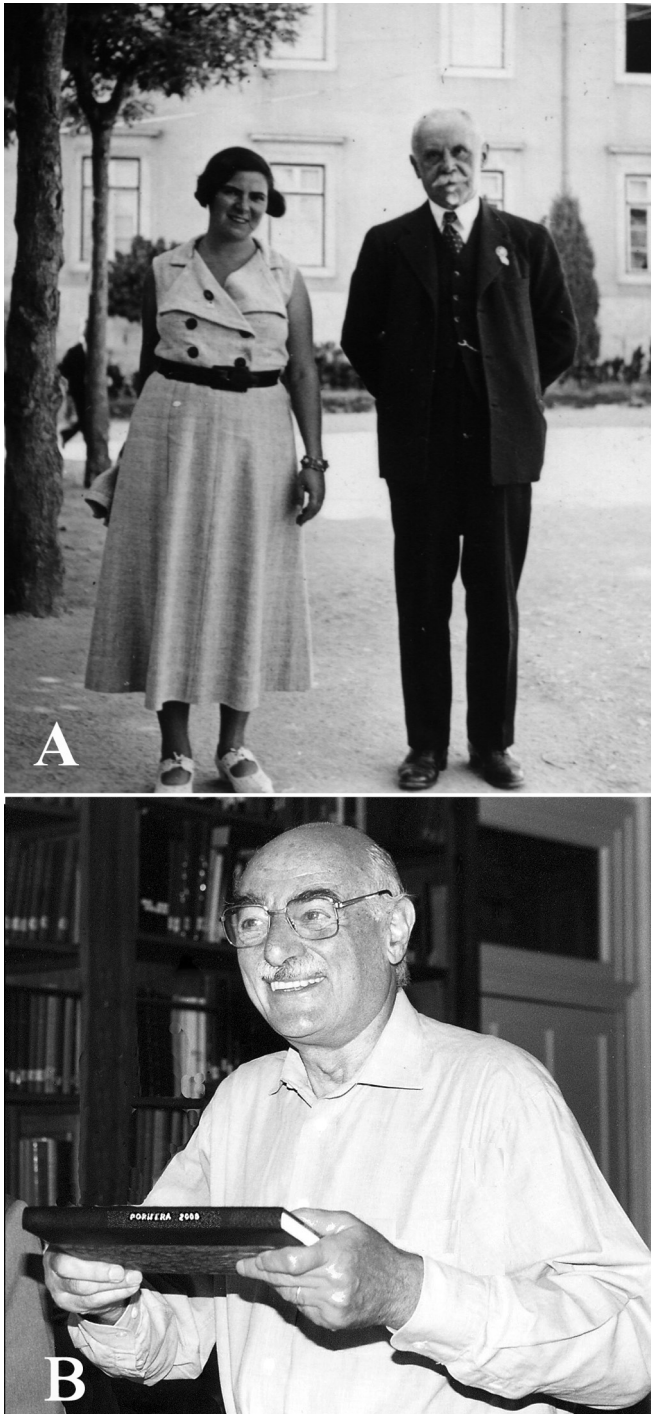
For instance, formation of sponge larvae is accompanied by almost all types of cell movements, characteristic of Eumetazoa (Efremova 1997, Leys 2004, Maldonado 2004, Ereskovsky 2005, Leys and Ereskovsky 2006, Ereskovsky and Dondua 2006): cell delamination (Hexactinellida – *Oopsacas minuta* Topsent, 1927) (Fig. 8A), morula delamination (Demospongiae: Dendroceratida, Dictyoceratida, Halichondrida, Haplosclerida) (Fig. 8B), invagination,



**Fig. 5:** Paul Brien (1894-1975), Brussels, 1968. (The photo is kindly given by Ph. Willenz).

unipolar and multipolar ingression (Demospongiae: Halisarcida – *Halisarca dujardini*) (Fig. 8C, D). At the same time, some unique morphogeneses, not found in other multicellular animals, have been described in sponges. They are, for example, multipolar egression in Homoscleromorpha (Demospongiae) (Fig. 8E), polarized delamination (Demospongiae: Poecilosclerida and Halichondrida) (Fig. 8F), excuvation in Calcaronea (Calcarea) (Fig. 8G), formation of blastula (pseudoblastula) by means of ingression of maternal cells into the embryo in *Chondrosia reniformis* Nardo, 1833 (Demospongiae: Chondrosida) (Fig. 8H), and unipolar proliferation (Demospongiae: Verticillitida – *Vaceletia crypta* (Vacelet, 1977) (Fig. 8I).

