Embryogenesis and larval differentiation in sponges

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REVIEW / SYNTHÈSE

Embryogenesis and larval differentiation in sponges[1]

S.P. Leys and A.V. Ereskovsky

Abstract: Having descended from the first multicellular animals on earth, sponges are a key group in which to seek innovations that form the basis of the metazoan body plan, but sponges themselves have a body plan that is extremely difficult to reconcile with that of other animals. Adult sponges lack overt anterior–posterior polarity and sensory organs, and whether they possess true tissues is even debated. Nevertheless, sexual reproduction occurs as in other metazoans, with the development of embryos through a structured series of cellular divisions and organized rearrangements of cellular material, using both mesenchymal and epithelial movements to form a multicellular embryo. In most cases, the embryo undergoes morphogenesis into a spatially organized larva that has several cell layers, anterior–posterior polarity, and sensory capabilities. Here we review original data on the mode of cleavage, timing of cellular differentiation, and the mechanisms involved in the organization of differentiated cells to form the highly structured sponge larva. Our ultimate goal is to develop interpretations of the phylogenetic importance of these data within the Porifera and among basal Metazoa.

Résumé : Descendants des premiers animaux multicellulaires de la terre, les éponges sont un groupe charnière pour la recherche des innovations qui forment la base du plan du corps des métazoaires, même si les éponges elles-mêmes possèdent un plan de corps qui est extrêmement difficile à réconcilier avec celui des autres animaux. Les éponges adultes ne possèdent pas de polarité avant–arrière évidente, ni d'organes sensoriels; il y a même un débat pour décider si elles possèdent de vrais tissus. Néanmoins, elles ont une reproduction sexuelle comme les autres métazoaires et leurs embryons se développent par une série structurée de divisions cellulaires et par un réarrangement méthodique du matériel cellulaire avec des déplacements à la fois du mésenchyme et de l'épithélium pour former un embryon multicellulaire. Dans la plupart des cas, l'embryon subit une morphogenèse qui produit une larve organisée dans l'espace qui possède plusieurs couches de cellules, une polarité antérieure–postérieure et des capacités sensorielles. Notre rétrospective examine des données inédites sur le mode de clivage, le calendrier de la différentiation cellulaire et le mécanisme impliqué dans l'organisation des cellules différentiées pour former la larve très hautement structurée d'éponge. Notre objectif final est de trouver des interprétations de l'importance phylogénétique de ces données dans le cadre des porifères et des métazoaires primitifs.

[Traduit par la Rédaction]

General introduction

Despite the fact that molecular data now confirm that the Metazoa, including sponges, is monophyletic (Cavalier-Smith and Chao 1995; Woollacott and Pinto 1995; Cavalier-Smith et al. 1996; Borchiellini et al. 2001; Medina et al. 2001; Snell et al. 2001; King et al. 2003), it is difficult to compare the unusual sponge body plan with that of other metazoans. This is where studies of development can help. The discovery of genes that are implicated in regulating anterior–posterior polarity and in specifying particular tissues during the development of other basal metazoans (Finnerty and Martindale 1998; Scholz and Technau 2003) has revived interest in comparative embryology in the Porifera and the debate of homology of cell or tissue layers among basal metazoans (Haeckel 1874). Molecular techniques are still in their infancy in the Porifera with the first gene expression patterns just being generated (Adell et al. 2003*a*, 2003*b*; Degnan et al. 2005, Larroux et al. 2006), but the attention on sponges as the most basal metazoan phylum, as well as suggestions that the Porifera may be paraphyletic (Kruse et al. 1998; Zrzavy et al. 1998; Borchiellini et al. 2001; Medina et al. 2001), have stimulated renewed investi-

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[1]This review is one of a series dealing with aspects of the biology of the phylum Porifera. This series is one of several virtual symposia on the biology of neglected groups that will be published in the Journal from time to time.

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gation and interpretation of embryogenesis of key groups of Porifera — Hexactinellida, Homoscleromorpha, Halisarcida, and Calcarea — and the development of model systems for molecular studies.

In general, much more is known about the development of viviparous sponges than of oviparous sponges. Even though brooded embryos are often difficult to encounter in sponges, many fewer species are oviparous and reports of spawning are rare. In this respect, our understanding of development in the Porifera differs from that of most other metazoan phyla, where in many groups development has been studied in vitro for over a century. How many sponges are oviparous is not exactly clear, but knowledge of viviparity is certainly biased by the relative abundance and easy access in littoral waters of sponges that readily release larvae in warmer summer months and by the often large size and robustness of these larvae. A quick survey of reproductive modes across the Porifera shows that all glass sponges (Hexactinellida) found to date are viviparous, as are calcareous sponges (Calcarea), and most oviparous sponges are currently grouped within the tetractinomorph Demospongiae.

Because of the key position occupied by sponges in the evolution of the Metazoa, many previous reviews have focused on the relevance of sponge developmental modes to basal metazoan phylogeny (Lévi 1963; Tuzet 1963; Borojevic 1970, 1979; Efremova 1997; Ereskovsky 2004, 2005; Leys 2004; Maldonado 2004). However, the original data are dispersed and sometimes difficult to obtain and such reviews often expect the reader to already possess knowledge of this literature to be able to understand the inferred relationships. Here we have decided to take a broader approach by providing readers with summaries of the original data on embryogenesis and larval development in each of the three main classes of sponges (Hexactinellida, Demospongiae, and Calcarea) and from each of the three main groups within the demosponges (Homoscleromorpha, Tetractinomorpha, and Ceractinomorpha). We follow the *Systema Porifera* (Hooper and van Soest 2002) in higher level classification, but in a final section we address the implications that comparative embryology and molecular developmental biology have for systematics within the Porifera and for concepts of gastrulation and homology of the germ layers among basal metazoans.

Hexactinellida

Because of the preferred deep-water habitat of glass sponges, knowledge of reproductive period, gametogenesis, and embryogenesis stems from very few species. Reproductive stages were reported in *Euplectella aspergillum* Owen, 1841 (Schulze 1880) and *Euplectella marshalli* Ijima, 1895 (Ijima 1901), but the most complete descriptions of development derive from only three species — two (*Vitrollula fertilis* Ijima, 1898 and *Farrea sollasii* Schulze, 1886) dredged by Japanese workers in the late-19th and early-20th centuries (Ijima 1901; Okada 1928) and one (*Oopsacas minuta* Topsent, 1927) that fortuitously was discovered inhabiting a submarine cave within reach by SCUBA in southern France (Boury-Esnault and Vacelet 1994; Boury-Esnault et al. 1999; Leys et al. 2006). Multiple collections for long-term studies have only been carried out with *F. sollasii* and *O. minuta*, and, whereas *F. sollasii* and *O. minuta* are reproductive year round, *V. fertilis* was found to be seasonally reproductive in early summer.

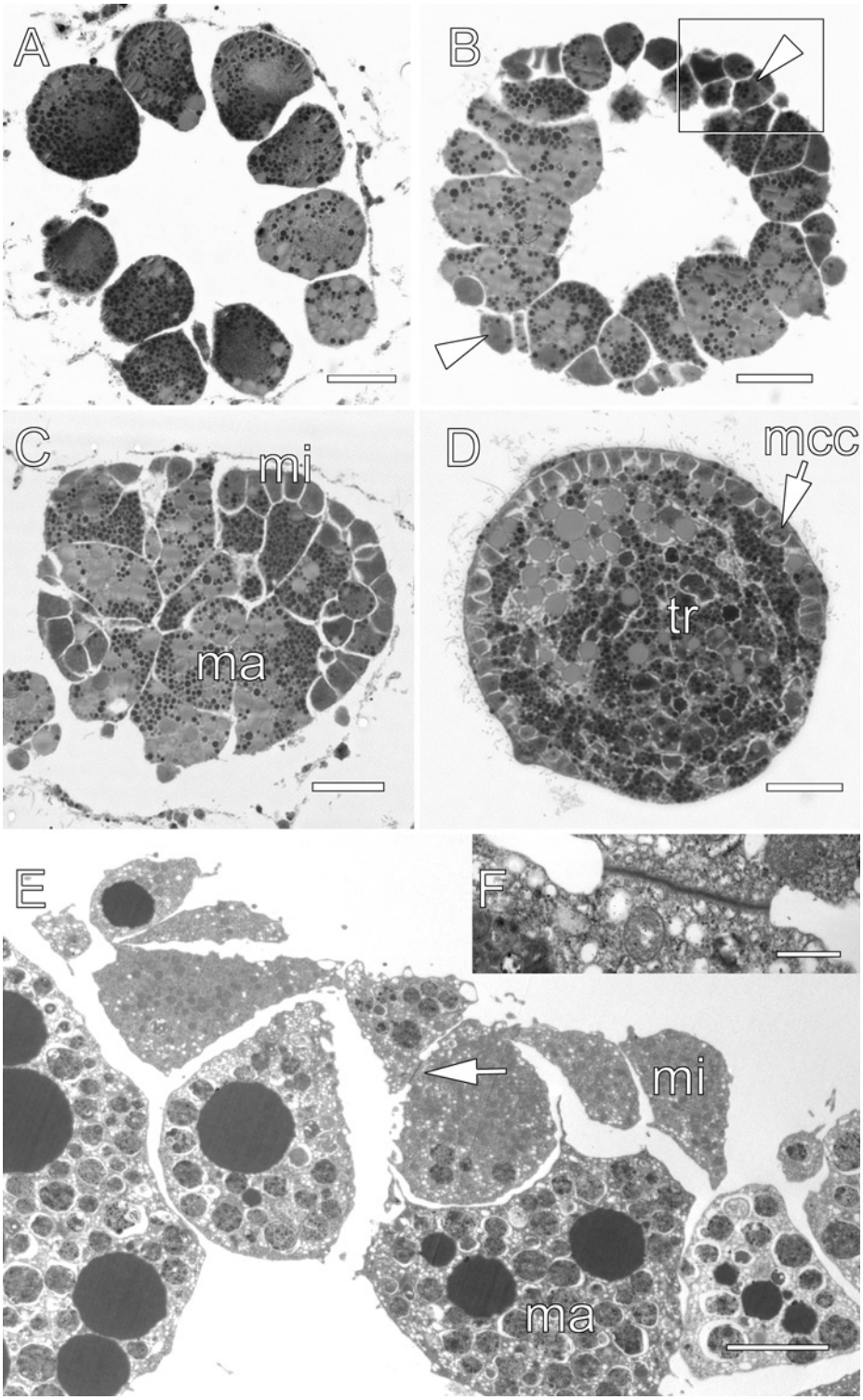
In all three species, gametes arise from groups of archaeocytes (called congeries) that lie suspended within the trabecular reticulum, a syncytial tissue that forms the bulk of the adult sponge. Spermatocysts in *O. minuta* are up to 30 μm in diameter; young sperm are round to ovoid, each has a long flagellum that coils two or three times around the cell and each cell is connected to others by cytoplasmic bridges. Oocytes in *O. minuta* and *F. sollasii* begin as a single archaeocyte that grows larger by the incorporation of lipid and yolk material presumably from neighbouring archaeocytes. All archaeocytes within a congery are connected by cytoplasmic bridges; however, the mature oocyte is an independent ovoid cell that is 100–120 μm long in *O. minuta* (Boury-Esnault et al. 1999) and 70–130 μm long in *F. sollasii* (Okada 1928). It is unknown how sperm find the oocytes or how fertilization occurs.

The following description of embryogenesis comes from *O. minuta* (Boury-Esnault et al. 1999; Leys et al. 2006). Cleavage up until the 5th cycle or 32-cell stage is total, equal, and asynchronous. The first cleavage is generally meridional and the second either equatorial or rotational. Daughter blastomeres lie above the previous cleavage plane and by the 16- and 32-cell stage the embryo is a hollow blastula (Figs. 1A, 1B, 1E, 1F). The next cleavages are unequal, producing smaller cells (micromeres) that lie on the outside and larger lipid-rich and yolk-rich cells (macromeres) on the inside. However, even the earliest micromeres are connected to one another by cytoplasmic bridges. The macromeres divide unequally, gradually filling the centre of the blastocoel, and at the same time, they envelop the micromeres with massive filopodia (Fig. 1C; Leys et al. 2006). Most unusually, these cells then fuse to form a single multinucleated giant cell, the new trabecular syncytium, which completely envelops the micromeres. In this way the embryo gains its outer epithelium (Fig. 1D). As epithelialization takes place, the new syncytial tissue also forms cytoplasmic bridges with each micromere; all of the cytoplasmic bridges are plugged with the characteristic osmiophillic "plugged junction" of glass sponges (Leys 2003).

The formation of micromeres was originally considered to represent gastrulation by cellular delamination (Boury-Esnault et al. 1999; see discussion under Gastrulation on p. 281). Another interpretation, however, is that epithelialization of the larva — the enveloping of the micromeres by the incipient trabecular reticulum — could be considered the true gastrulation event (Leys et al. 2006). Clearly, the concept of gastrulation in an animal that forms syncytial tissues is intriguing, and merits further investigation with molecular techniques to seek expression patterns of genes involved in gastrulation processes in other animals, as well as to study of the fate of larval tissues during metamorphosis.

Although the whole embryo is cytoplasmically connected from very early on, there are, as in adult sponges, uninucleate regions that are joined to the syncytial tissue by plugged cytoplasmic bridges. Because these uninucleate regions are thought to function independently, they are referred to as cells, as in the adult. Cellular differentiation

Fig. 1. Embryogenesis in the hexactinellid *Oopsacas minuta*. (A–F) Stages in morphogenesis. The embryo begins as a hollow cellular blastula (A). At the 5th cleavage stage (B), micromeres (arrowheads) are formed at the periphery of the embryo (boxed region is shown in E and F). (C, D) Large central macromeres (ma) fuse to form a giant syncytium, the trabecular reticulum (tr), of the future larva and adult sponge. (E) Micromeres are connected to macromeres by cytoplasmic bridges (arrow) with the characteristic hexactinellid plugged junction (F, inset). Scale bars: A–D, 20 μm; E, 5 μm; F, 0.5 μm. (S.P. Leys, unpublished data.)



begins soon after the micromeres are formed. Some of the first micromeres to form possess cilia. These cells are connected to macromeres by cytoplasmic bridges, and when the macromeres fuse to form the syncytial tissue, they remain connected to the syncytial tissue as they move to the equator of the embryo where they form a belt of cells whose cilia project out through the new epithelium. Other micromeres are non-ciliated, and soon after epithelialization these cells

become localized at the posterior-central region of the embryo where they differentiate into sclerocytes and choanocytes. Sclerocytes are amoeboid cells with numerous pseudopodia and a central vacuole that contains a distinct square proteinaceous axial filament around which the spicule is formed (Leys 2003). As the spicule elongates, the sclerocyte extends through the length of the larva, becoming multinucleate in the process. At all times, sclerocytes are connected to the syncytial trabecular tissue by plugged cytoplasmic bridges. Choanocytes are also connected to the trabecular tissue by cytoplasmic bridges. Early choanocytes are uninucleate and have a flagellum surrounded by a collar of microvilli, but in late larvae, choanocytes become branched so that one nucleated cell gives rise to several collar bodies at the end of long cytoplasmic bridges, as in the adult sponge. The last cell type to differentiate — a spherulous cell, or cell with inclusions — is the only cell type that appears to lack cytoplasmic bridges with the syncytial trabecular tissue. Thus, throughout early development, this unusual embryo manages to rearrange cellular regions that are tethered to the multinucleate syncytium via cytoplasmic bridges. Membrane continuity is maintained throughout the entire embryo and thus there are no typical cells, or cell–cell junctions.

