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Six major steps in animal evolution: are we derived sponge larvae?

Claus Nielsen

Zoological Museum (The Natural History Museum of Denmark, University of Copenhagen), Universitetsparken 15, DK-2100 Copenhagen, Denmark

Correspondence (email: cnielsen@snm.ku.dk)

SUMMARY A review of the old and new literature on animal morphology/embryology and molecular studies has led me to the following scenario for the early evolution of the metazoans.

The metazoan ancestor, "choanoblastaea," was a pelagic sphere consisting of choanocytes. The evolution of multicellularity enabled division of labor between cells, and an "advanced choanoblastaea" consisted of choanocytes and nonfeeding cells. Polarity became established, and an adult, sessile stage developed. Choanocytes of the upper side became arranged in a groove with the cilia pumping water along the groove. Cells overarched the groove so that a choanocyte chamber was formed, establishing the body plan of an adult sponge; the pelagic larval stage was retained but became lecithotrophic. The sponges radiated into monophyletic Silicea, Calcarea, and Homoscleromorpha. Homoscleromorph larvae show cell layers resembling true, sealed epithelia. A homoscleromorph-like larva developed an archenteron, and the sealed epithelium made extracellular digestion possible in this isolated space. This larva became sexually mature, and the adult sponge-stage was abandoned in an extreme progenesis. This eumetazoan ancestor, "gastraea," corresponds to Haeckel's gastraea. *Trichoplax* represents this stage, but with the blastopore spread out so that the endoderm has become the underside of the creeping animal. Another lineage developed a nervous system; this "neurogastraea" is the ancestor of the Neuralia. Cnidarians have retained this organization, whereas the Triploblastica (Ctenophora+Bilateria), have developed the mesoderm. The bilaterians developed bilaterality in a primitive form in the Acoelomorpha and in an advanced form with tubular gut and long Hox cluster in the Eubilateria (Protostomia+Deuterostomia).

It is indicated that the major evolutionary steps are the result of suites of existing genes becoming co-opted into new networks that specify new structures.

The evolution of the eumetazoan ancestor from a progenetic homoscleromorph larva implies that we, as well as all the other eumetazoans, are derived sponge larvae.

INTRODUCTION

Many questions about the origin and early radiation of the metazoans are still unanswered (Martindale 2005). It seems to be accepted that the Metazoa are monophyletic and have evolved from choanoflagellate-like ancestors (Ruppert et al. 2004; Steenkamp et al. 2006). However, there is no consensus about the evolution of the metazoans or of their body plans. Morphology and biology, especially feeding, of the ancestral metazoan and the establishment of the pelago-benthic life cycle with lecithotrophic larvae of the sponges must be considered in discussions of early animal evolution. Also, the recent molecular studies of animal phylogeny, which tend to regard the sponges as paraphyletic, call for a renewed consideration of early animal radiation.

THE NEAREST NEIGHBORS: THE CHOANOFLAGELLATES

Morphological and molecular studies now agree that the Metazoa is the sister group of the Choanoflagellata (Nielsen 2001; King 2004; Philippe et al. 2005; Steenkamp et al. 2006), although an in-group position within the Choanoflagellata is indicated in some analyses (Medina et al. 2003).

Most choanoflagellates are solitary and free-living or sessile (Fig. 1A), but several species form colonies (Leadbeater and Thomsen 2000) (Fig. 2A). Some colonies have cells on branched stalks, whereas others are free, flat, or spherical with the cells held together by the collars or situated in a gelatinous matrix. Some colonies are spherical with the collars facing the periphery, are but *Diaphanoeca sphaerica* has collars facing the lumen of the colony and resembles a free-swimming choanocyte chamber of a sponge (Thomsen 1982). *Proterospongia choanojuncta* shows a variety of forms, motile or sessile, solitary cells of normal size, minute swarmers, and free-living, plate-shaped colonies (Leadbeater 1983b). In some species of *Proterospongia*, certain cells may lose the collar and wander into the matrix, but their fate and function are unknown and their internal position can only be temporary, because they cannot feed. The collar complexes consist of an undulating cilium, which in some cases have a fibrillar vane (Leadbeater 2006), surrounded by a circle of long, contractile, actin-containing microvilli, which function as a sieve in