According to comparative embryological data on Porifera and Cnidaria, ancestors of Metazoa must have been able to form epithelial layers and to disaggregate these layers into individual cells. They were capable of epithelial morphogenesis and also had regulatory mechanisms controlling cell ingression and ensuring directed movement of cell masses. **It may be therefore concluded that mechanisms of morphogenetic movements emerged in the course of evolution prior to the separation of the covering cell layer as ectoderm and the internal digestive cell mass as endoderm.** This testifies to the independence of processes of spatial distribution of cells and their specification in the forming embryo as ectodermal and endodermal tissues (Ereskovsky and Dondua 2006). So, it is incorrect to apply the term “gastrulation” to sponge development.



**Fig. 6:** A. Odette Tuzet (1903-1976) and O. Duboscq (1868-1943), Banuls-sur-Mer Marine laboratory, 1937; B. Claude Lévi, Paris, 2000 (The photo is kindly given by J. Vacelet).

### *Evolutionary importance of larvae*

Evolutionary importance has been attached to larvae of Bilateria since A. Kowalevsky's studies on ascidia (1866). This idea has recently received molecular-biological support (Raff 1994, Peterson and Davidson 2000). Indeed, sponge

larvae have a strongly pronounced anterior-posterior polarity, distinct photoreception and other kinds of taxis (Maldonado 2004). Some demosponge larvae have desmosome-like cell junctions (Fig. 9). Finally, it has been shown that parenchymella of *A. queenslandica* possesses some of the transcription factor genes that appear to be characteristic of Metazoa. They are expressed during the development of this species (Larroux *et al.* 2006).

### **Cellular and molecular basis of embryonic morphogenesis in sponges**

#### *Cellular basis of embryonic morphogenesis*

During metazoan embryonic development, the cells can undergo changes either autonomously or in conjunction with their neighbors to form an embryo. Most of morphogenetic movements require that a subset of cells detach from their neighbors and acquire properties allowing them to migrate to new position. Obviously, the consequences of changes in cell shape and motility will be quite different if cells are joined in an epithelium or if they are unconstrained by neighbors. Cell motility is generated by contractile elements of the cytoskeleton. The following question requiring an answer arises: **What is the cytoskeleton dynamics during embryonic morphogenesis in sponges?**

#### *Cell-extracellular matrix adhesion*

One of the main molecules that mediate cell anchorage to the substratum during the morphogenesis is integrin, which are key molecules during early animal development (Darribere *et al.* 2000). (Integrins, members of the transmembrane linker proteins family, traverse the cell membrane, anchoring the actin microfilaments on the inside and may bind to the fibronectin and in other extracellular matrix proteins). Integrins were shown to be present in some adult demosponges: *Ophlitaspongia tenuis* and *Microciona prolifera* Ellis and Solander, 1786 (Brower *et al.* 1997, Kuhns *et al.* 2001, Sabella *et al.* 2004), *Geodia cydonium* (Jameson, 1811) (Pancer *et al.* 1997, Müller 1997) and in *Suberites domuncula* (Olivi, 1792) (Wimmer *et al.* 1999). The following questions arise: **Are integrins involved in embryonic development of sponges? Is their morphogenetic role the same in sponges and in other animals?**

#### *Intercellular adhesion*

Cell-cell interactions are also important for tissue formation during development. A remarkable feature of sponges is that when dissociated to single cells they can undergo species-specific reaggregation (Wilson 1907). This is mediated by an extracellular proteoglycan complex, known as aggregation factor (AF) that acts as a bridge between receptor proteins on neighboring cells (Schutze *et al.* 2001). The AF receptor also possesses an RGD (Arg-Gly-Asp attachment site) integrin-binding motif. RGD containing peptides will block AF-mediated aggregation. Both the RGD peptide and AF stimulate a range of intracellular responses (Wimmer *et al.* 1999). It was proposed that binding of AF promotes interaction between the RGD of the AF receptor

Fig. 7: The trend in sponge embryonic development publications between 1870 and 2006.