The fully differentiated glass sponge larva is called a trichimella. It is approximately 100 μm long in *O. minuta* (Boury-Esnault and Vacelet 1994), 70–130 μm long in *F. sollasii* (Okada 1928), rounded at the anterior pole, and conical at the posterior pole (Figs. 2A, 2C). The major tissue in the larva is the syncytial trabecular reticulum, which is continuous throughout the whole larva and forms the bulk of the inner mass of both anterior and posterior poles, as well as the surface epithelium of the whole larva (Figs. 2B, 2D, 2E). The trabecular reticulum contains massive lipid-dense inclusions at the anterior pole and small yolk inclusions at the posterior pole. Multiciliated cells form a belt around the equator of the larva, their cilia pierce or are surrounded by the syncytial eptithelium, and their cell bodies are connected to one another and to the trabecular tissue above and below by plugged cytoplasmic bridges. Strands of the unusual cobweb-like trabecular syncytium are separated from one another by a thin collagenous extracellular matrix. The complex nature of this reticular tissue can be appreciated by the fact that when the larva is dissociated membranes of separate pieces readily fuse; presumably, fusion can also occur in an organized and regulated fashion during embryogenesis.

In *F. sollasii*, 12 oxystauractin spicules are formed by sclerocytes that traverse the entire length of the larva (Okada 1928). Microscleres — discohexasters — are formed by sclerocytes that lie just under the multiciliated cells at the posterior pole. The *O. minuta* larva has 14 stauractin spicules and no microscleres (Fig. 3 in Leys 2003). The larva swims out of the parent osculum with rounded, lipid-filled anterior pole forward, rotating in a right-handed direction. Larvae swim for 12–24 h before settling on the anterior, lipid-filled pole and undergoing metamorphosis.

Demospongiae

Homoscleromorpha

The members of the subclass Homoscleromorpha, of which there are only seven genera, have long been considered the most primitive representatives of Porifera owing to their simple anatomic organization (Lévi 1956, 1970). Lévi and Porte (1962) considered *Oscarella lobularis* (Schmidt, 1862) to be the prototype of sponges because of its reduced mesohyl. These sponges appear to consist of only two epithelia (choanoderm and pinacoderm), most species lack a fibrous skeleton, and none has a mineral skeleton. Recently, however, it has been shown that homoscleromorph species share characters that are common to eumetazoans and absent in other poriferan clades. The presence of both an acrosome in spermatozoids (Baccetti et al. 1986; Boury-Esnault and Jamieson 1999), and a basal lamina with type IV collagen in adult tissue (Boute et al. 1996) and in the larvae (Boury-Esnault et al. 2003), suggest a higher level of evolutionary complexity than that expected from the simple organization in this subclass. This view is now confirmed by molecular data which suggest that the Homoscleromorpha are a clade that has no clear relationship with any Demospongiae (Borchiellini et al. 2004).

At the end of 19th century, the embryology of *O. lobularis* (currently known as *Oscarella tuberculata* (Schmidt, 1868)) was studied extensively to attempt to determine whether the simplicity of the adult tissue organization might represent the prototype pattern of development not only for the Porifera, but also for the whole Metazoa (Barrois 1876; Schulze 1877; Metschnikoff 1879; Heider 1886). Two early studies concern the reproduction and the larval structure of homoscleromorphs (Meewis 1938; Tuzet and Paris 1964). Ultrastructural studies have been limited to the description of the mature larva (Lévi and Porte 1962), egg development (Gaino et al. 1986), and spermatogenesis (Baccetti et al. 1986; Gaino et al. 1986). More recently, the early development from the egg to the coeloblastula stage in five species of the genus *Oscarella* (Ereskovsky and Boury-Esnault 2002) and the embryogenesis from coeloblastula to larva in eight species of Homoscleromorpha (Boury-Esnault et al. 2003) were described.

The homoscleromorph larva was originally called an "amphiblastula" because it was thought to possess two fairly distinct regions, one ciliated and the other not. However, morphogenesis in sponges of this group is not at all like that of sponges in the Calcaronea (a group for which the amphiblastula larva is characteristic), and the larvae are in fact completely ciliated (Borojevic 1970; Boury-Esnault et al. 2003). To avoid confusion between both types of larva, it was recently suggested that the homoscleromorph larva be called a "cinctoblastula", a term which more accurately describes the distinctive feature of the larva — cells with paracrystalline intranuclear inclusions that form a belt around the posterior pole (Boury-Esnault et al. 1995, 2003).

In homoscleromorph species, mature oocytes are ovoid and are located in the basal or central parts of the choanosome. The eggs are isolecithal, rich in yolk inclusions, and are completely surrounded by a follicle formed of parental endopinacocytes. Cleavage is holoblastic, equal, and synchronous during the first two divisions (Barrois 1876; Meewis 1938; Ereskovsky and Boury-Esnault 2002). At the third division, cleavage becomes irregular, asynchronous, and results in a solid morula of undifferentiated still yolk-rich blastomeres (Fig. 3A). When the embryo has

Fig. 2. Larvae of the hexactinellid *O. minuta*. (A, B) Longitudinal sections of the trichimella larva showing a rounded lipid-filled (li) anterior pole (ap) and pointed yolk-filled (y) posterior pole (pp). The trabecular reticulum (tr) extends throughout the larva. Young flagellated chambers (fch) lie in the mid-posterior section; the larva swims using multiciliated cells (mcc) whose many cilia pierce the syncytial epithelium. (C–E) Scanning electron micrographs of whole (C) and fractured larvae (D, E) show that the surface epithelium (e) is completely smooth (no cell boundaries) and is continuous with the underlying trabecular reticulum. (D) A fracture through the equator of a larva showing a flagellated chamber (fch) among the trabecular recticulum (tr); multiciliated cells (mcc) lie at the periphery under the syncytial epithelium (e); spicules (sp). (E) The cilia of two multiciliated cells (mc) at the equator of the larva project through the syncytial epithelium (e). Scale bars: A, 20 μm; B–D 10 μm; E, 2 μm. (S.P. Leys, unpublished data.)

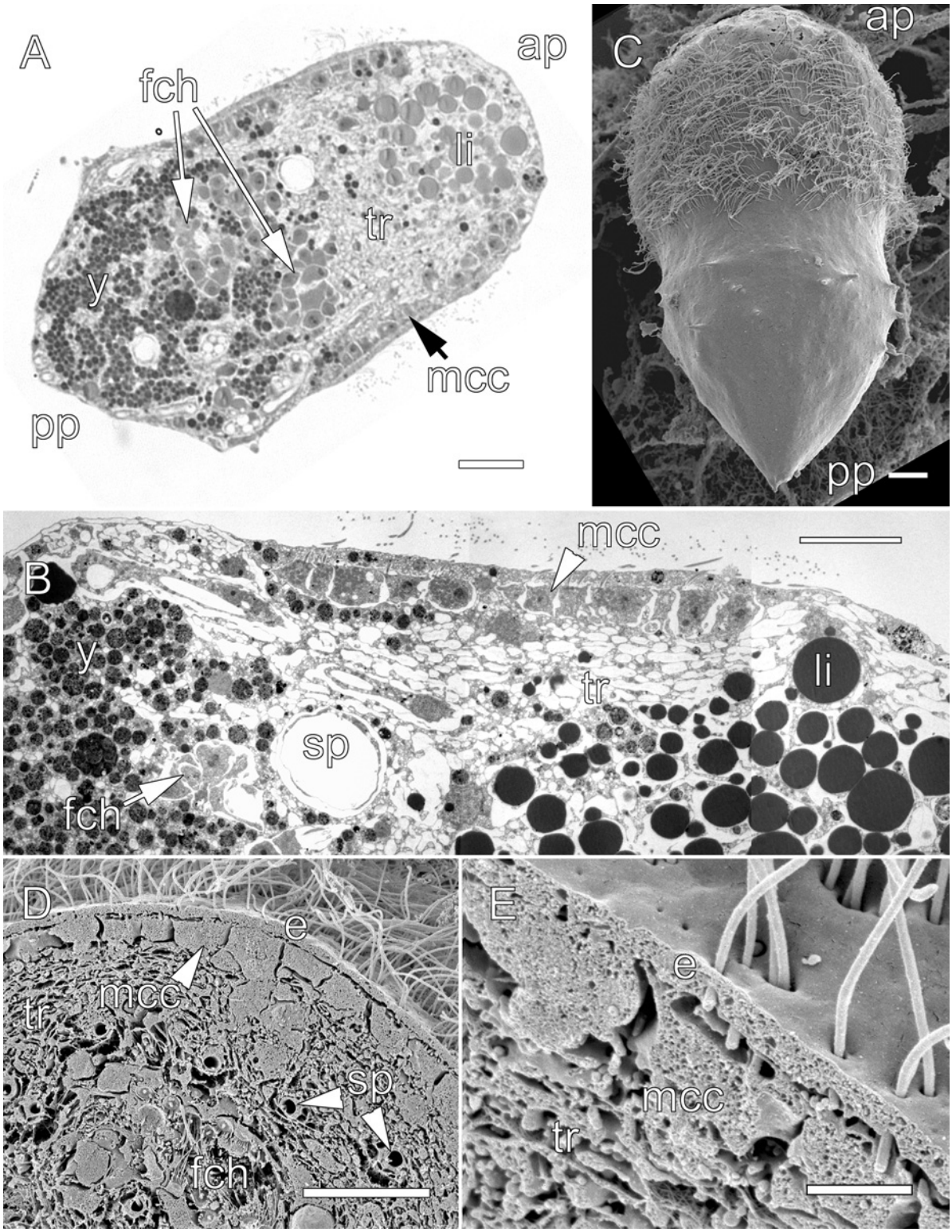
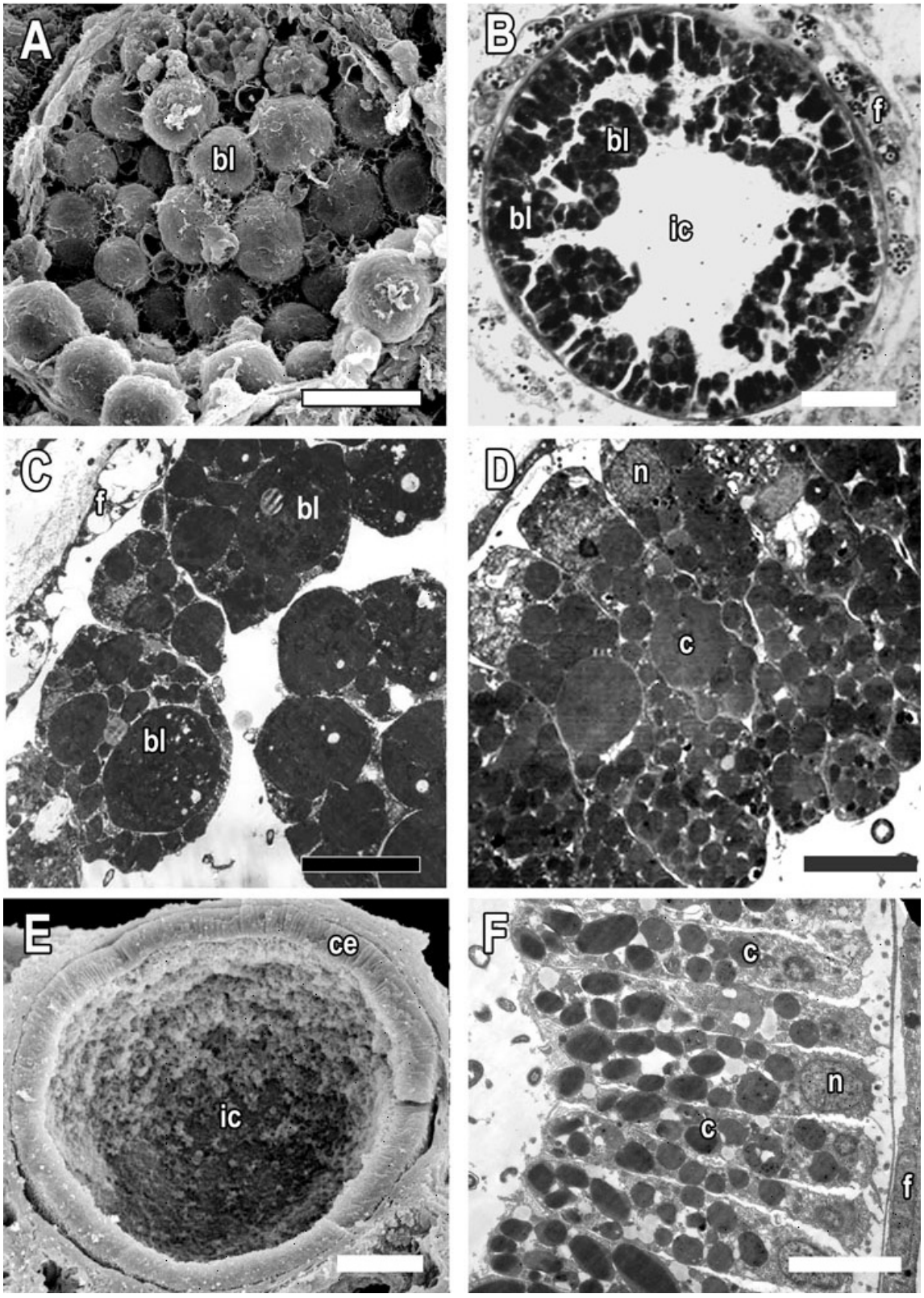


Fig. 3. Early development in homoscleromorph sponges. (A) Scanning electron micrograph (SEM) of the morula of *Oscarella lobularis*. (B) Thick plastic section showing multipolar egression in the embryo of *Oscarella microlobata*. (C, D) Transmission electron micrographs (TEM) showing successive stages of multipolar egression in *Oscarella tuberculata*. (E) SEM of the coeloblastula larva of *Oscarella lobularis*. (F) TEM of the ciliated cells of the coeloblastula of *Plakina trilopha*. bl, blastomere; c, ciliated cell; ce, ciliated epithelium; f, follicle; ic, internal cavity; n, nucleus. Scale bars: A, 24 μm; B, 25 μm; C and D, 10 μm; E, 25 μm; F, 5 μm. (A.V. Ereskovsky, unpublished data.)



approximately 64 cells, the outer epithelium begins to differentiate. The blastomeres close to the surface of the morula divide more actively, while the internal cells migrate to the periphery of the embryo and gradually form a monolayered larva with a central cavity that contains only symbiotic bacteria (Figs. 3B–3D). The resulting embryo is a hollow coeloblastula (Figs. 3E, 3F). The cilia begin to develop as the epithelium differentiates.