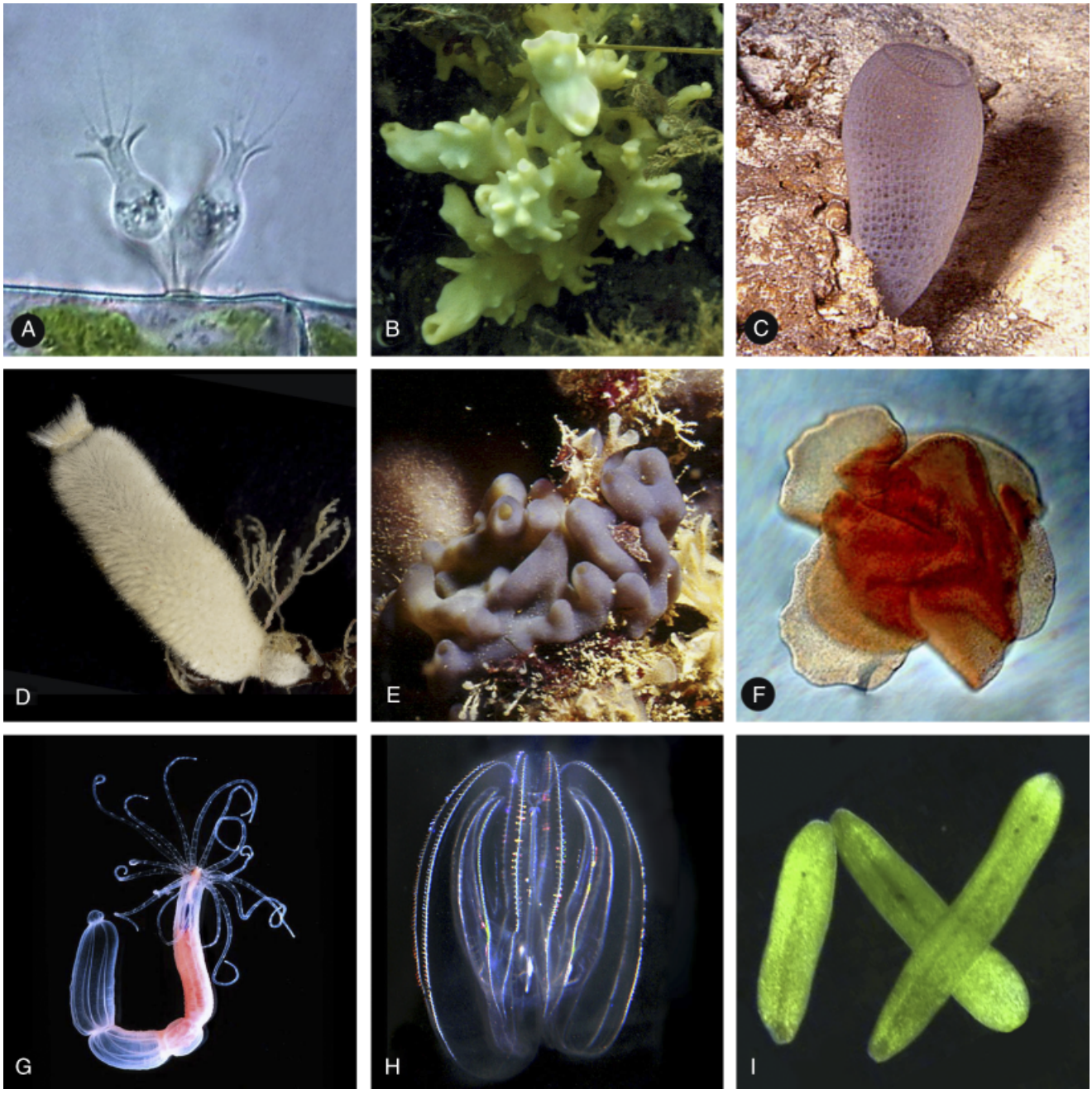

Fig. 1. "Dramatis personae": Representatives of the "lower animal groups" discussed in this paper. (A) Choanoflagellata: *Salpingoeca* (Michael Plewka, Plingfactory.de). (B) Silicea: Demospongiae: *Halichondria* (Martin Macnaughton, University of Copenhagen). (C) Silicea: Hexactinellida: *Euplectella* (Craig Young, University of Oregon). (D) Calcarea: *Sycon* (Fredrik Pleijel, Tjärnö Marine Biological Laboratory). (E) Homoscleromorpha: *Oscarella* (Wilfried Bay-Nouailhat, Mer and Littoral, Concarneau). (F) Placozoa: *Trichoplax* (Ana Signorovitch, Yale University). (G) Cnidaria: *Nematostella* (Timm Nüchters, University of Vienna). (H) Ctenophora: *Mnemiopsis* (Birgit Thorell, University Copenhagen). (I) Acoelomorpha: *Convoluta* (*Symsagittifera*) (Xavier Bailly, Station Biologique Roscoff).

particle collection (Hibberd 1975; Leadbeater 1983a). The ciliary basal system has various shapes, but some species have an accessory centriole and some a short striated root (Karpov and Leadbeater 1998). Some species are naked and others have an organic theca, but many species have a lorica consisting of costal strips impregnated with silica, which develop in membrane-bounded vesicles and subsequently become arranged into the basket-like lorica (Leadbeater 1987).

Sexual reproduction has not been reported, but gametes and fertilization may just have gone unnoticed because no one

has been looking for it. Practically all metazoan groups have sexual reproduction with eggs and sperm, and sexual reproduction is widespread in Fungi and many other eukaryote groups, so one must assume that the ancestral metazoan had sexual reproduction with eggs and sperm.

FIRST MAJOR STEP: THE EVOLUTION OF MULTICELLULARITY (METAZOA)

The evolution of multicellular metazoans from a colonial choanoflagellate (Figs. 2 and 3) was first suggested by Metschnikoff (1886) and has been taken up by a number of more recent authors (Remane 1963; Ivanov 1971; Buss 1987). However, the evolution from the early holopelagic ancestor to the sponges with indirect development and lecithotrophic larvae has not been much discussed.

If the metazoans are an in-group of the choanoflagellates, the ancestral metazoan (the urmetazoan (Müller 2001; King 2004)) was, of course, a specialized choanoflagellate, and if the living choanoflagellates are monophyletic, the common ancestor of the two groups may nevertheless have looked much like a colonial choanoflagellate (Steenkamp et al. 2006). The most basal metazoans, the sponges, feed with choanocytes, which both structurally and functionally are very similar to choanoflagellates (Maldonado 2004), and in agreement with almost all modern authors, I consider the collared units of choanoflagellates and sponges to be homologous. This indicates that the first metazoan consisted of choanocytes, which shared the nutrients with neighboring cells. The colony consisted of cells originating from one cell, which was probably a fertilized egg. This early metazoan (Fig. 2B) could appropriately be called choanoblastaea, to emphasize its structure and its feeding mode, which are both