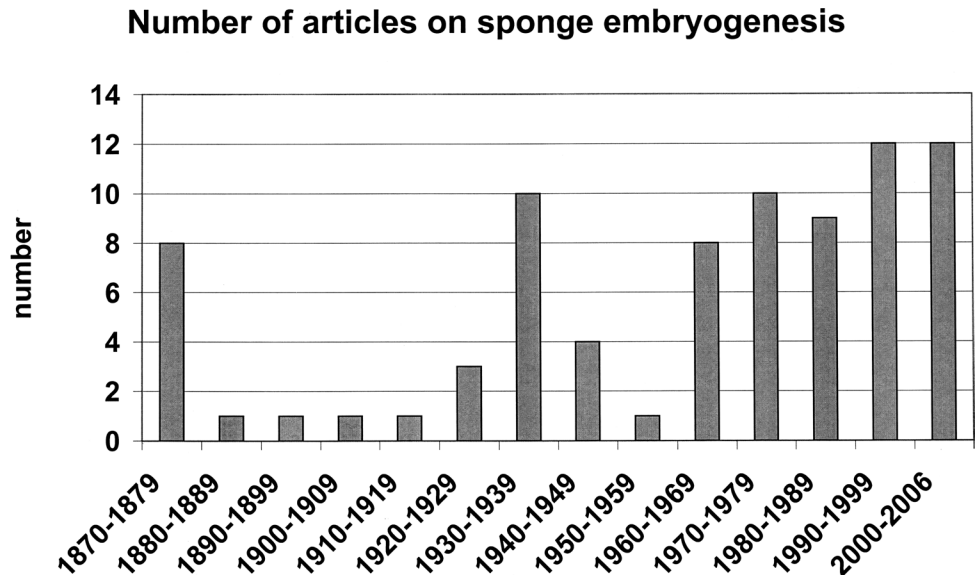
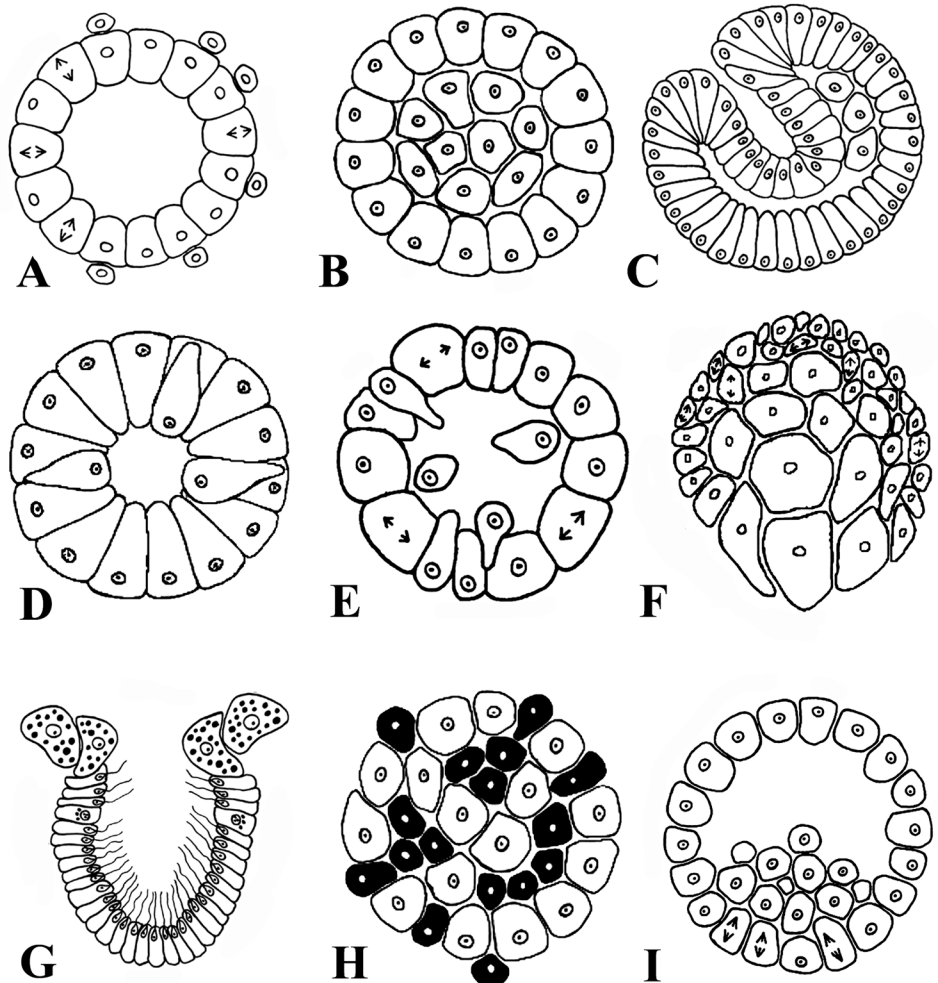