The centrifugal migration of cells from the centre to the periphery of a morula to form a coeloblastula is unique not only within the Porifera but also in the Metazoa and has been termed multipolar egression (Ereskovsky and Boury-Esnault 2002). The long columnar cells of the larval epithelium are closely linked by septate-like junctions. At the apical side of the cell, the intercellular space is approximately 14–22 nm and contains electron-opaque material; the cytoplasmic side of the cell membrane contains electron-opaque filamentous material (Fig. 4A). Below these apical junctions, other specialized junctions appear as regularly spaced membrane protrusions (Fig. 4B) (Boury-Esnault et al. 2003). Collagen fibrils fill most of the internal cavity of the developing larva; however, immediately below the epithelial cells, the extracellular matrix forms a tough mat of collagen fibrils, and beneath this layer, there is a loose net of collagen fibrils (Figs. 4C, 4D). These two layers of collagen constitute a basement membrane similar to the one found below the pinacoderm and choanoderm of the adult (Boury-Esnault et al. 1984). The presence of a highly defined basement membrane is not well documented in the Porifera, but a similar network of extracellular matrix is present at the base of the larval epithelium of at least one other demosponge larva (Maldonado 2004). The larval epithelium of homoscleromorphs, thus being a true columnar epithelium, could be considered homologous to the eumetazoan epithelium — the feltwork of collagen fibrils underlying the cells have been interpreted here as a basal lamina and the loose net of collagen fibrils below as a lamina reticularis (Boury-Esnault et al. 2003).

Throughout larval morphogenesis, cells actively proliferate. The multiplication of cells in the larval epithelium causes the larval surface to expand, but because the space to develop inside the parent follicle is limited, the epithelium becomes folded. These folds can be very numerous, giving larvae a highly convoluted appearance similar to the folds seen in some haplosclerids (Leys and Degnan 2002). As development continues, the whole central cavity is progressively filled by collagen fibrils. During the last stages of development, the larval epithelium becomes regionally differentiated. It consists of three types of ciliated cells distributed in the anterolateral, posterolateral, and posterior regions (Boury-Esnault et al. 2003). Some non-ciliated cells lie scattered among the ciliated cells without specific regional localization.

The free-swimming cinctoblastula larva is ovoid or pear-shaped, wider at the anterior pole than at the posterior pole, and the whole surface is ciliated (Figs. 4E, 4F). Cinctoblastulae are 150–200 µm long in *O. tuberculata*, are 220–260 µm long in *Corticium candelabrum* Schmidt, 1862, and are generally pigmented at the posterior pole (Boury-Esnault et al. 2003). The larva has a large central cavity that contains extracellular matrix and symbiotic bacteria, the latter being more abundant in the posterior region. All larvae for which observations are available rotate in a left-handed direction, i.e., in a clockwise direction when swimming forward and viewed from the anterior pole (Boury-Esnault et al. 2003). Figures 5A–5F diagram the whole sequence of embryogenesis in homoscleromorph sponges.

Tetractinomorpha

Reproduction in oviparous tetractinomorphs is very poorly known. Studies that follow the full progression of embryogenesis have been carried out on only one or two species within each family. Most information stems from studies on the development of representatives from three groups: Chondrosida (*Chondrosia reniformis* Nardo, 1847 and *Chondrilla australiensis* Carter, 1873), Spirophorida (*Tetilla japonica* Lampe, 1886 and *Tetilla serica* (Lebwohl, 1914)), and Hadromerida (*Polymastia robusta* (Bowerbank, 1861)). However, eggs and spermatocysts have been observed in many individuals (Nassonow 1883; Lévi 1956; Warburton 1961; Diaz et al. 1973; Diaz and Connes 1980; Sciscioli et al. 1991; Rosell 1993; Gaino and Sara 1994; Witte 1996; Usher et al. 2004).

Chondrosida: Chondrillidae

Chondrosia reniformis is a common subtidal sponge in the Mediterranean that is best known for its lack of a skeleton and a rather unusual ability to modify the stiffness and adhesive properties of its tissues (Bavestrello et al. 1998). Lévi and Lévi (1976) carried out the most detailed study on development at Banyuls-sur-Mer. *Chondrosia reniformis* is oviparous. Oocytes are found between May and September; 25%–30% of specimens have gametes in July, August, and into September. The mature oocyte lies within the mesohyl, where it is surrounded by follicular cells. Gametogenesis appears to be triggered by the lunar cycle because spawning of sperm — even in specimens maintained in laboratory aquaria — was observed in two separate years on the day of the new moon. Sperm were released in a single continuous "jet" at midday for 3 h. Release of oocytes has not been observed, but fertilized eggs were found on the same day and the first day of the first quarter moon.

Oocytes are highly adhesive after release and tend to aggregate and stick to the parent sponge, as well as to other substrates. Each oocyte is surrounded by a thick layer of follicle cells so that the entire diameter of oocyte and follicle sheath is approximately 90 µm. Spawned oocytes are also surrounded by a layer of sperm, which suggests that fertilization occurs outside the parent sponge (Lévi and Lévi 1976).

Larval development takes some 22–30 h after fertilization. After release, the egg is still quite small (40 µm) and is wrapped in a layer of follicle cells. Cleavage is total and equal, and continues until a solid morula of 32 cells is formed. Thereafter, however, the cells of the embryo become organized into a single epithelium, in essence forming a blastula; however, the central cavity is filled with bacteriocytes and small granular cells of the follicle layer. The larva gradually absorbs the outer follicle cells and the fully formed larva consists of a continuous ciliated epithelium surrounding a central cell mass of bacteriocytic and granular follicular cells. Furthermore, the outer epithelium forms two

Fig. 4. Details of the cinctoblastula larva of homoscleromorphs. (A) TEM of the apical parts of flagellated cells of the larva of *O. microlobata* showing two desmosome-like junctions (arrows). Scale bar: 1 μm. Inset, detail of a desmosome-like junction. Scale bar: 0.25 μm. (B) SEM of the cell junctions (arrows) in the middle part of ciliated cells (c) of a cinctoblastula of *P. trilopha*. Scale bar: 1.5 μm. (C, D) TEM and SEM, respectively, of the basement membrane (m) of larvae of *P. trilopha;* b, bacteria, c, ciliated cells, i, osmiophilic inclusions. Scale bars: C and D, 2 μm. (E) Longitudinal section through the free-swimming cinctoblastula of *Corticium candelabrum*. Arrows indicate the position of the posterolateral belt of cells with intranuclear paracrystalline inclusions; ap, anterior pole, pp, posterior pole. Scale bar: 50 μm. (F) SEM of the free-swimming cinctoblastula larva of *P. trilopha*; ap, anterior pole, pp, posterior pole. Scale bar: 50 μm. (A–C from Boury-Esnault et al. 2003, reproduced with permission of Invertebr. Biol., vol. 122, pp. 191 and 194, © 2003 Blackwell Publishing (http://www.blackwell-synergy.com).)

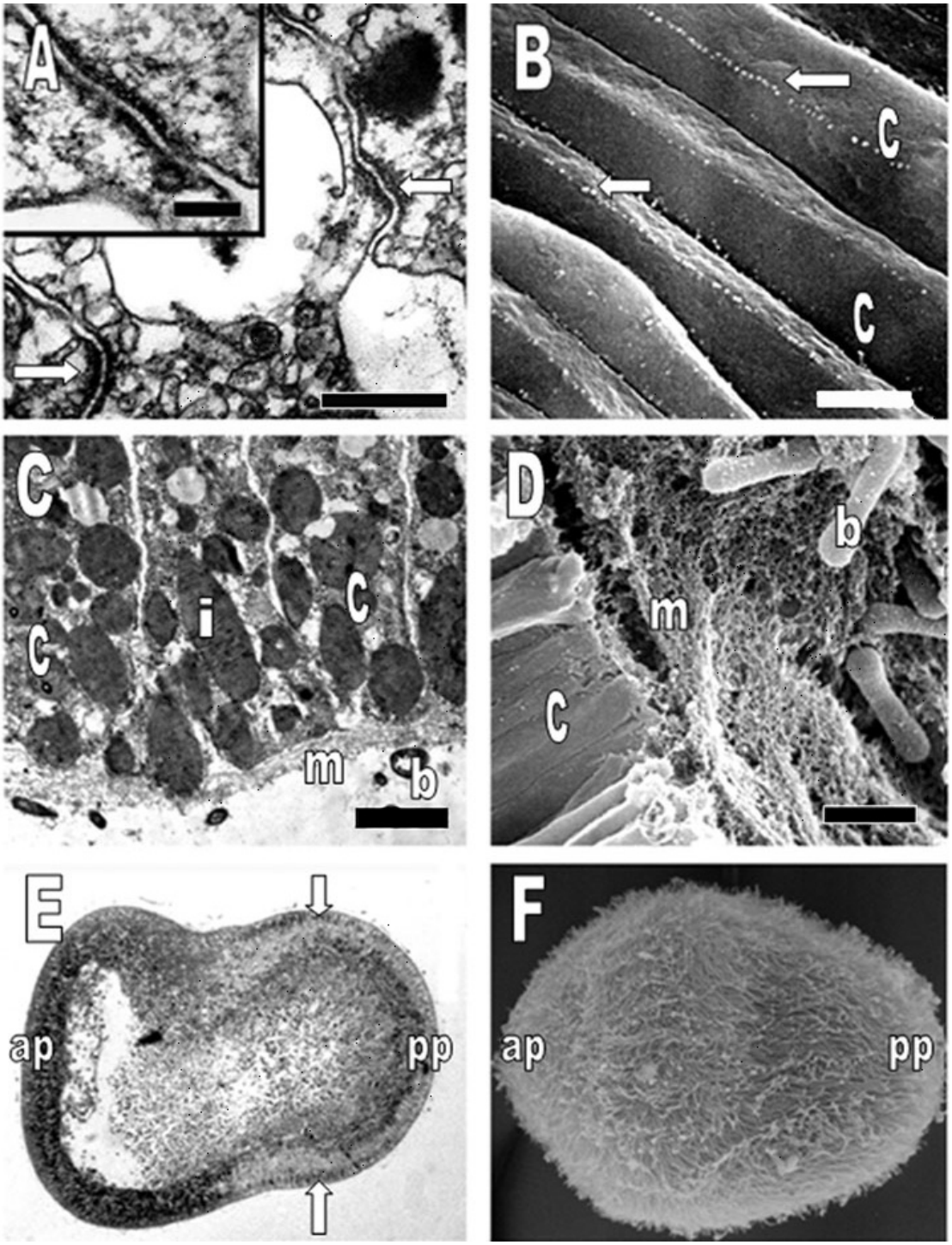
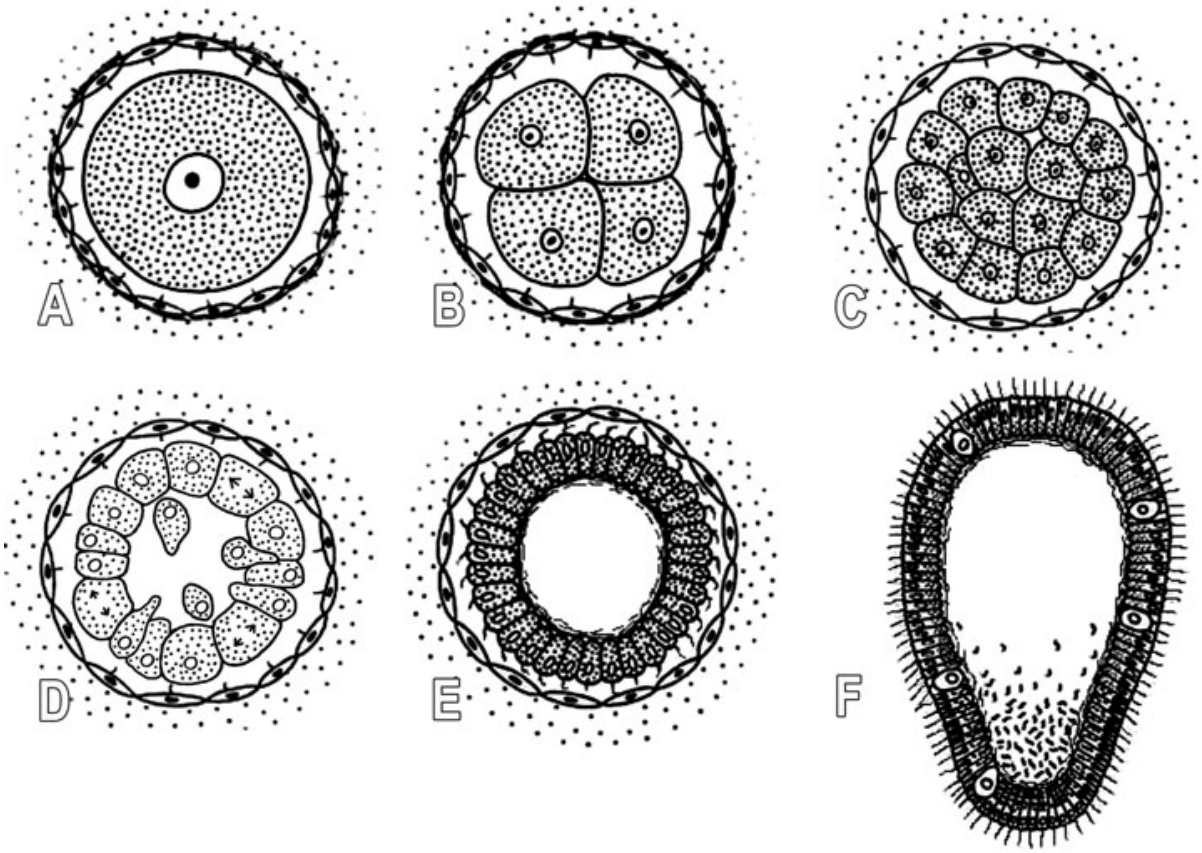


Fig. 5. Diagram of development in the Homoscleromorpha. (A) egg; (B) early cleavage; (C) morula; (D) multipolar egression; (E) coeloblastula; (F) cinctoblastula larva. (From Ereskovsky 2005, p. 191, reproduced with permission of Sanct-Petersburg University Press, © 2005.)



distinct regions: spherical ciliated cells cover the anterior two-thirds, while the cells of the posterior third are flattened ciliated cells (Lévi and Lévi 1976). The larva is considered a type of coeloblastula. It swims with the larger, anterior pole forward and rotates in a right-handed direction, i.e., counterclockwise when viewed from the anterior pole.

The recent description of gametes in *C. australiensis* (Usher et al. 2004) has provided an opportunity to compare development within the order Chondrosida. *Chondrilla australiensis* is an encrusting sponge that is often a dominant feature of tropical and temperate reefs around Australia. In Western Australia, *C. australiensis* spawns in late summer (February), and both internal and external fertilization appear to be possible. The zygote is surrounded by a sort of mucus coat. Its cytoplasm has two regions: the perinuclear region is packed with vacuoles and symbiotic bacteria, and the periphery is characterized by abundant yolk granules, lipid droplets, and a few phagosomes (Usher and Ereskovsky 2005).