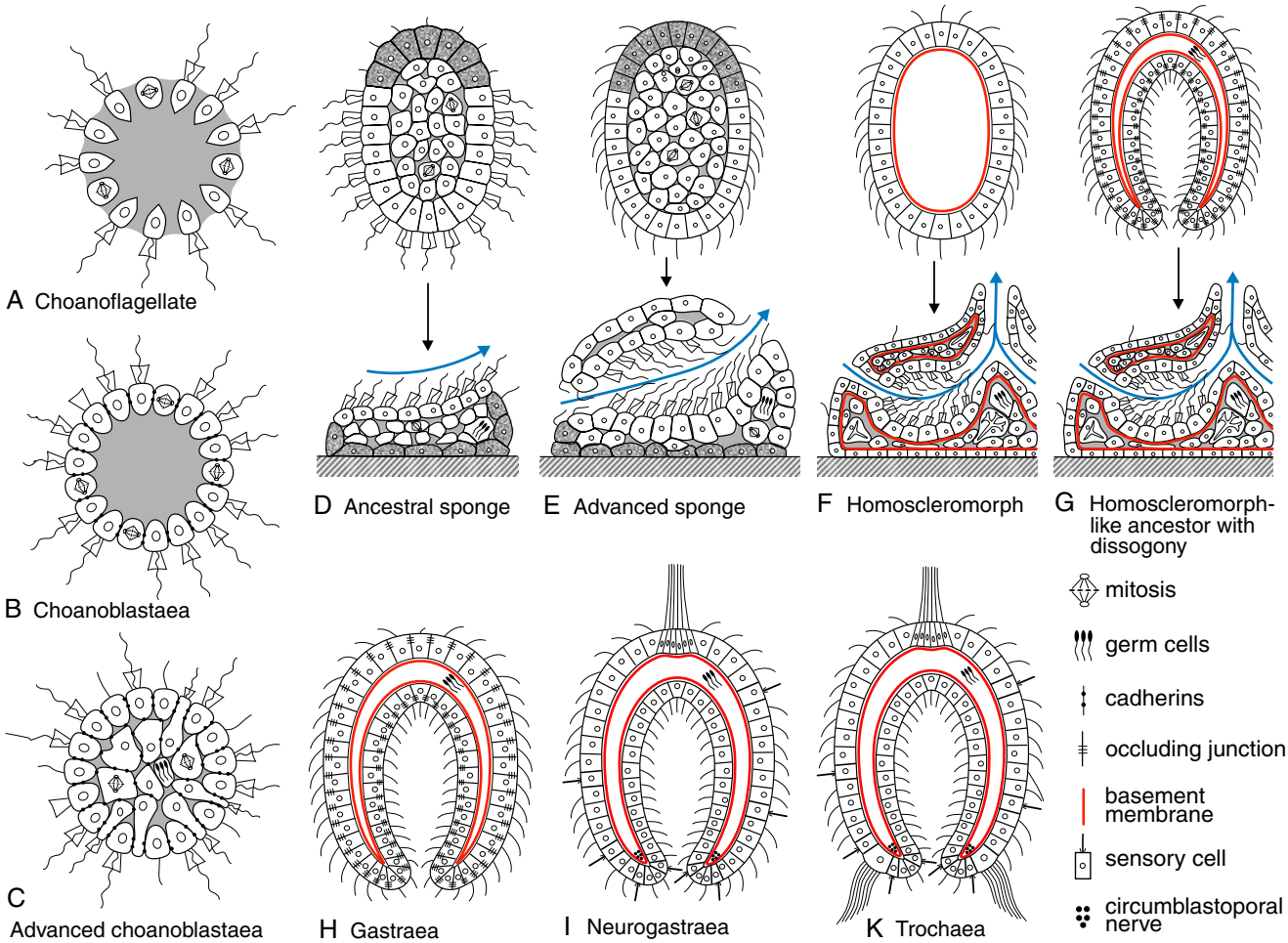

Fig. 2. Diagrammatic representations of various stages in the evolution of the bilaterians from a choanoflagellate ancestor to the major bilaterian groups as proposed here. Extracellular matrix gray. The characters related to cell contacts are only indicated in the first stages after appearance. The blue arrows indicate the major water currents of the sponges; the currents around the individual choanocytes are not drawn.

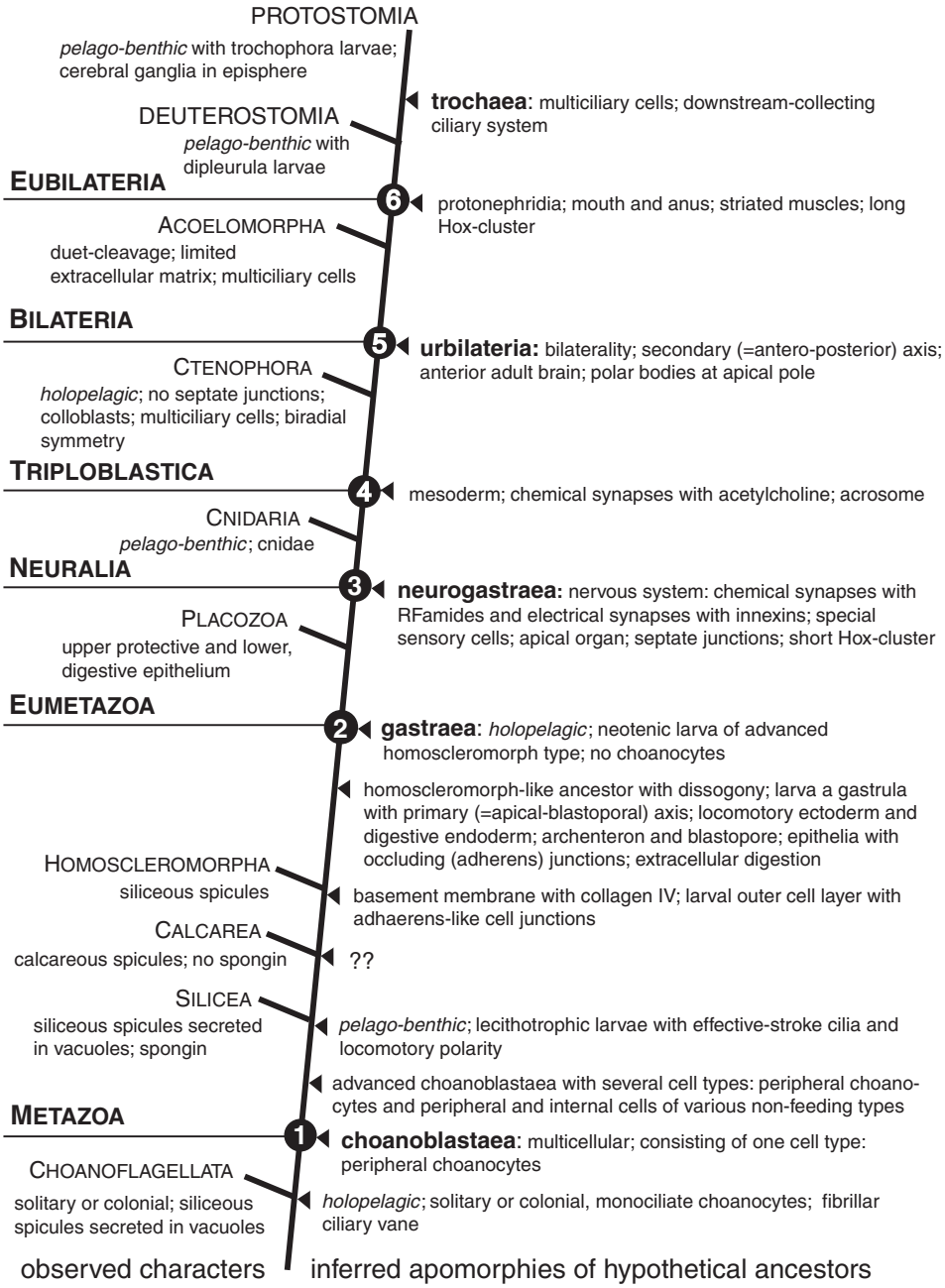

Fig. 3. Phylogenetic diagram of the origin and early evolution of the metazoans. Black arrowheads indicate apomorphies. The numbers indicate the six major evolutionary steps discussed in the text. The main ancestral stages are in boldface.

different from those of Haeckel's well-known blastaea (Haeckel 1874). Ciliated sensory cells or protonephridial cells with a circle of shorter or longer microvilli are here classified as collar cells, but not as choanocytes (Nielsen 2001). A character shared between some choanoflagellates (Leadbeater 1987) and the siliceous sponges (Leys 2003a) is the secretion of siliceous spicules in small vacuoles.