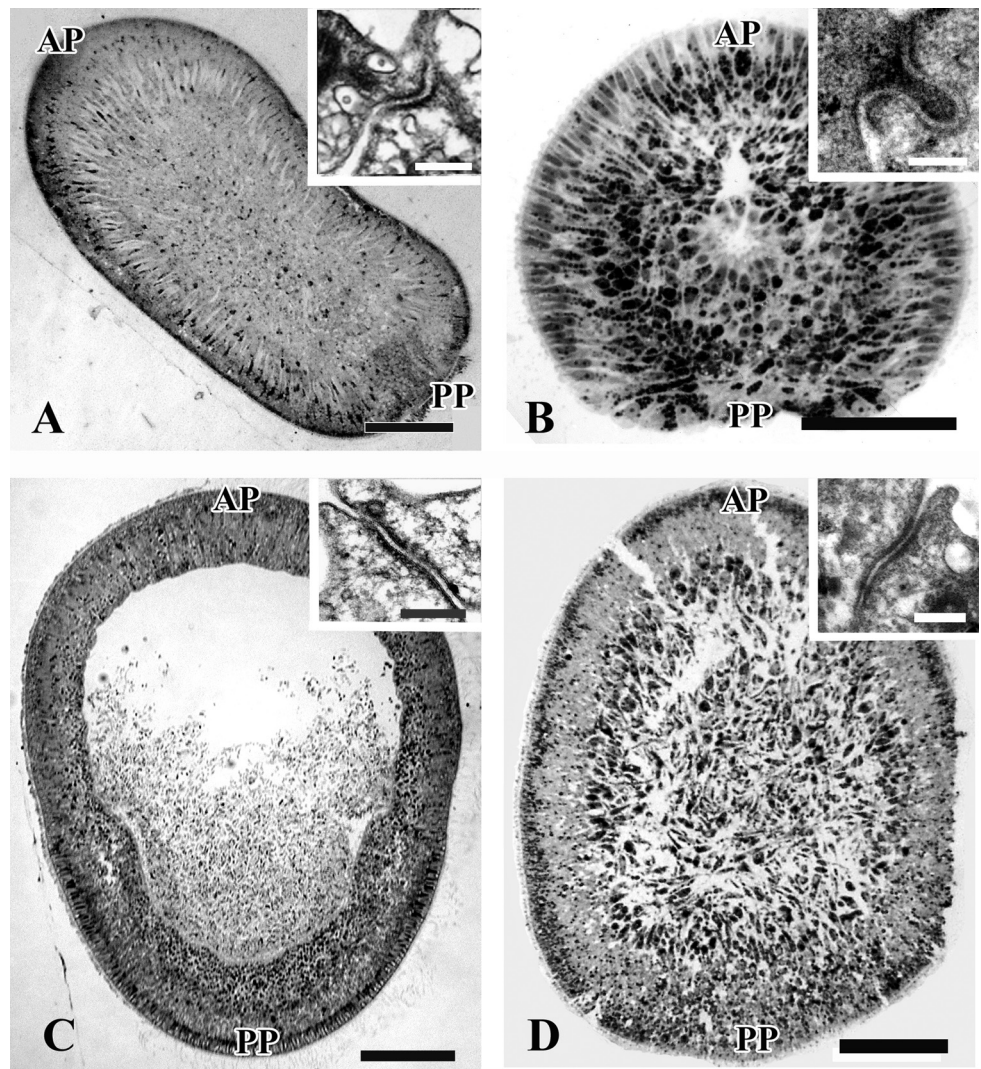