Cleavage is holoblastic, equal, and synchronous during the two first divisions and results in the formation of a small morula. Further development gives rise to a hollow blastula with a small blastocoel. Continued cell division and differentiation results in an entirely ciliated coeloblastula that is approximately 75 µm long and 50 µm wide. Like *C. reniformis* larvae (Lévi and Lévi 1976), *C. australiensis* larvae are spherical to ovoid and possess a flattened posterior pole. The outer epithelium consists of a layer of columnar monociliated cells that surrounds a central cavity. This epithelium is differentiated regionally, but unlike in the *C. reniformis* larva, the cilia are longer at the posterior pole than in the anterolateral regions of *C. australiensis* larva (Usher and Ereskovsky 2005). The centre of the blastula has numerous symbiotic bacteria and cyanobacteria within a loose network of collagen fibres.

Release of eggs has also been recently reported from northern Mediterranean populations of *Chondrilla nucula* Schmidt, 1862 (Sidri et al. 2005). Choanosomal tissue of sponges collected in August was dark gray, with oocytes 50–60 µm in diameter. Specimens maintained in aquaria at 18–20 °C released oocytes in a white fluid 2 days after the full moon. Because cleavage was observed soon after the eggs were collected, it was considered that fertilization had occurred in the parent. Cleavage was asynchronous and holoblastic, but all embryos died within a few hours of observation, thereby preventing further study.

Spirophorida: Tetillidae

The genus *Tetilla* consists of gonochoristic, oviparous sponges that have direct development. The two species in which reproduction has been studied — *T. japonica* and *T. serica* — are both small (1.5–2.0 cm), solitary, and have a single osculum. In Japan, *T. japonica* and *T. serica* are fertile from July through September, with most gametes being re-

leased in August. Both species are biennial and reports suggest that they can release so many eggs at one time that the surface of the sea turns red (Watanabe 1978). Because of the abundance of the sponges, Watanabe (1978) was able to harvest eggs and sperm from separately maintained male and female adult sponges; this is the only complete in vitro study of fertilization and development in the Porifera.

Oocytes form within the mesohyl and grow in size by engulfing nurse cells and storing yolk. Spermatogenesis has not been described. During peak season, *T. japonica* spawns from 0300 to 0700 daily, while *T. serica* spawns all day, but only a small amount of eggs and sperm are released at one time. The released egg is 130 µm in diameter and is surrounded by long, radiating fibre bundles. Within a minute of fertilization, a membrane rises from the point of sperm entry and continues around the zygote to completely enclose the fibre bundles within a perivitelline space (Figs. 6A–6D). During formation of the fertilization membrane, the egg rotates slowly, first in one direction and then in another; when all of the fibres are enclosed, the egg adheres to the substratum.

Watanabe (1978) suggests that the adherence of the embryo to the substrate dictates the cleavage planes. The first and second cleavage planes are meridional or perpendicular to the substrate, and the third and fourth cleavage planes are oblique to the substrate (Watanabe 1957). The fourth cleavage (16-cell stage) is equatorial and a solid morula is formed 16 h after fertilization (Figs. 6E–6H). Cells at the surface of the morula migrate into the perivitelline space and begin to form the surface epithelium (pinacoderm) of the developing sponge (Figs. 6I–6K). Once the epithelium has formed, two types of cellular migrations occur: first cells at the edge of the adhesive site form outgrowths that flatten the embryo and then cells at the site of adhesion migrate inwards in a type of invagination that increases the height of the embryo. Cellular differentiation begins 3 days after fertilization with the formation of sclerocytes and secretion of spicules. Choanocytes begin to form 4 days after fertilization and the aquiferous system (choanosome) is completed in the 7 day old sponge. Construction of the aquiferous system and skeletal framework is tied to morphogenesis of the whole sponge. As the sponge continues to develop its ovoid to spherical body, it gradually becomes raised on a short stalk or root (Watanabe 1978).

Hadromerida

Development in *P. robusta* has been described in detail (Borojevic 1967). Spawning in *P. robusta* was observed in September at Roscoff in the eastern Atlantic when eggs were released in a thick, adhesive mucus during a 24 h period. The eggs are 100 µm diameter, bright orange spheres, with a large central nucleus. A thin envelope enclosing a region of hyaloplasm surrounds each egg and persists until the morula stage. Cleavage is total, equal, and regular, and gives rise to a morula of equal-sized blastomeres.

During the next 12 h, cell divisions continue, the morula elongates and flattens, and the larva is fully formed 24 h after the release of gametes. The larva is an elongate, flattened blastula that is wider at the anterior pole than at the posterior pole. When the larva has 100–150 cells, the blastomeres differentiate cilia, and the larva then glides over the substrate with the aid of a mucous secretion. In the laboratory, larvae can exist like this for some time without much change except for the appearance of a few phagosomes and vacuoles within the blastomeres. The larvae observed by Borojevic (1967) attached 18–20 days after fertilization. At attachment, an outer epithelium was formed, but no other cellular differentiation was observed. One month after fertilization, the juvenile sponges had differentiated sclerocytes and produced spicules, but no flagellated chambers or canal system were present. Borojevic (1967) concluded that development is extremely attenuated in *P. robusta*.

Development differs considerably among other hadromerids. *Tethya aurantium* (Pallas, 1766) was found to spawn in July–August at Roscoff, France (Lévi 1956). Rapid division of cells at the periphery of the morula gives rise to a two-layered blastula whose outer layer is ciliated. The cells of the inner cell mass differentiate into archaeocytes and the free-living larva is a parenchymella (Lévi 1956). According to Nassonow (1883), Warburton (1961), and Mariani et al. (2000), the boring sponges *Cliona viridis* (Schmidt, 1862) and *Cliona celata* Grant, 1826 (Clionidae) all release eggs, but fertilization has already occurred in the parent sponge. A typical parenchymella larva develops in *C. viridis* (Mariani et al. 2000), but since timing of oocyte release is difficult to catch, no studies have described the complete stages of embryogenesis.

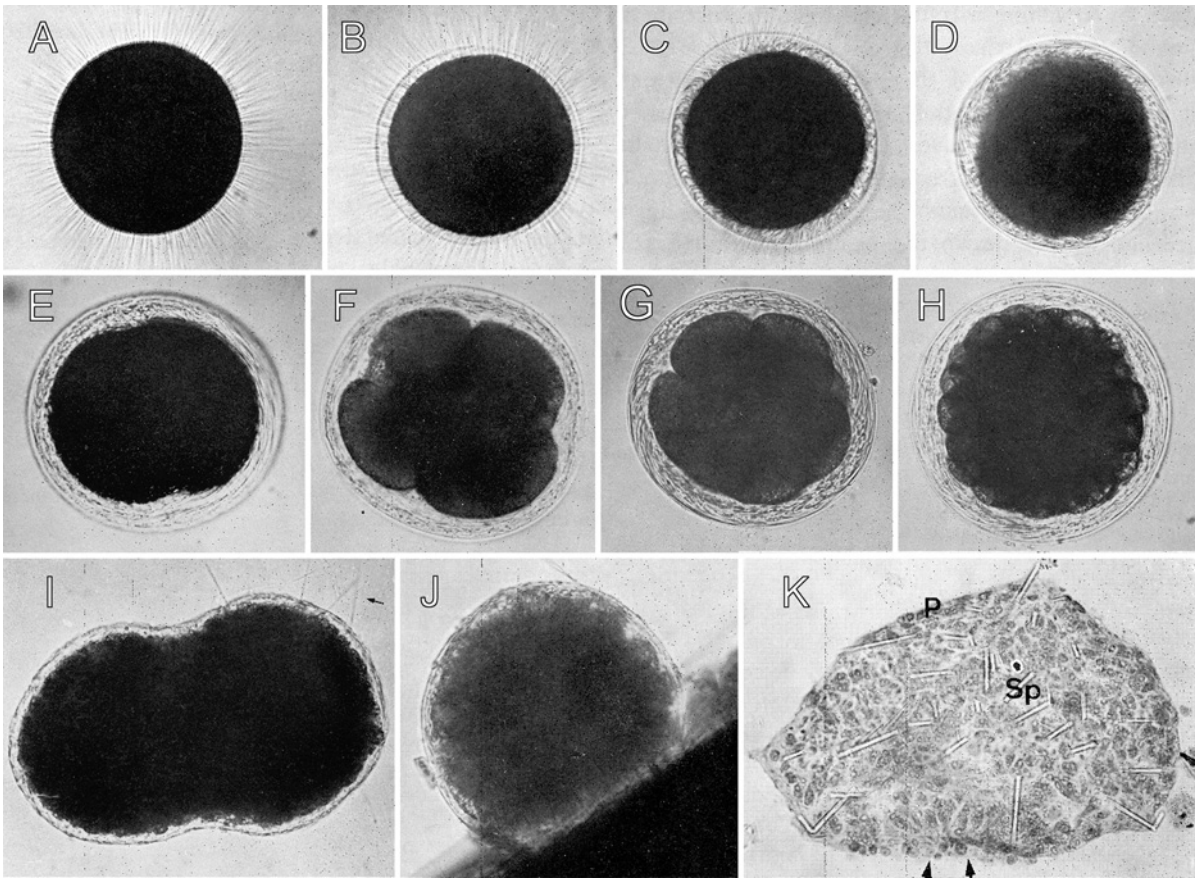
Astrophorida

An unusual astrophorid species, *Alectona wallichii* (Carter, 1874), that was recovered in the Indo Pacific and a new deep sea species, *Alectona mesatlantica* Vacelet, 1999, both brood embryos. This genus was previously of uncertain affiliation and the structure of the newly discovered embryos confirms the unusual character of this group of sponges, which is currently assigned to the Tetractinomorpha (Borchiellini et al. 2004*b*). The embryos of *A. wallichii* and *A. mesatlantica* develop into larvae that lack a ciliated epithelium, but instead develop a coating of special spicules that appear to function as armor (Vacelet 1999). Unfortunately, nothing is known of how the larvae are released, how they disperse, or how they metamorphose.

Ceractinomorpha

Ceractinomorph demosponges comprise a diverse group of marine and freshwater sponges. Despite the abundance of many groups in shallow and intertidal marine and freshwater habitats world wide, knowledge of development is extremely biased to the orders Haplosclerida and Halisarcida, with some notes on development in the Halichondrida, Poecilosclerida, Dictyoceratida, Agelasida, and Verongida (Fell 1969, 1976; Fell and Jacob 1979; Saller and Weissenfels 1985; Hoppe 1988; Saller 1988; Kaye 1991; Ereskovsky and Tokina 2004). The last comprehensive survey of development in marine groups was carried out by Lévi (1956). Ceractinomorphs are largely viviparous. However, there are several oviparous genera that have a wide distribution in the tropical Atlantic, and synchronous release of gametes has been reported in several of these (Reiswig 1976; Hoppe and Reichert 1987). Here we specifically focus on development in the Haplosclerida and Halisarcida as two distinct developmental modes, and

Fig. 6. Development in the tetractinomorph sponge *Tetilla japonica*. (A) Egg with surface filaments prior to fertilization (egg diameter 130 μm). (B) At fertilization a membrane is raised. (C) One minute and (D) 4 min after fertilization. (E–K) Embryo attached to the substrate. (E) Two-cell stage, 1.5 h after fertilization. (F) Four-cell stage, 3 h after fertilization. (G) Eight-cell stage, 4.5 h after fertilization. (H) Morula, 16 h after fertilization. (I) Morula formed by two fused eggs, 2 days after fertilization. (J) Side view of a 3 day old attached "larva". (K) Longitudinal section through a 4 day old "larva". (From Watanabe 1978, reproduced with permission of Y. Watanabe and Nat. Sci. Rep. Ochanomizu Univ., vol. 29, pp. 86, 88, and 89, © 1978 Ochanomizu University.)



refer to spawning and embryogenesis in other orders where significant differences are known.

Haplosclerida

The bright colours and easy access of many haplosclerids in shallow marine and freshwater habitats is likely why they have been such common subjects for studies of development (Barrois 1876; Brien and Meewis 1938; Lévi 1956; Fell 1969; Saller and Weissenfels 1985; Saller 1988; Leys and Degnan 2002). General features of haploserid development can be summarized as follows: many show alternative sexual and asexual stages through gemmules or types of overwintering cysts; specialized nurse cells are often involved in oogenesis and gemmulogenesis; yolk is often formed during cleavage, as well as during oogenesis; cellular differentiation is precocious; the larva is a parenchymella with a high level of cytological differentiation, some with flagellated chambers, with larval skeletons, and with distinct larval behaviours (Ereskovsky 1999; Maldonado 2006). Reproduction generally occurs during the warmer months of the year (Saller 1988); however, in temperate regions, there may be two reproductive seasons (i.e., spring and fall) (Fell 1969), whereas in tropical regions, species may be reproductive year round (Leys and Degnan 2002).

Spermatocytes develop in special cysts within the mesohyl. In freshwater sponges, they derive from choanocytes; however, in marine haplosclerids, their origin is not known (Paulus and Weissenfels 1986). Oocytes arise from archaeocytes in the mesohyl in most species that have been studied; only in the freshwater sponges of Lake Baikal, family Lubormirskiidae, are they thought to derive from choanocytes (Ereskovsky 1999). From early on, oocytes are accompanied by specialized yolk-rich nurse cells, which form a thick follicle around the oocyte. As the oocyte matures, it begins to engulf the nurse cells, incorporating the yolk into its own cytoplasm, but often leaving other components of the nurse cell (membrane and even nucleus) intact (Meewis 1939; Fel 1969; Saller 1988; Ereskovsky 1999).

Cleavage planes are difficult to describe accurately owing to the presence of massive amounts of yolk inclusions in the egg (Fell 1969; Leys and Degnan 2002). In most species, cleavage is complete, unequal, and usually asynchronous. In species of the genus *Reniera* Schmidt, 1862, unequal cleavage produces two sizes of cells, smaller micromeres and larger macromeres that are distributed throughout the embryo (Figs. 7A–7C) (Leys and Degnan 2002). The former develop a cilium wherever they are within the embryo, and then both cell types (ciliated micromeres and non-ciliated

macromeres) sort out until the micromeres are only at the periphery of the embryo, a process that has been termed multipolar delamination. At approximately the same time, sclerocytes become evident at the periphery of the embryo (Leys 2003).

The clearest observations of cleavage come from freshwater sponges. In *Ephydatia fluviatilis* (L., 1758) and *Spongilla lacustris* (L., 1758), planes of cleavage are variable (i.e., sometimes equatorial and other times rotational), and frequently, daughter blastomeres are multinucleated (Saller and Weissenfels 1985; Saller 1988). The result of cleavage is a solid blastula of uniformly sized undifferentiated cells (Figs. 7D–7F). In other halplosclerids, sclerocytes are also the first identifiable cell type (Fell 1969; Saller and Weissenfels 1985; Saller 1988); they also appear at the periphery of the embryo in some sponges, while they are deep within the embryo in others. In *Reniera* sp., sclerocytes appear to be migratory because they possess numerous filopodia; in later larvae, sclerocytes are found in the posterior central region of the larva where they are aligned along the anterior–posterior axis of the embryo (Leys and Degnan 2002; Leys 2003). Meewis (1939) first noted that the larval epithelium develops at one pole before it covers the entire embryo. In *Haliclona ecbasis* de Laubenfels, 1930, a ciliated epithelial plate becomes organized at one pole, while a non-ciliated plate forms at the opposite pole (Fell 1969). The latter becomes surrounded by a ring of ciliated cells that contain brown pigment as the ciliated epithelium knits together around the remainder of the embryo.