The principal evolutionary step leading to the metazoan grade of organization is the establishment of multicellularity, where nutrients can be transported between cells. The choanoblastaea was most probably a small hollow sphere with cells organized as in an epithelium. This organization must depend on molecules that hold the cells together, such as cadherins (Tyler 2003), molecules that attach the basal side of the cell to an extracellular matrix, such as integrins (Burke 1999), and molecules that make cell recognition, cell communication, and transport between the cells possible. Several adhesion and signaling protein families, such as tyrosine

Table 1. Recent molecular-based phylogenies of the basal metazoan groups

Sponges:	
Peterson and Eernisse (2001)	18S: Hexactinellida (Demospongiae (Ctenophora (Clacarea (Placozoa (Cnidaria+Bilateria)))))
Medina et al. (2001)	18S+28S: ((Demospongiae+Hexactinellida) Calcarea)+(Ctenophora (Cnidaria+Bilateria))
Borchiellini et al. (2001)	18S: Hexactinellida (Demospongiae (Calcarea (Ctenophora (Placozoa + Cnidaria))))
Cavalier-Smith and Chao (2003)	18S: (Calcarea (Hexactinellida+Demospongiae))+(Ctenophora (Placozoa+Cnidaria))
Medina et al. (2003)	18S: (Demospongiae+Hexactinellida)+(Calcarea (Ctenophora ((Cnidaria+Placozoa)+Bilateria)))
Manuel et al. (2003)	18S: (Demospongiae+Hexactinellida)+(Calcarea (Ctenophora (Cnidaria+Bilateria)))
Borchiellini et al. (2004)	18S: Calcarea+Demospongiae+Homoscleromorpha+(Cnidaria + Ctenophora)
Glenner et al. (2004)	18S: ((Calcarea+Demospongiae)+Ctenophora+((Placozoa+Cnidaria)+Bilateria)
Wallberg et al. (2004)	18S: Silicea+Calcarea+(Ctenophora (Cnidaria (Placozoa+Bilateria)))
Wang and Lavrov (2007)	mitochondrial: (Placozoa (Homoscleromorpha+Demospongiae) + Cnidaria))+Bilateria
Sperling et al. (2007)	nuclear: Demospongiae (Calcarea (Homoscleromorpha (Cnidaria+Bilateria)))
Placozoa:	
Peterson and Eernisse (2001)	18S: Hexactinellida (Demospongiae (Ctenophora (Clacarea (Placozoa (Cnidaria + Bilateria)))))
Podar et al. (2001)	18S: Calcarea (Ctenophora (Placozoa (Cnidaria+Bilateria)))
Borchiellini et al. (2001)	18S: Hexactinellida (Demospongiae (Calcarea (Ctenophora (Placozoa+Cnidaria))))
Cavalier-Smith and Chao (2003)	18S: (Calcarea (Hexactinellida+Demospongiae))+(Ctenophora (Placozoa+Cnidaria))
Medina et al. (2003)	18S: (Demospongiae+Hexactinellida)+(Calcarea (Ctenophora ((Cnidaria+Placozoa)+Bilateria)))
Wallberg et al. (2004)	18S: Silicea+Calcarea+(Ctenophora (Cnidaria (Placozoa+Bilateria)))
Glenner et al. (2004)	18S: ((Calcarea+Demospongiae)+Ctenophora+((Placozoa+Cnidaria)+Bilateria)
Wang and Lavrov (2007)	mitochondrial: (Placozoa (Demospongiae+Cnidaria))+Bilateria
Dellaporta et al. (2006)	proteins: (Placozoa (Demospongiae+Cnidaria))+Bilateria
Ctenophora:	
Peterson and Eernisse (2001)	18S: Hexactinellida (Demospongiae (Ctenophora (Clacarea (Placozoa (Cnidaria+Bilateria)))))
Medina et al. (2001)	18S+28S: ((Demospongiae+Hexactinellida) Calcarea)+(Ctenophora (Cnidaria+Bilateria))
Borchiellini et al. (2001)	18S: Hexactinellida (Demospongiae (Calcarea (Ctenophora (Placozoa+Cnidaria))))
Podar et al. (2001)	18S: Calcarea (Ctenophora (Placozoa (Cnidaria+Bilateria)))
Cavalier-Smith and Chao (2003)	18S: (Calcarea (Hexactinellida+Demospongiae))+(Ctenophora (Placozoa+Cnidaria))
Medina et al. (2003)	18S: (Demospongiae+Hexactinellida)+(Calcarea (Ctenophora ((Cnidaria+Placozoa)+Bilateria)))
Manuel et al. (2003)	18S: (Demospongiae+Hexactinellida)+(Calcarea (Ctenophora (Cnidaria+Bilateria)))
Borchiellini et al. (2004)	18S: Calcarea+Demospongiae+Homoscleromorpha+(Cnidaria + Ctenophora)
Wallberg et al. (2004)	18S: Silicea+Calcarea+(Ctenophora (Cnidaria (Placozoa+Bilateria)))
Glenner et al. (2004)	18S: ((Calcarea+Demospongiae)+Ctenophora+((Placozoa+Cnidaria)+Bilateria)
Steenkamp et al. (2006)	several: Demospongiae (Ctenophora (Cnidaria+Bilateria))
Total evidence	
Peterson and Eernisse (2001)	18S+morphology: (Hexactinellida+Demospongiae)+Calcarea (Ctenophora (Placozoa (Cnidaria+Bilateria)))
Eernisse and Peterson (2004)	18S+morphology: Silicea (Calcarea (Ctenophora (Cnidaria (Placozoa+Bilateria))))
Steenkamp and Baldauf (2004)	18S+proteins+morphology: Hexactinellida+Demospongiae+(Calcarea (Ctenophora (Cnidaria+Bilateria)))
Glenner et al. (2004)	18S+morphology: Calcarea (Demospongiae (Ctenophora ((Placozoa+Cnidaria)+Bilateria)))
Peterson et al. (2005)	18S+morphology: Demospongiae (Calcarea (Ctenophora (Cnidaria+Bilateria)))
Peterson and Butterfield (2005)	several+morphology: Demospongiae (Clacarea (Cnidaria+Bilateria))

The respective groups are indicated by underlining. The data use are indicated as 18S and 28S rRNA, mitochondrial genes, nuclear genes, protein genes, several genes, and morphology.

kinases and cadherins, are present both in choanoflagellates and in metazoans, showing that these molecules have already existed with other functions before the multicellular grade and have been co-opted for their present functions in the metazoans (King et al. 2003).