Fig. 8: Different types of morphogenesis in sponges resulting in larva formation: **A.** Cell delamination (Hexactinellida – *Oopsacas minuta*); **B.** Morula delamination (Demospongiae: Dendroceratida, Dictyoceratida, Halichondrida, Haplosclerida); **C.** Invagination (*Halisarca dujardini*, Demospongiae); **D.** Multipolar ingression (*H. dujardini*, Demospongiae); **E.** Multipolar egression (Homoscleromorpha, Demospongiae); **F.** Polarized delamination (Demospongiae: Poecilosclerida and Halichondrida); **G.** Excurvation (Calcareonea, Calcarea); **H.** Formation of blastula (pseudoblastula) by means of ingression of maternal cells into the embryo (*Chondrosia reniformis*, Demospongiae: Chondrosida); **I.** Unipolar proliferation (Demospongiae: Verticillitida – *Vaceletia crypta*). (From: Ereskovsky and Dondua 2006).



**Fig. 9:** Semi-thin micrographs of demosponges larvae with the desmosom-like cell junctions (insets). **A.** Parenchymella of *Ircinia oros* (Dictyoceratida); **B.** Disphaerula of *Halisarca dujardini* (Halisarcida); **C.** Cinctoblastula of *Corticium candelabrum* Schmidt, 1862 (Homoscleromorpha); **D.** Parenchymella of *Pleraplysilla spinifera* (Schulze, 1879) (Dictyoceratida). Abbreviations: AP – anterior pole, PP – posterior pole. Scale bar, A – 100  $\mu$ m; Inset – 0,2  $\mu$ m; B - 50  $\mu$ m; Inset – 25 nm; C - 50  $\mu$ m; Inset – 0,2  $\mu$ m; D – 50  $\mu$ m; Inset – 0,2  $\mu$ m.



and sponge integrin proteins (Harwood and Coates 2004). Many excellent studies dealt with the fine mechanisms of cell-cell interactions in sponge cell cultures (see: Fernandez-Busquets *et al.* 2002, Misevic *et al.* 2004). Nevertheless, no cadherin, catenin or related proteins have been identified in sponges (Harwood and Coates 2004).

**However, there is not a single work demonstrating either specific or differential cellular adhesiveness in sponge embryonic development.**