Haplosclerid larvae are parenchymellae. They are polarized and typically possess a ciliated epithelium at all but the posterior, and in some the anterior, poles. The bare posterior pole is fringed by a ring of pigmented cells with longer cilia. Most larvae have bundles of spicules at their posterior poles, and in most there are three layers: a pseudostratified columnar epithelium, a sub-epithelial layer of cells that lie parallel to the larval surface as a "girdle" around the larva, and an inner cell mass consisting of several types of amoeboid cells in a collagenous mesohyl (e.g., Fell 1969; Woollacott 1993; Leys and Degnan 2001, 2002). This tri-layered structure is also found in the parenchymella larvae of other demosponges (Woollacott and Hadfield 1989; Woollacott 1990). The cells of the columnar epithelium are joined at their apical side by dense junctions that have not been characterized but which nevertheless are considered to form a seal against the outside fluid environment.

Freshwater sponge larvae are unusual in possessing a large apical cavity that is connected by pinacocyte-lined canals to nonfunctional choanocyte chambers (Figs. 7G) (Saller and Weissenfels 1985; Saller 1988). Development of choanocyte chambers in the larva is considered a heterochronic event, one rarely found in the larvae marine haplosclerids (Ilan and Loya 1990). Flagellated chambers are otherwise only known from two other larval types: the hexactinelid trichimella larva (Boury-Esnault et al. 1999; Leys et al. 2006) and the hoplitomella larva of the astrophorid genera *Alectona* and *Thoosa* Hancock, 1849 (Garrone 1974).

Other ceractinomorphs

The development of other ceractinomorph groups is very similar (Ereskovsky 2004). These species possess isolecithal or telolecithal (in Poecilosclerida) eggs and total but chaotic cleavage that usually results in the formation of a stereoblastula. Segregation of the cells of the blastula into external and internal layers usually occurs by delamination; further development leads to the formation of a parenchymella larva.

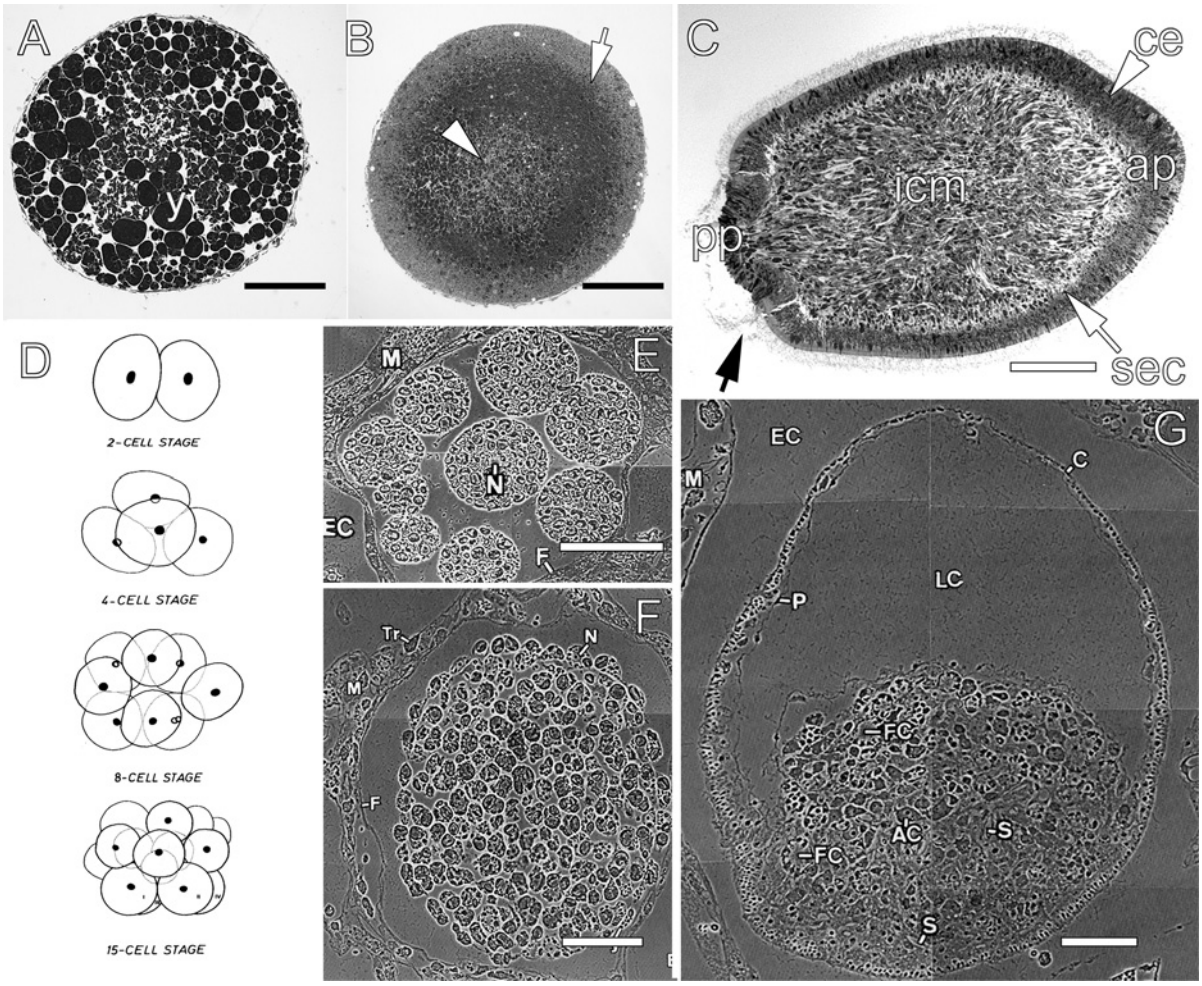
Natural release of sperm has been reported in half a dozen ceractinomorph genera from shallow Caribbean reefs, but only five species from three genera are known to be oviparous. Nevertheless, predictable synchronous spawning has been reported on numerous occasions in species of *Agelas* Duchassaing and Michelotti, 1864 (Agelasida), *Hemectyon* Topsent, 1920, and *Neofibularia* Hechtel, 1965 (both poecilosclerids) (Reiswig 1976; Hoppe and Reichert 1987). Sperm release in the genus *Agelas* may occur throughout the year, but release of both gametes in synchrony is more seasonal and was observed over several years from the hours of 1500 to 1700 on the date of the waning quarter moon in late July and early August (Reiswig 1976). Hoppe and Reichert (1987) reported spawning by *Agelas clathrodes* (Schmidt, 1870) during early morning hours throughout the summer, and by *Neofibularia nolitangere* Duchassaing and Michelotti, 1864 over a 2 month period with two predictable spawnings exactly one lunar month apart. While sperm are released from the osculum as a synchronous behavioural event, in all of these sponges female gametes are released through the body wall (dermis) near the osculum and are enclosed in gelatinous strands. Although spawning is synchronous, it is unknown whether oocytes are fertilized prior to or after release from the parent. Reiswig (1976) provides the only observations on embryogenesis. The oocytes of all species observed were nearly identical, 120 µm diameter, orange spheres. Unlike *Hemectyon ferox* Duchassaing and Michelotti, 1864, oocytes in species of *Agelas* have no follicles. Cleavage in both genera is holoblastic and equal, and gives rise to a stereoblastula. In the genus *Agelas*, gastrulation results in a bilayered, polarized, ciliated parenchymella of 1–2000 cells (Reiswig 1976). Later developmental stages of the genus *Hemectyon* were not found.

Halisarcida

The genus *Halisarca* Johnston, 1842 stands out among the Demospongiae in lacking a mineral skeleton. Many authors have repeatedly noted significant differences between *Halisarca* and other keratose sponges including those in the order Dendroceratida into which this genus was placed for many years (see Ereskovsky and Gonobobleva 2000). Citing these differences, Bergquist (1996) created the monogeneric order Halisarcida with 10 valid species (Bergquist and Kelly 2004). The embryological peculiarities of *Halisarca* also support the separation of these sponges from other demosponges at the order level (Ereskovsky and Gonobobleva 2000; Ereskovsky 2004). The embryonic development of *Halisarca* has been studied for more then 130 years (Gonobobleva and Ereskovsky 2004*a*, 2004*b*; Ereskovsky 2005).

Halisarca dujardini Johnston, 1842 is a dioecious viviparous sponge (Ereskovsky 2000). Spermatogenesis is triggered by low temperatures during winter months and mature sperm can be found in sponges starting in March or April and lasting for about 4 months (Chen 1976; Ereskovsky

Fig. 7. Development in haplosclerid demosponges. (A–C) *Reniera* sp. and (D–G) *Spongilla lacustris*. (A, B), Cleavage of a yolk-dense (y) egg gives rise to a stereoblastula. (B) Cells of the stereoblastula differentiate and sort to form outer (arrow) and inner (arrowhead) regions. (C) A parenchymella larva with three distinct cell layers and anterior–posterior polarity. ap, anterior pole; pp, posterior pole; ce, ciliated epithelium; sec, sub-epithelial cells; icm, inner cell mass. Scale bars: A and B, 200 µm; C, 100 µm. (D) Schematic of the early-cleavage stages of *S. lacustris*. (E) Plastic section through a 15-cell-stage embryo. N, nucleus; M, mesenchyme; EC, excurrent canal; F, follicle epithelium. (F) Stereoblastula with yolk-filled cells. (G) Young larva with hemispherical larval cavity (LC) lined by pinacocytes (P). AC, archaeocyte; S, spicule; FC, flagellated chamber; C, ciliated epithelium. Scale bars: E, 50 µm; F and G, 30 µm. (A–C from S.P. Leys, unpublished micrographs; D–G from Saller and Weissenfels 1985, reproduced with permission of Zoomorphology (Berl.), vol. 105, pp. 369 and 370, © 1985 Springer Science and Business Media.)

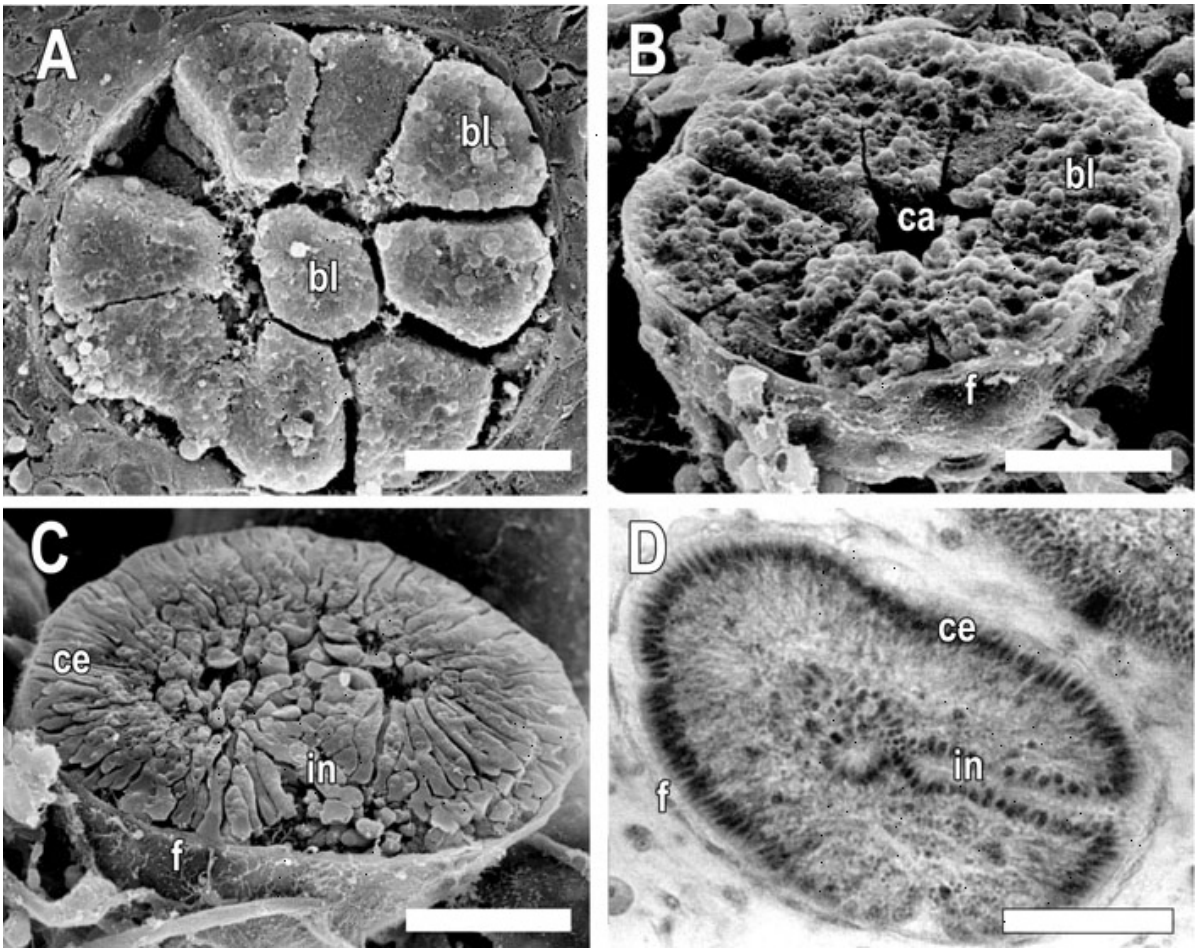


2000). Spermatocytes appear to originate from choanocytes, which form special spermatic cysts that between January and March can occupy as much as 65% of the volume of the choanosome of male specimens. The periphery of the sponge retains the normal organization of canals and feeding chambers. Oocytes also derive from choanocytes (Korotkova and Apalkova 1975), and during vitellogenesis, as in haposclerids, symbiotic bacteria characteristic of the mesohyl of adult sponges are incorporated in the oocyte (Ereskovsky et al. 2005). Cleavage is total and equal. The first cleavage is meridional relative to the polar body, but all subsequent cleavages are perpendicular to the surface of the embryo (radial) so as to produce a hollow blastula of elongate cells (Figs. 8A, 8B) (Ereskovsky 2002). In many embryos, cells now invade the blastocoel by both multipolar and unipolar ingression (Lévi 1956; Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004*b*). Cilia differentiate on the external cells of the 32- to 64-cell-stage blastula, while internal cells differentiate to become amoeboid cells or archaeocytes of the larva.

When the embryos have about 800 cells, two important events occur: first, the maternal granular eosinophilic cells migrate into the embryo (Fig. 8B) and, second, the outer epithelium of the blastulae becomes folded or convoluted as it increases in size within the confines of the follicular epithelium (Figs. 8C, 8D). The arrangement and depth of folds are different in each embryo in the same parent sponge, and in some embryos, one of the deep invaginations of external ciliated cells becomes internalized as a small ciliated chamber.