Multicellularity enables the evolution of "the advanced choanoblastaea" (Fig. 2C) consisting of peripheral, feeding choanocytes, and nonfeeding cells with parallel orientation and interconnecting molecules, like the cells of an epithelium but without occluding junctions, and internal cells of various structures and functions. This ancestor resembles the "early phagocytella" pictured by Ivanov (1971, fig. 11), although cell contacts were not specified. Various types of cell junctions, some characterized as transient (Green and Bergquist 1979), have been described from sponges (Maldonado 2004), but none of them are of the permanent, occluding type characteristic of true epithelia (Tyler 2003). The scattered observations of "septate junctions" in a few sponges are all from

specialized cell groups and not from the pinacoderm of the adults or the ciliated outer layer of the larvae, which would be more comparable to the true epithelia of the eumetazoans.

Ontogeny of the early metazoan involved multiple divisions of choanocytes, but neither choanoflagellates nor monociliate animal cells divide while ciliated (Margulis 1981; Buss 1987), possibly because both centrioles are needed for the organization of the mitotic spindle. A dedifferentiation of choanocytes obviously impedes both movement and feeding of the organism. Growth, including multiplication of choanocytes, could be eased if internal cells would account for the division of cells, some of which could then migrate to the periphery and differentiate. Similar thoughts led Margulis (1981, p. 272) to propose that "the failure to solve the problem of simultaneous division and motility on the single-cell level may have led, in several groups, to the origin of eukaryotic multicellularity."

The planula-like advanced choanoblastaea was feeding with the peripheral choanocytes, and it would be quite misleading to describe the internalization of some cells as a gastrulation, which in the eumetazoans is the process separating the digestive endoderm from the locomotory and protective ectoderm (Ereskovsky and Dondua 2006). None of the sponges have an absorptive/digestive inner epithelium like the eumetazoan gut.

MOLECULAR AND COMBINED STUDIES OF EARLY METAZOAN RADIATION (TABLE 1)

Almost all recent molecular studies agree on the monophyly of Metazoa and Bilateria. However, there is no consensus about the topology of the lower part of the metazoan phylogeny. Several different "trees" have been presented, but many of the older studies are based on limited taxon sampling and statistical methods that are now considered insufficient. I have therefore limited the discussion to papers from this century, with emphasis on studies including several sponges and *Trichoplax*; older studies are summarized in Wallberg et al. (2004). Special emphasis has been placed on the recent study of Sperling et al. (2007), which is one of the few studies that includes the homoscleromorphs, but unfortunately not *Trichoplax* and the ctenophores. It is based on a very large selection of nuclear-coded genes analyzed with the newest statistical methods.

Several older and a few more recent analyses show a clade called Diploblastica, comprising sponges, *Trichoplax*, cnidarians, and ctenophores (e.g., Zrzavý and Hypša 2003; Dellaporta et al. 2006; Wang and Lavrov 2007). The topology of this clade is quite variable and it is not supported by morphology. It will not be discussed here.

The various sponge groups are situated at the base of the tree in almost all analyses, but the traditional "phylum Porifera" is usually not monophyletic. Demosponges and hexcatinellids are usually sister groups and occupy a basal position. The position of Calcarea is more uncertain, but a number of analyses place them as the sister group to the Eumetazoa. Homoscleromorpha are only included in a few analyses; the study of Sperling et al. (2007) shows them as a sister group of Eumetazoa, and this finds support from morphology.

Trichoplax is in almost all analyses found to be closely related to the cnidarians, although the exact position is not firmly indicated. The morphological characters indicate a position as the sister group of the Cnidaria+Triploblastica.

The most problematic group is the Ctenophora. Most analyses place them as the sister group of the remaining eumetazoans, whereas morphological and embryological characters suggest that they are the sister group of Bilateria.

EVOLUTION OF THE EARLIEST METAZOANS, THE SPONGES

The sponges have always been considered to be the most "primitive" group of animals, as also indicated by the old name Parazoa. They are multicellular but have only a comparatively low number of cell types, and epithelia with occluding cell junctions and Hox genes are not found (Tyler 2003; Richelle-Maurer et al. 2006). All sponges have ciliated, lecithotrophic larvae, and sessile adults with choanocytes situated in internal chambers. The few exceptions, such as the carnivorous *Asbestopluma*, are clearly specializations (Vacelet and Duport 2004).

The evolution of the pelago-benthic life cycle from the holopelagic cycle of the advanced choanoblastaea must have gone through a stage where pelagic adults acquired a polarity and settled with the pole without choanocytes. This enabled the internalization of the choanocytes, which were no longer locomotory (Lameere 1901; Ivanov 1971). The first stage of the internalization could have been a groove with choanocytes that propelled the water along the groove (Fig. 2D); this shape of the choanocyte layer would ensure a unidirectional common current that prevented recirculation of already filtered water. The groove could then become overarched by cells to form a tube, finally with the choanocytes forming a small chamber (Fig. 2E). This restructuring would both enhance the feeding currents and give the collar complexes a more protected position. The pelagic larval stage could then lose the choanocytes and become lecithotrophic. They developed a new type of ciliation, with locomotory effective-stroke cilia coordinated in the metachronal pattern seen in modern sponge larvae and in larvae and adults of many eumetazoans (Nielsen 1979).

The sponges are generally regarded as a monophyletic group, the phylum Porifera, but newer morphological and

especially the molecular studies indicate a more complicated story. There seem to be four monophyletic groups, Demospongiae, Hexactinellida, Homoscleromorpha, and Calcarea, but their relationships are still debated.

The Demospongiae (Fig. 1B) (exclusive of Hexactinellida and Homoscleromorpha) are generally accepted as monophyletic. Their skeleton usually consists of siliceous spicules embedded in a meshwork of spongin, which is a demosponge-specific collagenous protein (Aouacheria et al. 2006); both spicules and spongin may be absent, and some types have a heavily calcified basal structure (Hooper and Van Soest 2002). The siliceous spicules are secreted in vacuoles both in larvae and adults (Leys 2003a). Choanocytes are arranged in chambers with incurrent and excurrent canals. A fibrillar ciliary vane has been reported from some species (Brill 1973; de Vos et al. 1991). The cilia lack a striated root; in some species, they show an accessory centriole (Woollacott and Pinto 1996). Cells are held together by cadherin–catenin complexes, as seen in the eumetazoans (Tyler 2003). Cell junctions of other types have been characterized as transient, and permanent occluding junctions have not been reported (Green and Bergquist 1979; Tyler 2003). Cells are attached to the extracellular matrix through integrins as in the eumetazoans (Brower et al. 1997; Tyler 2003). There is no report of dedicated sensory cells, and nerve cells are not present; the photosensitive–ciliated cells of the demosponge larvae are at the same time effectors, which, by changing the posture of the cilia, change the direction of swimming (Leys and Degnan 2001). The sperm lacks an acrosome, although a somewhat acrosome-like structure has been pictured from *Crellomima* (Ereskovsky 2005).