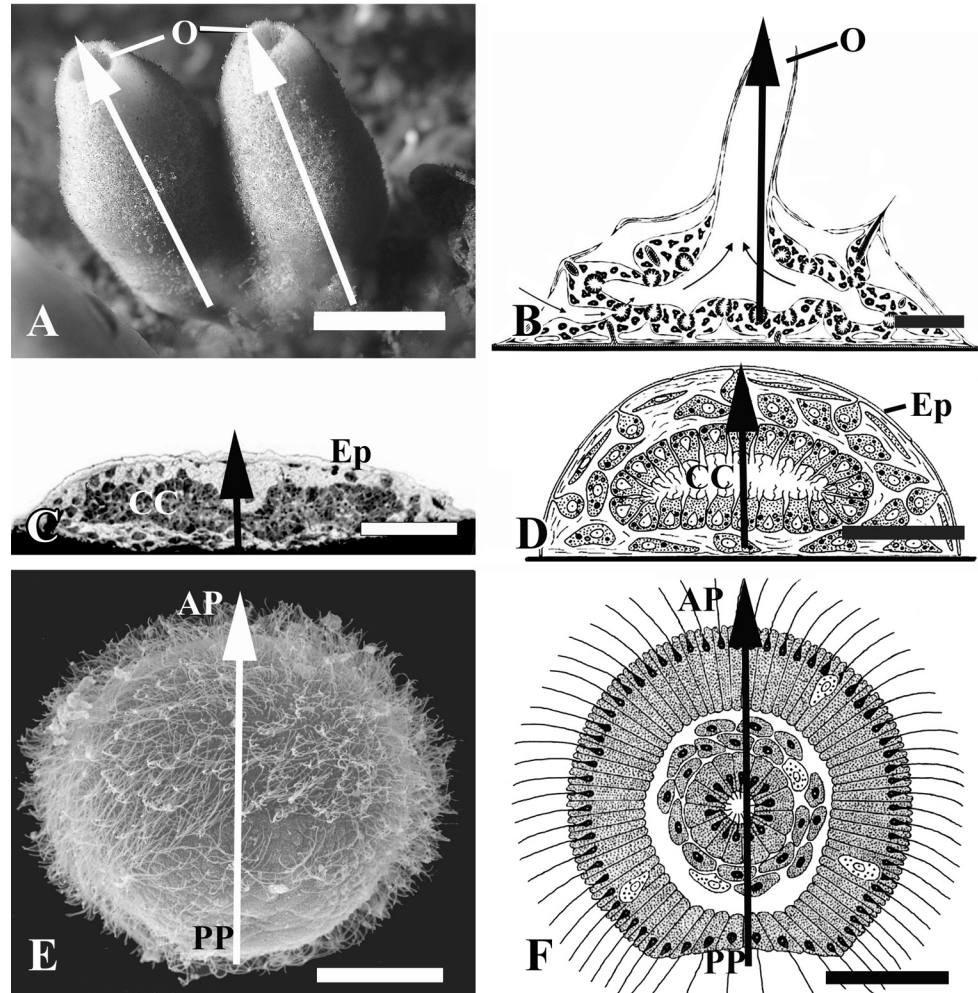
#### *Developmental genes in sponges*

The presence of metazoan developmental genes in demosponge genomes has been shown (e.g., Degnan *et al.* 1993, 1995, Coutinho *et al.* 1994, 2003, Seimiya *et al.* 1994, 1997, Hoshiyama *et al.* 1998, Richelle-Maurer *et al.* 1998, Manuel and Le Parco 2000, Adell *et al.* 2003, Perovic *et al.* 2003, Wiens *et al.* 2003a, b, Adell and Muller 2004, Hill *et al.* 2004, Manuel *et al.* 2004, Richelle-Maurer *et al.* 2006, Larroux *et al.* 2006, 2007). However, their roles in

embryogenesis and metamorphosis are unknown. To date, our understanding of sponge gene expression is restricted chiefly to asexual reproductive processes, such as gemmule germination, cell aggregation, and primorphs formation.

Hill *et al.* (2004) followed the expression of non-*Hox Antp*-class Bar-/Bsh-like gene during larva releasing, larva swimming and metamorphosis. Laroux *et al.* (2006) demonstrated that an extensive range of metazoan transcription factor genes, including members of the ANTP class (outside Hox, ParaHox, and extended-Hox clades), Pax, POU, LIM-HD, Sox, nuclear receptor (NR), Fox (forkhead), T-box, Mef2, and Ets gene classes are expressed during *A. queenslandica* (Haplosclerida) development. These data combined with developmental gene expression patterns from other animals suggest that these genes may have played important regulatory roles in the embryos of the first metazoans. These works will probably now trigger an explosion of studies on the role of developmental genes in sponge development.

**Fig. 10:** The basal-apical and posterior-anterior axis of sponges on different stage of its life cycle. **A.** *Haliclona* sp. from White Sea. **B.** Diagram of demosponges organization. **C, D.** Semi-thin micrograph (C) and diagram (D) of *Halisarca dujardini* (Halisarcida) rhagon. **E, F.** SEM micrograph (E) and diagram (F) of *Halisarca dujardini* (Halisarcida) disphaerula larva. The arrows indicate the basal-apical (A – D) and posterior-anterior (E, F) axis. Abbreviation: AP – anterior pole, CC – choanocyte chamber; Ep – exopinacoderm; O – osculum; PP – posterior pole. A – 2 cm; B – 250  $\mu$ m; C – 50  $\mu$ m; D – 50  $\mu$ m; E – 50  $\mu$ m; F – 50  $\mu$ m.



### Axis formation

An important characteristic distinguishing sponges from higher metazoans is the nature of body symmetry. Higher animals have two obvious body axes, anterior-posterior and dorsal-ventral, and are therefore bilaterally symmetrical (Bilateria). All young (rhagon or olynthus) and monoscular sponges, by contrast, have a single overt axis (apical-basal) defined by the presence of an osculum at one end (Fig. 10 A-D). The question is: **Does the apical-basal axis of an adult sponge correspond to the posterior-anterior axis of higher animals?** Since the larvae of all the sponges investigated also possess an apical-basal axis, the answer to this question may be yes (Ereskovsky 2005) (Fig. 10E, F).

### Development: phylogeny and evolution

The following question is very important in this respect: **Can embryological data be applied to sponge phylogeny and evolution?**

According to the paraphyletic hypotheses, based on molecular data, Porifera consists of four groups: Calcarea, Hexactinellida, Demospongiae and Homoscleromorpha (Borchellini *et al.* 2001, 2004). I proposed seven

developmental types in recent Porifera: I - “trichimella” (Hexactinellida); II - “calciblastula” (Calcinea); III - “amphiblastula” (Calcaronea); IV - “cinctoblastula” (Homoscleromorpha); V - “disphaerula” (Halisarcida); VI - direct development (Tetilla, Spirophorida); VII - “parenchymella” (Ereskovsky 2004).