A peculiar characteristic of development in the genus *Halisarca* is that embryogenesis can result in three types of larvae with very different morphologies (Figs. 9A–9D, 10A–10J). The first is a coeloblastula that consists of a single layer of wedge-shaped cells that border a small lumen with

Fig. 8. Development in *Halisarca dujardini*. (A) SEM of a blastula with interior blastomeres. (B) SEM of an early blastula with a small central cavity (ca). (C) SEM and (D) light micrograph of the formation of a disphaerula larva by invagination of a fold of the external ciliated epithelium. bl, blastomere; ce, ciliated epithelium; f, follicle; in, invagination. Scale bars: A–D, 20 μm. (A.V. Ereskovsky, unpublished data.)



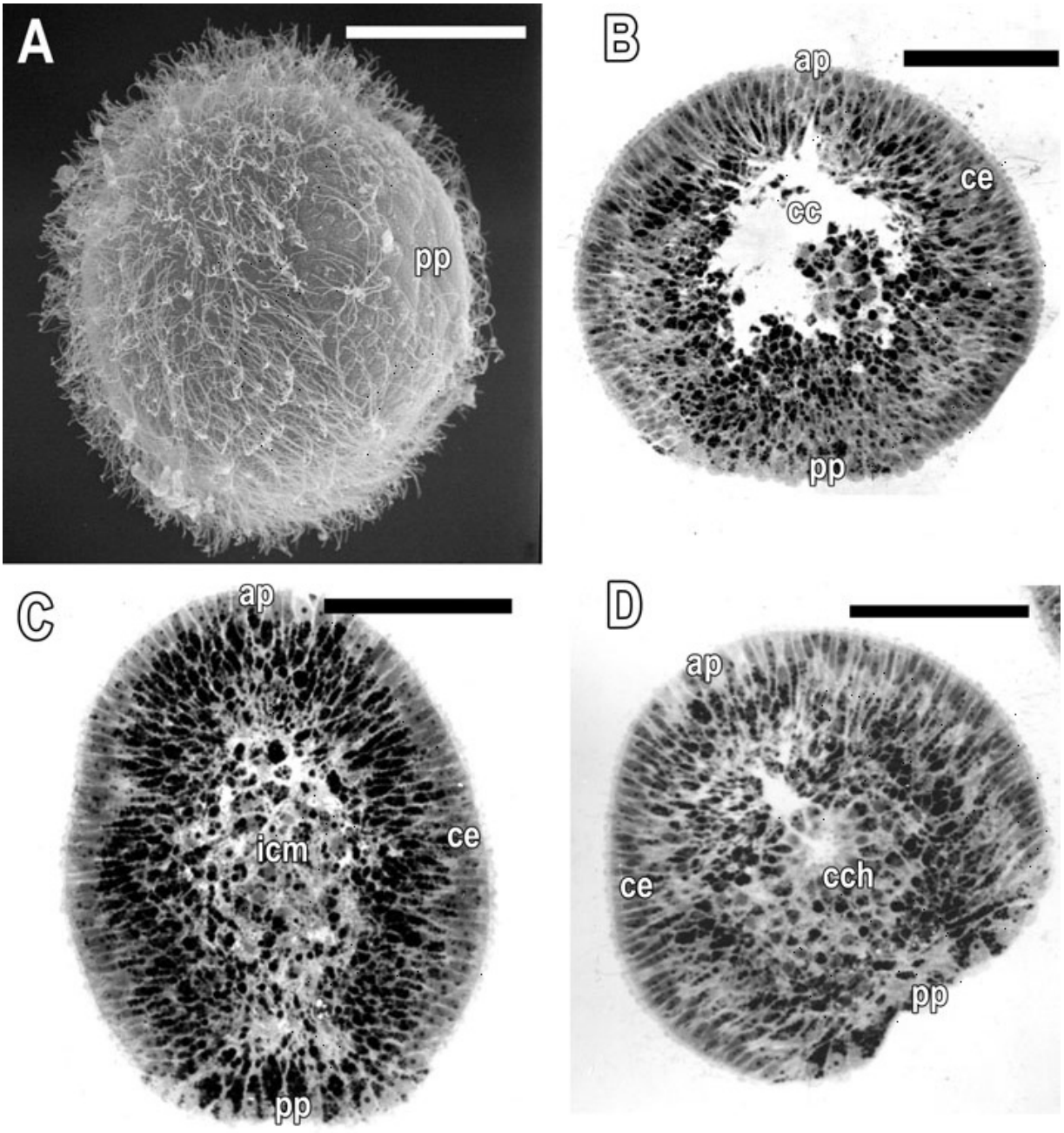
only a few amoeboid cells (Figs. 9B, 10I). The second is a parenchymella that has an outer epithelium surrounding an inner cell mass of amoeboid cells (Figs. 9C, 10J). The third has two layers of epithelial cells, one external and the other internal, that line a small lumen; this type of larva is called a disphaerula (Figs. 9D, 10H) (Ereskovsky and Gonobobleva 2000). All three types of larvae may be found in the same parent. The larvae are wider than they are long (120–150 μm diameter and 110–140 μm long), but are polarized by external patterns of ciliation (Ereskovsky and Gonobobleva 2000). All the larvae of *H. dujardini* are milky white and swim by rotating in a left-handed direction, i.e., clockwise as seen from the anterior pole.

The unusual structure of larvae in the genus *Halisarca* has been the focus of some discussion by different authors. Although internal ciliated chambers were described in the larvae of *Halisarca metschnikowi* Lévi, 1953, *H. dujardini*, and *Halisarca nahantensis* Chen, 1976, some authors have interpreted all larvae as parenchymellae (Lévi 1956; Chen 1976), while others have considered them to be coeloblastulae. The unusual invagination of the larval epithelium to form a ciliated chamber has also been compared with gastrulation movements in other animals (Maldonado 2004). A name for the third larval type, the disphaerula, was only recently formalized (Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004*a*, 2004*b*).

Development in Calcareous sponges

The Calcarea are an intriguing group. They are distinguished from other sponges because they secrete a spicule skeleton of calcium carbonate, which is in contrast to the siliceous spicules of all other sponges. (Other sponges can also secrete a firm calcium carbonate base, but these also have a loose skeleton of siliceous spicules, and are thus currently considered to be siliceous sponges (Hooper and van Soest 2002).) The Calcarea comprise less than 5% of all sponges and are widely used in teaching because of their small size, their accessibility, and because they illustrate grades of morphological complexity within the Porifera — ascon, sycon, and leucon. These grades of organization are thought to reflect stages in evolutionary complexity of sponges (Borojevic et al. 2002; Manuel et al. 2002), even though the Calcarea are the only sponges that possess all three grades.

Fig. 9. SEM (A) and light micrographs (B, C, D) of different morphotypes of larvae from *H. dujardini*. (A) External view of the larva showing the posterior pole (pp). (B) Longitudinal section through a coeloblastula-like larva showing the anterior pole (ap), posterior pole (pp), and inner cavity (cc). (C) Longitudinal section through the parenhymella-like larva showing the anterior pole (ap), posterior pole (pp), and inner cell mass (icm). (D) Longitudinal section through the disphaerula larva showing the anterior pole (ap), posterior pole (pp), and inner ciliated chamber sphere (cch). Scale bars: A–D, 20 μm. (A.V. Ereskovsky, unpublished data.)

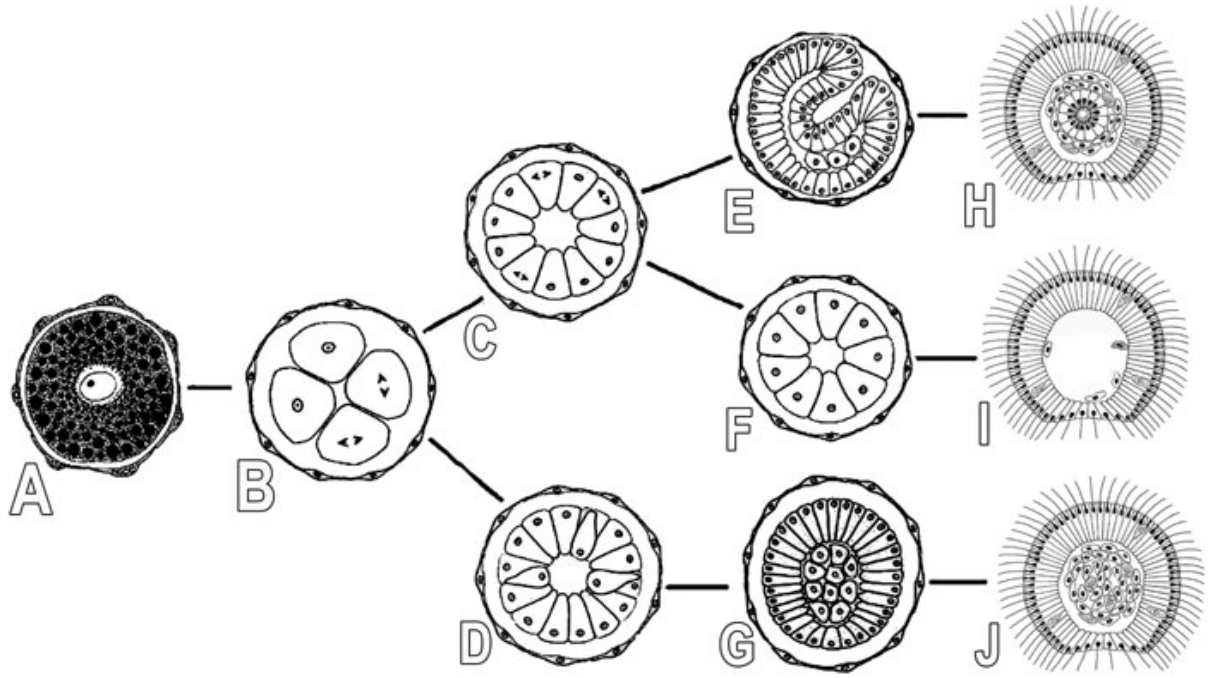


Calcareous sponges have long been considered basal within the Porifera, but recent molecular data suggest that the Porifera may be paraphyletic and that the Calcarea is more closely related to other metazoans — Cnidaria and Ctenophora — than it is to other sponges (Lafay et al. 1992; Cavalier-Smith et al. 1996; Collins 1998; Kruse et al. 1998; Zrzavy et al. 1998; Borchiellini et al. 2001). These data imply that a sponge-like animal may be ancestral to all other metazoans.

Calcinea

Embryogenesis in calcinean sponges is known from earlier studies (Haeckel 1872; Barrois 1876; Metschnikoff 1879; Minchin 1900). Only two other recent studies (Borojevic 1969; Fischel Johnson 1979) have reassessed development using light microscopy. *Clathrina blanca* (Miklucho-Maclay, 1868) and *Clathrina coriacea* (Montague, 1818) inhabit submarine caves at Santa Catalina Island, California, where specimens are reproductive between April and August (Fischel

Fig. 10. Diagram of development in *H. dujardini*. (A) Egg; (B) early cleavage; (C, F) coeloblastula; (D) multipolar ingression; (E) invagination; (G) morula (stereoblastula); (H) disphaerula; (I) coeloblastula larva; and (J) parenchymella larva. (From Ereskovsky 2005, p. 186, reproduced with permission of Sanct-Petersburg University Press, © 2005.)



Johnson 1979). Oocytes develop within the mesohyl and grow in size by phagocytozing neighbouring eosinophillic amoebocytes. Oogenesis does not appear to be synchronized among the population, and no spermatic cysts were found in any specimens. Cells with two nuclei were seen in the mesohyl near oocytes, and some oocytes had two darkly staining masses that were suggestive of transport of sperm to the oocyte by a choanocyte carrier cell.

In both genera *Ascandra* Haeckel, 1872 and *Clathrina*, cleavage is total and equal and gives rise to an 8-cell-stage blastula that is about 100 μm in diameter. In some species, cleavage is synchronous within a population of embryos, but in the genus *Ascandra*, brood chambers contain embryos at many different stages of development (Fischel Johnson 1979). Each embryo is enveloped in a sheath of elongate parental cells, but after the 16- or 32-cell stage, the embryo moves out of the mesohyl into the parent choanoderm and further cleavages occur within the choanocyte chamber of the parent sponge. The resulting larva is a hollow blastula (coeloblastula) consisting of two types of cells: ciliated epithelial cells and one or two posterior granular cells. Although some authors have considered the posterior cells to contain the germ lineage (Borojevic 1969), cell lineage studies have not yet been carried out.

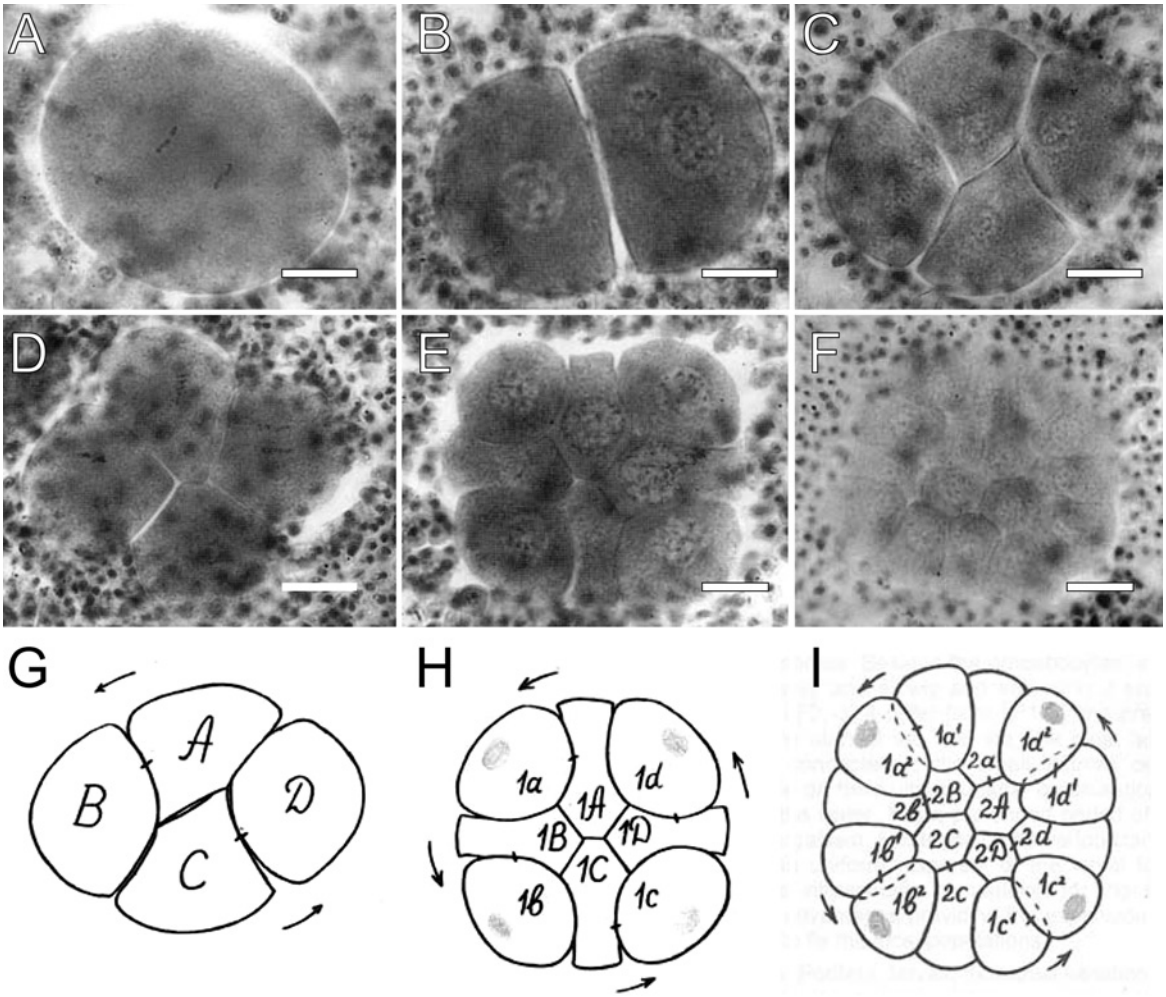
Prior to and after larval release, some blastomeres loose their cilia and ingress into the blastocoel. The rate at which ingression fills the blastocoel varies, and in some species ingression never occurs (Amano and Hori 2001). In *C. coriacea* and *C. blanca*, the blastocoel may be relatively free of cells, partially filled, or completely filled with cells at larval release (Fischel Johnson 1979). In *Clathrina reticulum* (Schmidt, 1862), only a few cells migrate into the blastocoel during the first 3 days of larval life; at the 4th day, a massive immigration starts at the equator and moves progressively towards the posterior pole, and then anteriorly, filling up the blastocoel (Borojevic 1969). The whole process takes several hours; at the end, the larva begins to metamorphose into a juvenile.