The tetractinomorphs are predominantly oviparous, whereas the ceractinomorphs are mainly viviparous (Hooper and Van Soest 2002). The larvae are planuloid, almost totally ciliated, with the effective-stroke cilia beating in a metachronal pattern, which makes the larvae rotate around the longitudinal axis (Nielsen 2001; Leys et al. 2002). The ciliated cells lack a striated ciliary root, but an accessory centriole is found in some species (Woollacott and Pinto 1996). A weak collagenous basement membrane is seen in some species, but it is apparently without collagen IV (Aouacheria et al. 2006). Nonfunctioning choanocyte chambers develop already in the embryos in several species (Meewis 1940; Saller 1988), and siliceous spicules are secreted in vacuoles in embryos of many species (Leys 2003a). After a short pelagic period, the larvae settle with the anterior pole. The ciliated cells dedifferentiate, become internalized and redifferentiate as choanocytes, for example, in *Amphimedon* (Leys and Degnan 2002, as *Reniera*), but are cast off or resorbed in other species (Woollacott and Pinto 1996).

The Hexactinellida (Fig. 1C) have a very unusual structure with a syncytial body with partially isolated "collar complexes" instead of choanocytes (Mackie and Singla 1983; Leys 2003b). The exclusively siliceous skeleton is initially secreted in vacuoles in the syncytium (Leys 2003a). A ciliary fibrillar vane has been reported in *Aphrocallistes* (Mehl and Reiswig 1991). The cilia lack accessory centriole and striated root both in larvae and adults (Leys et al. 2006). The fully developed sperm has not been described.

All species appear to be viviparous. The embryology is mainly known through studies of *Oopsacus* (Boury-Esnault et al. 1999; Leys et al. 2006). The first cleavages are holoblastic, and the 32-cell stage is a hollow blastula, which becomes two-layered and finally compact through delamination of large interior macromeres. The outer cells become connected, and an equatorial band of cells is at first monociliate but later becomes multiciliate. Lamellipodia from the macromeres extend over the outer cells to form a thin outer layer penetrated by the cilia. Some micromeres ingress in the posterior region and differentiate into choanocytes, which subsequently fuse with the inner syncytium. Finally, the whole larva is a syncytium. Spicules develop already at the embryonic stage (Leys 2003a). Settling has not been described.

The embryology indicates that the hexactinellids are derived from cellular ancestors and that the majority of the molecular analyses indicate a sister-group relationship with the demosponges. The two groups are here treated together under the name Silicea (Leys et al. 2006) (Fig. 3).

The monophyly of Calcarea (Fig. 1D) appears unquestioned (Dohrmann et al. 2006). The skeleton consists of calcareous spicules in a mesenchymatous tissue without spongin (Aouacheria et al. 2006). The pinacocytes are tightly joined but septae are generally absent (Eerkes-Medrano and Leys 2006). Septate-like junctions between sclerocytes have been observed in *Sycon* (Ledger 1975) and between choanocytes in *Clathrina* (Green and Bergquist 1979). However, the cell junctions are generally described as transient (Green and Bergquist 1979). Cilia of the larvae have an accessory centriole and a long striated root, but these structures are missing in the adult choanocytes (Woollacott and Pinto 1996). A fibrillar ciliary vane has been reported in *Sycon* (Simpson 1984).

All species are viviparous, and the fertilization and development of the calcaronean *Sycon*, with a modified choanocyte functioning as a carrier cell for the sperm and the development through an amphiblastula stage, are shown in most textbooks (see also Franzen 1988; Leys and Eerkes-Medrano 2005). The planktonic larva has an anterior region with long cilia and a posterior region with granular cells. The larvae usually settle with the anterior pole and immediately invaginate the ciliated cells, which de-differentiate but rapidly redifferentiate as choanocytes or amoebocytes (Leys and Eerkes-Medrano 2005). Spicules are only found in the adult stage. However, this type of development is only known with certainty from species of the Calcaronea. In the less well-studied Calcinea, some observations suggest the presence of a

carrier cell (Johnson 1979), but the embryology resembles that of some demosponges (Leys and Ereskovsky 2006).

The small group Homoscleromorpha (Fig. 1E) comprises "primitive" types, such as *Oscarella*, with very little mesohyl and no spongin skeleton or spicules, and more complex types, such as *Plakina*, which have a skeleton of siliceous spicules (Muricy and Díaz 2002). They show a number of characters not seen in other sponges (Boury-Esnault et al. 1984; Muricy and Díaz 2002). A basement membrane with collagen IV underlies both choanoderm and pinacoderm of the adults and lines the blastocoel of the larvae (Boute et al. 1996; Boury-Esnault et al. 2003). The fully developed sperm has an acrosome-like structure (Baccetti et al. 1986; Boury-Esnault and Jamieson 1999). The ciliated cells of the larvae show desmosome-like junctions. There is an accessory centriole in all ciliated cells and a striated root in the larval ciliated cells (Boury-Esnault et al. 2003).

The acrosome-like structure is reminiscent of the acrosome of the Triploblastica, but if these structures are interpreted as homologous, the acrosome must have been lost in the cnidarians. This appears less likely, although the acrosome has been lost, for example, in some chitons (Franzén 1987). The structure of *Trichoplax* sperm could cast light on this question. *Oscarella* has internal fertilization, and development goes through a completely ciliated coeloblastula with a well-developed basement membrane with collagen IV (Boury-Esnault et al. 2003). Its cells are a little taller than wide and the blastula is highly folded. Just before hatching, the blastula unfolds and the cells become tall and narrow. The newly hatched larva is completely ciliated, and the cilia probably beat in the usual metachronal pattern (Boury-Esnault et al. 2003). There is a zone of "desmosome-like" cell junctions in the apical zone of the ciliated cells and longitudinal rows of other junctions between the middle parts of the cells (Leys and Ereskovsky 2006). Larvae of *Oscarella* (Meewis 1938, as *Halisarca*) settle with de-ciliated cells at the anterior pole; these cells degenerate while the body flattens and the whole upper side of the body de-ciliates too. The settling larva then attaches with the peripheral zone enclosing the ring of ciliated cells. These cells later lose the cilia, infold, and differentiate into choanocytes, whereas the excurrent canals develop from the upper (posterior) cell layer. Other homoscleromorphs, such as *Plakina* and *Corticium*, show variations over this theme (Ereskovsky et al. 2007).

Both the molecular studies and the morphological evidence summarized above indicate that the old "phylum Porifera" consists of three monophyletic groups and that the eumetazoans are the sister group of one of these groups, the Homoscleromorpha. The siliceous spicules found in Silicea and Homoscleromorpha, as well as in some of the choanoflagellates, are probably an ancestral metazoan character, which has been lost independently in Calcarea and Eumetazoa. The phylogeny of the basal part of the metazoan tree, indicated in Fig. 3, is based on a combination of these indications. The relative position of the Silicea and Calcarea is indicated by some molecular studies, but no firm morphological synapomorphy of Calcarea and Homoscleromorpha+Eumetazoa has been found. If this phylogenetic scheme is accepted, the term Porifera must disappear, but the vernacular term "sponges" can still be used, just like "invertebrates."