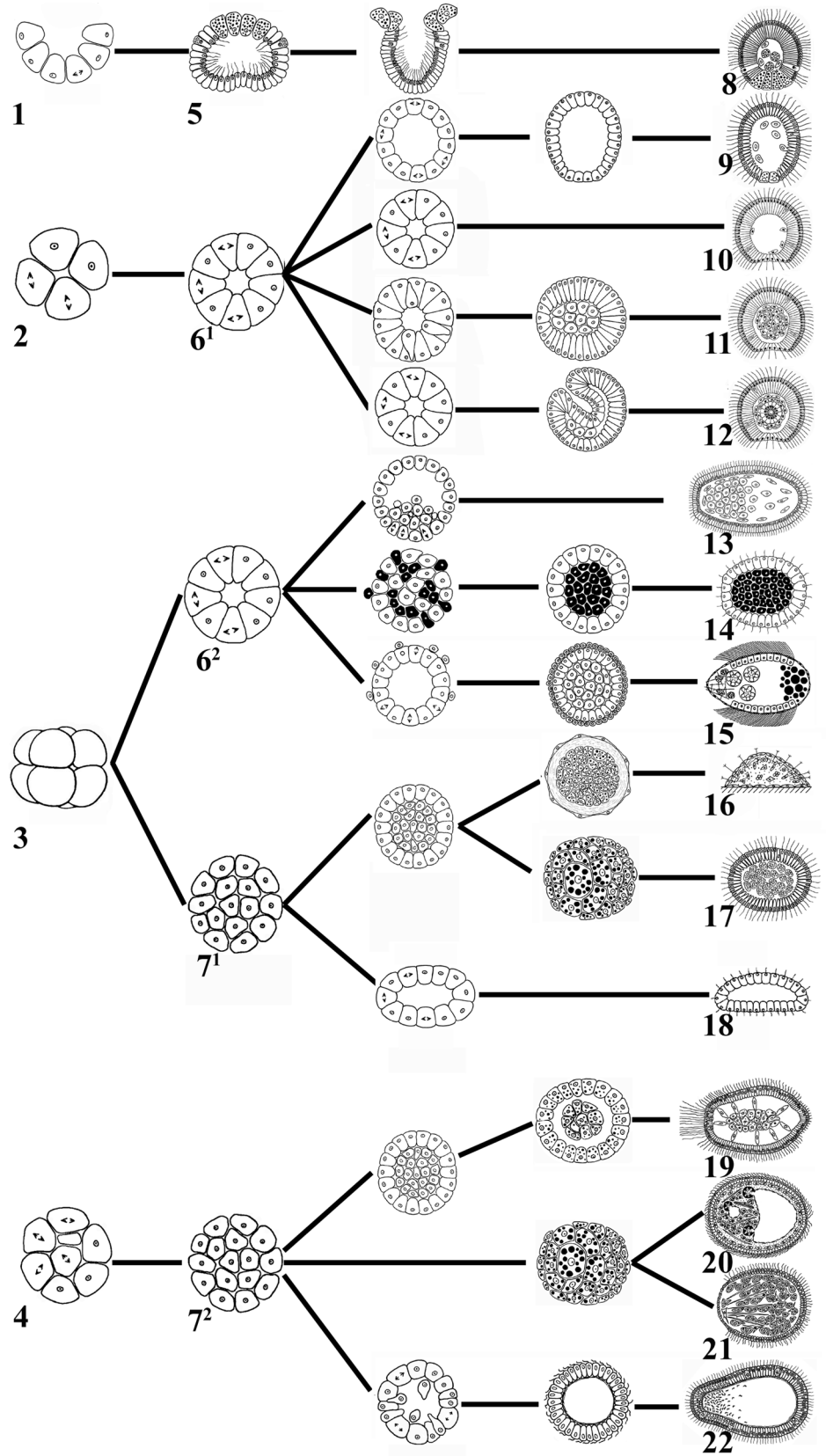
There are four principal cleavage patterns in sponges: incurvational, polyaxial, radial and chaotic (Fig. 11: 1-4). They result in formation of three main types of blastula: stomoblastula, coeloblastula and stereoblastula (Fig. 11: 5-7). The latter two types are derived from different cleavage patterns. Different embryonic morphogeneses lead to 8 or 9 larval types (Fig. 11: 8-22).