Calcaronea

Embryogenesis in calcaronean sponges has been well studied in the past, but little recent work exists. Because of two hallmark inversion/invagination events that occur during embryogenesis, the subject merits re-investigation. Detailed studies have been carried out on the development of the genera *Sycon* Risso, 1826 (= *Scypha* Gray, 1821) (Schulze 1875, 1878; Duboscq and Tuzet 1935; Tuzet 1963, 1970; Franzen 1988; Leys and Eerkes-Medrano 2005) and *Grantia* Fleming, 1828 (Gatenby 1920; Gallissian 1983; Gallissian and Vacelet 1992), which are both syconoid (heterocoel) sponges.

Oocytes differentiate from choanocytes and move into the mesohyl (Gatenby 1920; Franzen 1988). In some species, young oocytes occur near the atrial wall of the chambers, and after a period of growth by phagocytosis of trophocytes, they migrate to the periphery of the sponge (Franzen 1988). Cleavage is total and equal, but may be regular or asynchronous, and the first and second cleavages can result in blastomeres with multiple nuclei. The first to third cleavages are meridional, cleaving the oocyte and blastomeres perpendicular to the choanoderm. According to authors from the 19th century, in syconoid sponges the fourth cleavage is equatorial, producing two tiers of 8 cells, which at the fifth cleavage give rise to another two tiers of cells to form a hollow blastula of approximately 32 cells (Schulze 1875; reviewed in Leys and Eerkes-Medrano 2005). But a recent

Fig. 11. Development in the homocoel (asconoid) calcaronean sponge *Leucosolenia complicata*. (A) Anaphase of the first cleavage division. (B) Two-cell stage. (C) Second cleavage division. (D, G) Four-cell stage. (E, H) Third cleavage division. (F, I) Sixteen-cell stage. Scale bars: 2 µm. (From Anakina 1997, pp. 51 and 52, reproduced with permission of Berliner Geowissenschaftliche Abhandlungen, © 1997.)

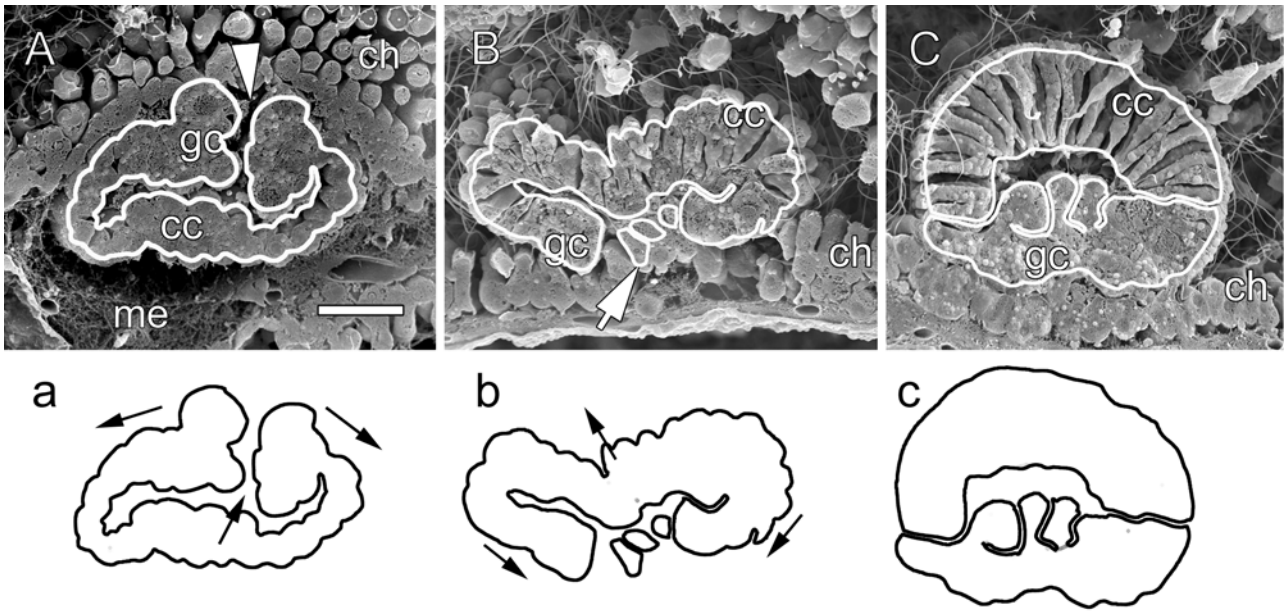


study of development in the asconoid sponge *Leucosolenia complicata* (Montagu, 1818) shows that from the fourth division cleavage is oblique, almost exactly like cleavage in the colonial alga *Volvox* L., 1758; a type of cleavage referred to as palintomy (Figs. 11A–11I; Anakina 1997). The resulting embryo is a cup-shaped blastula, with a small opening (the phyalopore) at the side closest to the choanoderm. Images from an earlier study by Duboscq and Tuzet (1937) suggest that similar divisions may also occur in the genus *Sycon* and other heterocoels. While most of the cells continue to cleave in the same manner, forming a single-layered epithelium, a few that lie directly under the choanoderm do not; these remain much larger than the other cells, with their cytoplasm filled with large yolk granules. The most unusual feature of the calcaronean embryo is that cilia, which differentiate on the micromeres, project into the centre of the blastocoel, i.e., its orientation is inverted compared with those in all other sponge embryos.

To reach the correct orientation, the embryo turns inside out, either within a follicle in the mesohyl (Lufty 1957) or as it moves out of the mesohyl and into the flagellated chamber (Franzen 1988). In the latter case, the large granular cells form junctions with the choanocytes above them and an opening appears in the choanoderm (Franzen 1988). At this stage, the embryo is referred to as a stomoblastula. The ciliated blastomeres move upwards through the opening as a single epithelium, into the choanocyte chamber (much as a bag is turned inside out; Figs. 12A–12C, 12a–12c). The large granular cells remain closely associated with the parent choanoderm, and as the blastula fully inverts, cells from the parental mesohyl migrate into the new larval cavity where it is suggested that they have a nutritive role (Figs. 12B, 12b) (Gallissian 1983).

The larva is an amphiblastula, composed of anterior ciliated cells, posterior granular cells, and inner nutritive amoebocytes. Four unusual cells also lie around the equator of *Sycon* and early *Grantia* larvae — the cross cells, which were first described as presumptive photoreceptors (Duboscq and Tuzet 1941). Although *Sycon* larvae are phototactic (Elliott et al. 2004), there is no evidence yet that these cells are the photoreceptors. In the genus *Grantia*, the cross cells are difficult to find in larvae in the parent; in free-swimming larvae, they have already degenerated (Gallissian 1983).

Fig. 12. SEM of the stages in inversion of the calcaronean sponge *Sycon raphanus*. (A, a) A stomoblastula lying in the mesohyl (me) with ciliated cells (cc) whose cilia face into the blastocoel and granular cells (gc) that form an opening (arrowhead) to the choanoderm (ch). (B, b) After inversion into the choanocyte chamber, the ciliated cells have externally facing cilia and the granular cells remain in contact with the choanoderm. Cells from the mesohyl migrate into the new larval blastocoel (arrow). (C, c) The amphiblastula larva with two distinct hemispheres of ciliated and granular cells in the choanocyte chamber. Scale bar: 10 μm. (S.P. Leys, unpublished data.)



Comparative embryology and molecular developmental biology

In this paper, we have shown that sponges pass though a series of developmental events that are characteristic of Eumetazoa. The fertilized egg undergoes successive mitotic divisions to form a multicellular organism. Profound, but well-ordered, rearrangements of cells occur in the blastula. Both mesenchymal and epithelial movements are involved in forming different sponge embryos, and cellular differentiation, morphogenesis of larval structures, and formation of an anterior–posterior axis all take place during sponge embryogenesis.

The fact that it is possible to identify a suite of events during early ontogenesis, which is typical for a species, genus, family, or order, has allowed developmental characters to be used for classification within the Porifera (Lévi 1956; Borojevic 1970; Brien 1973; Korotkova 1981; Fell 1989; Ereskovsky 2004, 2005). However, interpretation of the morphogenetic events that occur during sponge embryogenesis in the larger context of metazoan development, in particular with respect to what series of events might be conceived as gastrulation, is more contentious (Efremova 1997; Ereskovsky and Korotkova 1997; Leys and Degnan 2002; reviewed in Leys 2004).

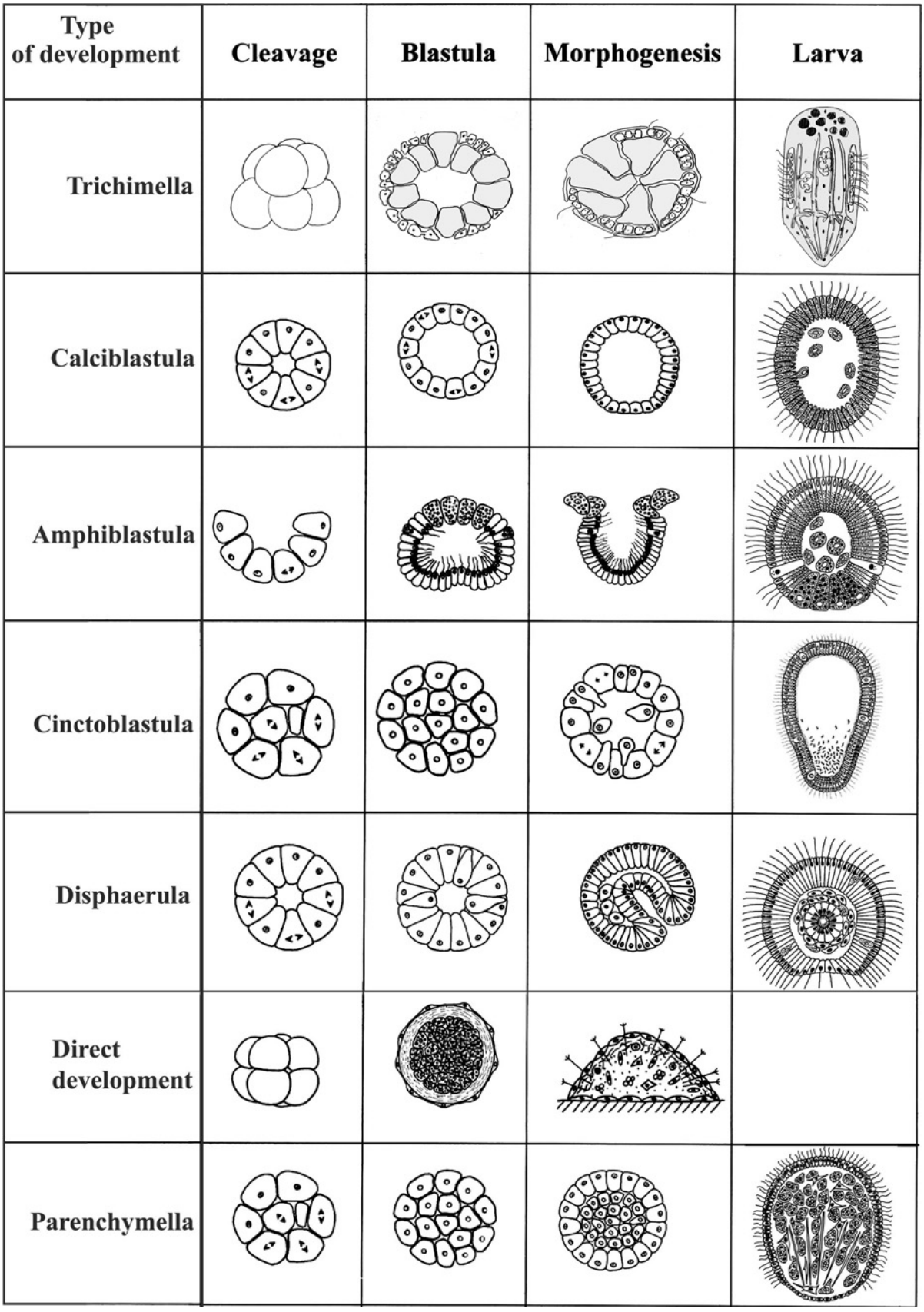
Within the Porifera, seven types of sexual development are currently recognized by their resultant larval form: (1) "trichimella" (Hexactinellida); (2) "calciblastula" (Calcinea); (3) "amphiblastula" (Calcaronea); (4) "cinctoblastula" (Homoscleromorpha); (5) "disphaerula" (Halisarcida); (6) direct development (genus *Tetilla*, Spirophorida); (7) "parenchymella" (most of Demospongiae) (Fig. 13; Maldonado and Bergquist 2002; Ereskovsky 2004). Each of these developmental types represents an established sequence of invariant stages that is characteristic of the development of animals within high taxonomic groups (above the order level). The reason that so many different larval types have been identified is partially due to the plasticity or variability which occurs in development. In some instances, the same cleavage pattern and type of blastula may be characteristic of several different larval types; e.g., both genera *Polymastia* (Hadromerida) and *Tetilla* (Spirophorbida) have radial cleavage and form a morula, but the former gives rise to a coeloblastula larva and the latter has direct development (Borojevic 1967; Watanabe 1978). On the other hand, the same larval type may arise from different cleavage patterns; e.g., the parenchymella of the genus *Reniera* (Haplosclerida) develops as a result of chaotic cleavage and multipolar delamination, while the parenchymella larva of the genus *Halisarca* (Halisarcida) arises by polyaxial cleavage and multipolar ingression (Leys and Degnan 2002; Gonobobleva and Ereskovsky 2004*b*).