SECOND MAJOR STEP: THE ORIGIN OF SEALED EPITHELIA AND EXTRACELLULAR DIGESTION (EUMETAZOA)

The decisive evolutionary steps leading to the eumetazoans are formation of a true epithelium and gastrulation (Figs. 2G and 3). The scattered cadherin molecules that join the cells of the sponges become organized in belts near the apical pole if the epithelial cells, where they form occluding adherens junctions, which seal the true epithelia of the organism (Tyler 2003). The sponges are microphagous and capture small particles and digest them intracellularly. The evolution of sealed epithelia made extracellular digestion possible, but the digestive processes can only function in an enclosed space, and such a space could be formed by an invagination of the epithelium. This could be the origin of the archenteron, where larger captured particles could be digested by enzymes secreted by the endoderm, which became specialized as the digestive epithelium, whereas the ectoderm retained the locomotory function (Peterson et al. 2005; Rieger 2007; Sperling et al. 2007). The ciliated epithelia were probably able to reverse the effective stroke, as observed in many larval and adult eumetazoans (Holley and Shelton 1984; Lacalli and Gilmour 1990), so the transport of particles in and out of the archenteron could be carried out by the cilia.

The few molecular and combined analyses indicate that the Homoscleromorpha are the sister group of the eumetazoans. Adult sponges show none of the features characteristic of the eumetazoans, whereas ciliated "epithelia" with effective-stroke cilia with metachronal waves are found in the sponge larvae, which also show the accessory centriole and striated root characteristic of eumetazoan ciliated cells (Nielsen 2001). It seems impossible to derive eumetazoans from an adult sponge, so if the eumetazoans evolved from a sponge, it was probably through progenesis of a larva of a homoscleromorph-like organism (Maldonado 2004; Sperling et al. 2007).

The first step in the evolution toward the eumetazoans could have been that the larval stage of the homoscleromorph-like ancestor became sexually mature. This could have been through the process called dissogony. This "repeated" sexual maturity is seen in ctenophores, where the tiny, just-hatched stage is already sexually mature. The older juveniles have reduced gonads, which again become ripe in

the adults. If the eumetazoan ancestor had a similar reproductive cycle, the way was paved for the loss of the adult sponge stage and the establishment of the eumetazoan ancestor usually called gastraea (Fig. 2H).

PLACOZOA: *TRICHOPLAX*

The structure of the adult (Fig. 1F), with an underside of cells that are digestive and an upper side with cells with peculiar shiny spheres (Schierwater 2005), resembles an unfolded gastraea with the endoderm in contact with the substratum. This agrees well with the presence of Hox/Parahox (the gene *Trox-2*), Pax gene expression (Jakob et al. 2004; Hadrys et al. 2005; Schierwater 2005), and RFamide (Schuchert 1993) along the periphery, which should then represent the boundary between ectoderm and endoderm, i.e., the blastopore rim. However, no special sensory cells or nerve cells have been described. The structure of the *TriPaxB* gene indicates that it is basal to all *PaxA, PaxB*, and *PaxC* genes in cnidarians and bilaterians (Hadrys et al. 2005), in agreement with the phylogenetic position of *Trichoplax* as the sister group of the Neuralia (Fig. 3). Additional support is found in the presence of the Hox/Parahox-type gene *Gsx* in *Trichoplax* and a cnidarian, but not in sponges (or ctenophores) (Martinelli and Spring 2005). Extracellular digestion in the isolated space between the substratum and the lower epithelium has been demonstrated (Grell and Ruthmann 1991), but intracellular digestion has been observed too (Wenderoth 1986). Many cells of both epithelia are monociliate, and each cilium has an accessory centriole and a striated root. The cells are connected with simple zonula adherens. There is no basement membrane. A layer of more or less fluid extracellular matrix with interconnected fiber cells separates the two epithelia (Grell and Ruthmann 1991).

Sexual reproduction has been suggested by observations of oocyte/egg-like cells and putative early embryos, but sperm, later embryos, or larvae have never been observed. Genetic analyses indicate the presence of outbreeding (Signorovitch et al. 2005).

Trichoplax can be interpreted in two ways, either as the ancestral eumetazoan, which gave rise to the gastraea by infolding of the digestive "endoderm" (this is the "plakula theory" which derives all metazoans from a flat, two-layered plakula (Bütschli 1884; Grell 1974; Schierwater 2005)) or as a specialized gastraea that has become unfolded to digest benthic microorganisms. A flat, two-layered ontogenetic stage is not seen in any eumetazoan, which makes the "flattened gastraea" interpretation more likely. The molecular phylogenetic studies show no consistency about the position of *Trichoplax* (Table 1). The mitochondrial genome is more than twice as large as the average metazoan mitochondrial genome (Dellaporta et al. 2006), which could influence the molecular phylogenetic analyses. I have chosen to follow the phylogenetic indications from morphology (Fig. 3) and place *Trichoplax* as the sister group of Cnidaria, Ctenophora, and Bilateria (sometimes called Gastraeozoa, but this obviously depends on the interpretation of *Trichoplax*). However, its phylogenetic position at the base of the eumetazoans agrees with both interpretations, and it seems impossible to make a clear choice between the two theories as long as the ontogeny is unknown. The idea that *Trichoplax* could be a "derived cnidarian" is refuted by molecular analyses (Ender and Schierwater 2003).

THIRD MAJOR STEP: THE ORIGIN OF A NERVOUS SYSTEM (NEURALIA)

The absence of a nervous system in all sponges and *Trichoplax*, and the presence of a nervous system with both electrical and chemical synapses in all cnidarians, ctenophores, and bilaterians, mark an important step in metazoan evolution and sets *Trichoplax* aside from the remaining eumetazoans (Lichtneckert and Reichert 2007) (Fig. 3). Animals with a nervous system form a monophyletic unit, which to my knowledge has no formal name, and I therefore propose the name Neuralia. It seems important to distinguish the evolutionary stages of a gastraea without a nervous system from the more advanced stage having a nervous system with an apical organ and electrical and chemical synapses. To facilitate the discussion, I propose the name neurogastraea for this ancestral neuralian (Fig. 2I), which was probably a small, holopelagic ciliary particle-feeder, much like some anthozoan larvae. The evolution of a nervous system must have made more complicated lifestyles possible.