Difficulties of using embryological data for phylogenetic interpretation of Porifera are associated with a high degree of polymorphism of their development. The same cleavage pattern and blastula type may be characteristic of several different larval types. For example, radial cleavage, resulting in coeloblastula and stereoblastula, leads to parenchymella, trichimella, direct development, and coeloblastula (Fig. 11: 3-6<sup>2</sup>-13, 14, 15; 7<sup>1</sup>-16, 17, 18).

On the other hand, the same larval type may be the result of different cleavage patterns and modes of morphogenesis.



**Fig. 11:** Diagram of sponges cleavage and morphogenesis, leading to the larvae. 1-4 - Cleavage patterns in sponges: incurvational (1), polyaxial (2), radial (3), and chaotic (4). Three main form of sponges blastula: stomoblastula (5), coeloblastula (6), and stereoblastula (7). Different larval types of sponges: amphiblastula of *Calcareonea* (*Calcarea*) (8); calciblastula of *Calcinea* (*Calcarea*) (9); coeloblastula (10), parenchymella (11), and disphaerula (12) of *Halisarca* (*Halisarcida*); parenchymella of *Vaceletia crypta* (*Verticillitida*) (13); pseudoblastula of *Chondrosia reniformis* (*Chondrosida*) (14); trichimella of *Oopsacas minuta* (*Hexactinellida*) (15); juvenile of *Tetilla* under direct development (16); parenchymella of *Tethya aurantium* (Pallas, 1766; *Hadromerida*) (17); coeloblastula of *Polymastia robusta* Bowerbank, 1866 (*Hadromerida*) (18); parenchymella of *Dictyoceratida* (19); parenchymella of freshwater *Haplosclerida* (20); parenchymella of *Poecilosclerida* (21); cinctoblastula of *Homoscleromorpha* (22).



For example, parenchymella may originate from polyaxial, radial and chaotic cleavage, using different modes of morphogenesis (Fig. 11: 11, 13, 17, 19-21). Coeloblastula larva can result from polyaxial (Halisarcida and Calcinea) as well as radial cleavage (Hadromerida), through coeloblastula or stereoblastula stages (Fig. 11: 9, 10, 18).

The fact that similar characters can result from different developmental pathways means that ontogenetically earlier stages can be evolutionarily altered. The opposite case showing early similarity with later occurring differences is more common. However, both aspects taken together reveal that in the course of evolution developmental stages may be altered at all levels, from the molecular to the morphogenetic, regardless of whether a stage occurs early or late during the ontogenetic process.

Results of the comparative analysis of the cleavage and embryonic morphogenesis testify that these characters taken separately cannot form a basis for phylogenetic constructions within the Porifera. For example, Calcinea (Calcarea) embryogenesis (Fig. 11: 2-6<sup>l</sup>-9) is much closer to the development of Halisarcida (Demospongiae) (Fig. 11: 2-6<sup>l</sup>-10) than to Calcaronea (Fig. 11: 1-5-8).

Thus, a variety of cleavage patterns, types of blastulae and morphogenesis, leading to larvae formation in sponges, does not allow making a conclusion about certain linear ways of developmental evolution for all Porifera. It testifies to an early divergence of sponge macrogroups or, more likely, paraphyly and their long parallel evolution.

## The future

We are now on the threshold of the **fourth** period of sponge developmental studies. To enhance our knowledge on this topic, the following steps are currently necessary:

- To select some model sponge species with different types of development;
- To investigate their development from egg to juvenile at ultrastructural level;
- To decode morphogenetic mechanisms of development using ultrastructural and molecular methods.

Studies of cellular and molecular basis of embryonic morphogenesis in sponges will provide answers to the following important questions:

- What is the role of intercellular contacts and cytoskeleton dynamics in embryonic and postembryonic morphogenesis?
- What is the role of cell-cell and cell-extracellular matrix interactions?
- Are integrins, laminins, and signaling molecules involved in development the same in sponges and other metazoans?
- Which “developmental genes” work during sponge embryonic development?
- Does the apical-basal axis of adult sponge correspond to the anterior-posterior axis of higher animals?

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