However, the use of developmental characters in phylogenetic interpretation is most hampered by the paucity of precise embryological studies on sponge groups. Currently, there are developmental data on only about 100 sponge species. These studies offer a mosaic of information and many only provide a description of certain stages of development, often only of the larva. Two features that have been used for phylogenetic analysis within the Porifera, and for understanding the construction of the sponge body plan within the Metazoa, are cleavage patterns and gastrulation (morphogenetic) movements.

Cleavage

Cleavage is the first morphogenetic process in metazoan ontogenesis. It is a very well coordinated, species-specific

Fig. 13. Schematic diagram summarizing the various types of development and their resultant larvae in the different groups of Porifera. (S.P. Leys and A.V. Ereskovsky, unpublished data.)



process of diagnostic significance and is often used for establishing phylogenetic links in different groups of animals (Siewing 1979; Ivanova-Kazas 1995; Gilbert and Raunio 1997; Valentine 1997). Cleavage plays two important roles: the creation of multicellularity and ooplasmic segregation. The cytoplasmic segregation of morphogenetic determinants results in the distribution of qualitatively different areas of the zygote cytoplasm between the daughter cells.

Cleavage is poorly studied in sponges because many sponges brood their embryos, thus making observations more difficult. In sponges, four main holoblastic cleavage patterns have been described: chaotic (anarchic), table palintomy, radial, and polyaxial (Ereskovsky 2005). Chaotic is characteristic for many groups of Demosponges (Fell 1993; Ereskovsky and Boury-Esnault 2002) (Fig. 14A). Table palintomy is a type of cleavage in which the cleavage furrows pass obliquely relatively to the egg's animal–vegetal axis. It is characteristic of the genus *Volvox* and a number of colonial protists, and occurs in some Calcaronea (Calcarea) (Anakina 1997) (Fig. 14B). Radial cleavage has been described in some demosponges: *T. aurantium* (Hadromerida, Demospongiae) (Lévi 1956), *P. robusta* (Hadromerida, Demospongiae) (Borojevic, 1967), *C. reniformis* (Chondrosida, Demospongiae) (Lévi and Lévi 1976), and the genus *Tetilla* (Spirophorida) (Watanabe, 1978) (Fig. 14C). The polyaxial cleavage pattern is characteristic for the genus *Halisarca* and possibly also for the Calcinea (Calcarea) (Borojevic, 1969). This type of cleavage is characterized by cleavage furrows that are perpendicular to the surface of the embryo from the 8- to 16-cell stage until cytodifferentiation. Throughout all cleavages, the embryo has several axes of symmetry (equal to the number of blastomeres), which radiate at certain angles from the centre of the embryo (Ereskovsky 2002) (Fig. 14D). However, often after the 16-cell stage, division becomes chaotic.

Discussions of which cleavage type may have been ancestral within the Metazoa often ignore sponges, the most basal metazoan phylum. Most authors consider radial cleavage to be basal within the Metazoa (Siewing 1979; Davidson et al. 1995; Valentine 1997). Ivanova-Kasas (1995) treats the table palintomy of Calcaronea (Calcarea) as the ancestral cleavage type. However, others contend that development of Calcaronea by inversion of the blastula is aberrant, and thus interpretations of this cleavage pattern as ancestral should be treated with caution (A.V. Ereskovsky, personal comment). Another scenario of the evolution of early metazoan embryogenesis involves anarchic and polyaxial cleavage as the most primitive cleavage types, from which radial cleavage could have originated.

Gastrulation

The concept of gastrulation is problematic for sponges because these animals are considered to lack a gut in the normal sense and a gut is the purported product of gastrulation in animals (see Leys 2004). Three opinions have been put forward on the problem of gastrulation in sponges. The first suggests that there is no gastrulation because the fate of specialized larval cells in sponges is not homologous to that of germ layers in Eumetazoa, i.e., the sponge cells do not form tissues homologous to a gut (Rasmont 1979; Ereskovsky and Korotkova 1997; Ereskovsky 2005). Second is the idea that gastrulation occurs at metamorphosis when the sponge forms an internal cavity which houses the feeding chambers and epithelia that are equated with a gut (Lévi 1963; Tuzet 1963, 1973; Brien 1967; Fell 1974). And the third opinion is that the formation of two and sometimes three layers during the development of many sponge larvae does comprise gastrulation. Some authors have suggested that these layers invert at metamorphosis, making sponge germ layers inverted with respect to other animals (Delage 1892; Brien 1967). Other authors regard the changes that occur at metamorphosis as a rearrangement of already differentiated layers (Borojevic 1970; Amano and Hori 1996; Efremova 1997; Leys and Degnan 2002). Interestingly, as the reader will have seen, the authors of this review have two completely opposing views on the question.

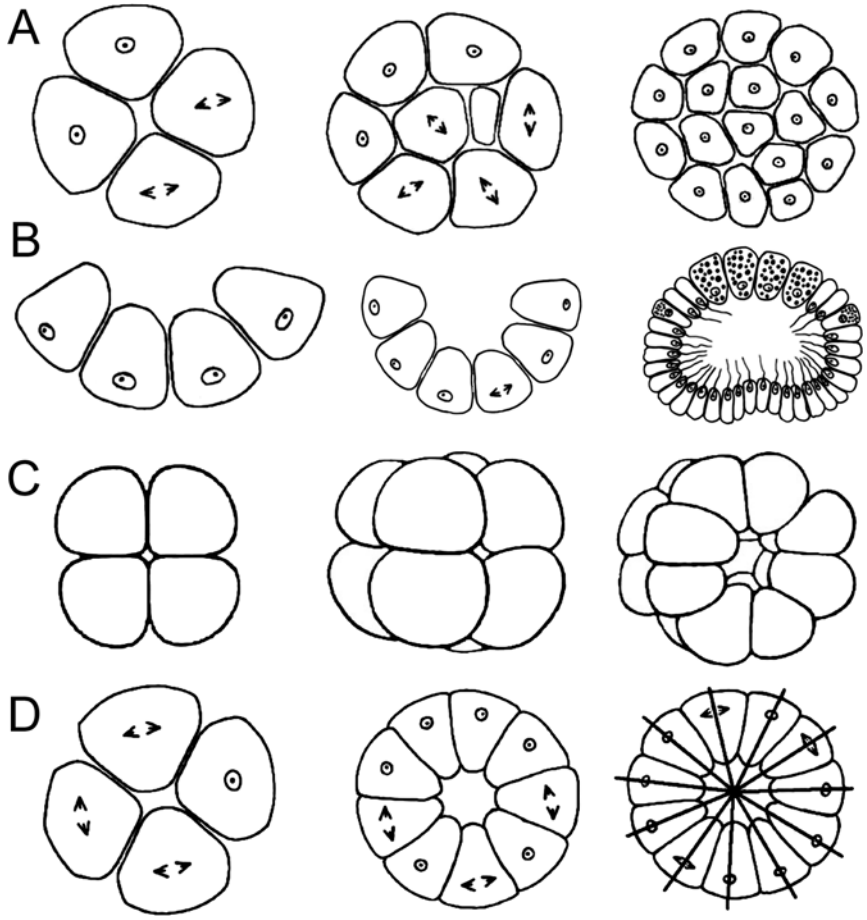
The term gastrulation itself derives from Haeckel's description of the calcareous sponge larva as it undergoes metamorphosis into the sponge adult (Haeckel 1872). Thus, for many years gastrulation in sponges has been associated with metamorphosis, the time at which the choanocyte chambers are formed; yet in other animals, gastrulation is an embryonic process during which the inner and outer layers of the animal are formed, the inner usually gives rise to the gut and the outer gives rise to the skin and nervous tissue. However, in many cnidarians, the formation of the two layers and the formation of a gut are often dissociated (Byrum and Martindale 2004) so that gastrulation is in fact a two-step process, as it may be understood in sponges. Yet another viewpoint altogether is that gastrulation is really just the epithelialization of embryos (Cherdantsev and Krauss 1996). If epithelialization is the true gastrulation event, then metamorphosis concerns the transdifferentiation of already differentiated cells (Amano and Hori 1996; Leys and Degnan 2002).

However, whereas comparisons of gastrulation based on morphology are difficult even in many model systems, comparison of gene expression patterns and functions suggests that some general molecular pathways are used to pattern bilaterian embryos (Price and Patel 2004). Similar molecular approaches may help in understanding homology in poriferan and metazoan development.

Molecular developmental biology

Comparison of transcription factors that regulate the expression of genes during development has provided an evolutionary perspective to relationships among basal metazoan phyla. Relatively few homologues of developmental genes are known in the Porifera, but the situation should change with a genome project currently underway to sequence *Reniera* sp., a ceractinomorph demosponge (Rokhsar, Joint Genome Institute), and the production of numerous EST databases. Transcription factors that have been identified in different species of Porifera include several homeoboxes of the POU class (Seimiya et al. 1994), PAX class (Hoshiyama et al. 1998), NK-2 class (Seimiya et al. 1994), Msx class (Seimiya et al. 1994), *Six* gene class (Bebenek et al. 2004), *Bsh* and *Bar* gene families (Hill et al. 2004), *Iroquois* gene (Perovic et al. 2003), and several new classes such as *Prox* (Seimiya et al. 1997) and *Sycox* (Manuel and Le Parco 2000). More recently, homologues of the *T-box* gene have also been identified (Adell et al. 2003*a*; Manuel et al. 2004).

Fig. 14. Different patterns of cleavage in the Porifera. (A) Chaotic cleavage, (B) table palintomy, (C) radial cleavage, and (D) polyaxial cleavage (straight lines depict the axes of symmetry of the embryo). (A.V. Ereskovsky, unpublished data.)



The presence of a diversity of homeobox genes in sponges suggests that these genes diverged very early during the evolution of the Metazoa (Manuel and Le Parco 2000), but remarkably the function of at least some is highly conserved. Phylogenetic studies suggest that the *EmH-3* gene from the freshwater sponge *Ephydatia muelleri* (Lieberkühn, 1856) (Coutinho et al. 1994) belongs to the *Tlx* homeobox gene family (Coutinho et al. 2003). Expression of the promoter region encoded by the *EmH-3* gene in a human cell line (erytholeukemia K562 cells), associated with T-cell leukemias, demonstrated that the sponge promoter was able to carry out the same pattern of expression and down-regulation upon differentiation as the endogenous promoter (Coutinho et al. 2003). *Ephydatia muelleri* is a sponge that can be hatched in the laboratory from 1 mm diameter overwintering cysts called gemmules (Kilian 1952). In the sponge, *EmH-3* gene is up-regulated after hatching of the gemmule and is strongly expressed in pluripotent archaeocytes, but not at all in pinococytes or choanocytes both differentiated cell types that form epithelial layers (Richelle-Maurer and Van De Vyver 1999). Furthermore, treatment with retinoic acid (RA), a known morphogen and regulator of gene expression in vertebrates, causes down-regulation of the *EmH-3* gene (Nikko et al. 2001). In RA-treated developing sponges, archaeocytes appear as they do immediately upon hatching, while choanocytes and an aquiferous system are entirely absent. These morphological characteristics, as well as *EmH-3* gene expression, are regained upon removal of RA.

Another approach has been to examine gene and protein expressions in primorph cultures, long-term aggregates of the sponge tissue that were developed as a model system for understanding gene expression during the construction of the adult sponge body plan (Le Pennec et al. 2003). The homoebox gene *Iroquois* is expressed in the canals and chambers of the developing sponge (Perovic et al. 2003). Expression is up-regulated in response to increased water current, a stimulus which causes the formation of pore-like canals in the primorph. A similar result was found for expression of the Brachyury protein. *Brachyury* gene is a T-box transcription factor that is known to have a role in notochord and mesoderm formation in vertebrates, and is suspected to be involved in axis determination in more basal metazoans (Manuel et al. 2004). Phylogenetic analysis of the T-box genes *Sd-bra* and *Sd-Tbx2* from the marine hadromerid sponge *Suberites domuncula* (Olivi, 1792) suggests that these genes are basal within the Metazoa (Adell et al. 2003*a*). The Sd-bra protein is expressed in adult sponges and in adherent primorph cultures, but is down-regulated in primorphs that lack canals or choanocyte chambers, suggesting that it is involved in the formation of these structures (Adell et al. 2003*a*, 2003*b*).

The lack of a reliable oviparous sponge with which to explore the expression of genes during development has hampered efforts to develop in situ hybridization protocols for this phylum. Even in brooding sponges, the reproductive period is often uncertain or embryos and larvae are not numerous. *Reniera* sp. is a model system that overcomes these difficulties. *Reniera* sp. is a haplosclerid demosponge that is closely related to many intertidal haplosclerids of the genus *Haliclona* world-wide. The warm-water habitat of *Reniera* sp. at the southern tip of the Great Barrier Reef means that it is reproductive year-round (Leys and Degnan 2001). Embryos are brooded in large chambers within the adult, and mature larvae are released when the sponge is left in still, warm water.

The ability to obtain large numbers of larvae has allowed development of the first in situ hybridizations that show patterns of genes expressed during larval development (Degnan et al. 2005). EST analysis reveals that a range of structural, biosynthetic, and cell communication genes are expressed during the development of *Reniera* sp. (Larroux et al. 2006). Whole-mount in situ hybridization analysis shows that a number of these genes are expressed in complex spatial patterns. Ferritin and procollagen lysyl hydroxylase (E.C. 1.14.11.4) are expressed in different regions of the outer columnar epithelium, and procollagen lysyl hydroxylase is the only gene of those studied that is expressed in the middle sub-epithelial layer, a collagen-rich region where cells form a band around the circumference of the larva (Leys and Degnan 2001). Galectin and DEAD nuclear RNA helicase (E.C. 3.6.1.-) appear to be expressed by different cells of the inner cell mass. Similarly, B-ZIP-1 is expressed in only a few cells at the outer rim of the inner cell mass (Larroux et al. 2006).

Unlike studies of development from gemmules or aggregates, the study of development in *Reniera* sp. allows comparison with eumetazoan embryogenesis and metamorphosis (Leys and Degnan 2002). Based on the type of embryogenesis observed in *Reniera* sp. — formation of anterior–posterior polarity and simple tissue layers — and the expression of a wide range of transcription factors and their restricted spatial and temporal expression patterns in the sponge larva, it is inferred that the last common ancestor to the Metazoa had both a fixed body axis and organized, albeit simple, multicelluar tissues (Degnan et al. 2005).

Summary

Developmental data from the *Reniera* larva, primorph aggregates, and hatched gemmules all suggest that patterning systems are widespread within the Porifera. Interpreting gene expression patterns and function will nevertheless depend on a solid understanding of the events that occur during embryogenesis, larval differentiation, and metamorphosis in a wide range of sponges. The results of the present review demonstrate that comparative embryology is a necessary part of modern sponge phylogenetics and is important to new views of the evolution and development of basal metazoans.

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