It is important to remember that a number of genes (and their proteins) generally considered to be characteristic of an organ or structure, for example, the synapse of neuralians, can be found in its sister group and therefore presumably evolved in their common ancestor, where they must have been involved in other processes. A very good example is the presence in sponges of most of the genes of the postsynaptic scaffold (Sakarya et al. 2007), although the sponges lack a nervous system and therefore synapses. Their function in the sponge is unknown, but it appears that only very few genes are needed for completing the network characteristic of the synapse of the sea-anemone *Nematostella* and further of the bilaterians.

Nervous systems comprise both sensory cells and cells specialized for communication and coordination (Lichtneckert and Reichert 2007). Most sensory cells have a rudimentary cilium, and receptor molecules involved in sensation are usually located in the ciliary membrane (Singla and Reiter 2006). Sensory cells of neuralians send information to other cells and are integrated in the nervous system. The nerves communicate

through gap junctions with innexins and chemical junctions with FMRFamides (Lichtneckert and Reichert 2007).

It appears that almost all ciliated neuralian larvae have an apical ganglion, which degenerates at metamorphosis (Nielsen 2005). The homology of apical organs in cnidarians and the various bilaterian groups has been taken for granted by most authors, but this is put in question by some new studies of gene expression. There is an expression of a posterior Hox gene (AntHox1) at the apical pole of *Nematostella* (Matus et al. 2006), whereas the anterior Hox1 is expressed in apical tuft cells of the polychaete *Platynereis* (Kulakova et al. 2007). Transcription factors necessary for the correct organization of the ciliated apical cells in the sea urchin *Strongylocentrotus* were not found in this region in the gastropod *Haliotis* (Dunn et al. 2007). Further studies are clearly needed.

CNIDARIA

The clearly monophyletic Cnidaria comprise Anthozoa and Medusozoa (Collins et al. 2006). Morphological characters indicate that the anthozoan life cycle, with a ciliated swimming planula larva and a sessile adult, is the ancestral one (Werner 1973), and this is supported by the fact that they have a circular mitochondrial DNA, like almost all other metazoans, whereas the medusozoans have a linear mitochondrial DNA (Bridge et al. 1992). The medusa is therefore interpreted as an added sexual stage (Collins et al. 2006). A unique feature of all cnidarians is the presence of cnidae (including nematocysts), which are highly organized intracellular structures that differentiate in interstitial cnidoblasts (Tardent 1995). Development of cnidae in the holopelagic cnidarian ancestor enabled the capture of larger prey, which could then be digested in the archenteron. With cnidae on tentacles, the early cnidarians could develop a sessile adult while retaining the pelagic developmental stage as a larva.

All cnidarians are of the gastraea-type organization with epithelia with septate junctions; the endoderm is an archenteron with extracellular digestion (Tyler 2003). Polyps have been described as essentially "two-dimensional sheets folded to produce three-dimensional animals" (Fautin and Mariscal 1991), and only the medusae have a more extensive mesogloea between these epithelia. Both ectoderm and endoderm are epitheliomuscular, usually with smooth myofilaments. However, striated myofilaments are found in cells of the subumbrellar zone in hydromedusae, where they originate from the so-called entocodon during budding. This structure has been interpreted as mesoderm in a number of papers by Schmid (see, e.g., Seipel and Schmid 2005), but it is never situated between the ectoderm and the endoderm, and in the medusae it forms the ectodermal subumbrella. It is not likely that these epitheliomuscular cells of the highly specialized medusae are homologous of the mesoderm of bilaterians (Burton 2008). The mesogloea is a more or less extensive extracellular matrix with collagens, fibrillin, and a few cells (Shaposhnikova et al. 2005). Scyphopolyps have ectodermally derived myocytes in the mesogloea in addition to the epitheliomuscular cells (Lesh-Laurie and Suchy 1991). Some authors have interpreted the mesogloea as a mesoderm, but the mesogloeal cells do not form organs. Further, a study of "mesodermal" genes in *Nematostella* showed expression only in the endoderm, which indicates that the mesoderm (endomesoderm) of the bilaterians is derived from the endoderm of the eumetazoan ancestor and that there is no separate mesoderm in cnidarians (Martindale et al. 2004). The adult nervous system is a network with concentrations of nerve cells around the blastopore/mouth and along the periphery of the bell of the medusae (Grimmelikhuijzen and Westfall 1995). Sensory structures include ciliated chemosensory or mechanosensory epidermal cells, and ocelli and statocysts, or a combination of these occur in many medusae (Skogh et al. 2006). Gap junctions with innexin have now been found both in the anthozoan *Haliplanella* (Mire et al. 2000) and in the medusozoan *Hydra* (Alexopoulos et al. 2004). The chemical synapses contain FMRFamide (Anderson et al. 2004) but lack acetylcholine (Grimmelikhuijzen et al. 1996).

Cnidarians are traditionally described as radially symmetrical, and the medusozoans generally show tetraradial symmetry, but with a few examples of bilaterality, as in various siphonophores. However, the anthozoans are biradial with bilateral tendencies in the arrangement of the septa and their musculature and in the presence of one or two siphonoglyphs, but without a head with a brain like that of the bilaterians. Bilaterality could have become established in the latest common ancestor of cnidarians and bilaterians, but this finds no support from morphology. Recent genetic analyses have shown the presence in *Nematostella* of several genes involved in organizing bilaterian body axes. The interpretation of these findings is controversial. Martindale's group (e.g., Matus et al. 2006) tends to believe that the bilaterian symmetries and axes can be recognized in the cnidarians, although organ homologies cannot be pointed out, whereas the groups of Ball (e.g., de Jong et al. 2006) and Technau (e.g., Rentsch et al. 2006) conclude that there is no simple relationship between the axes and symmetries in the two groups. Analyses of the Hox-like cnidarian genes indicated that the split between cnidarians and bilaterians predated the origin of the full bilaterian (eubilaterian) Hox cluster with anterior, group 3, central, and posterior genes (Garcia-Fernàndez 2005a,b; Chourrout et al. 2006; Kamm et al. 2006; Ryan et al. 2007). It should be emphasized that the expression of a gene in two structures is not a "proof" of historical homology of the structures (Nielsen and Martinez 2003) and that many genes are found in more "primitive" groups where they must have different functions.

Cnidarian sperm shows no acrosome, but a number of small vesicles anterior to the nucleus may facilitate the

"acrosomal" cell contact at fertilization (Franzén 1987). Most cnidarians are free spawners, and the first cleavage stages are highly characteristic, resembling those of the ctenophores, and the polar bodies are situated at the blastoporal pole (Freeman 1990). The endoderm develops through many different forms of gastrulation (Nielsen 2001; Byrum and Martindale 2004) to a planula larva, which is compact and lecithotrophic in many species, but which in many anthozoans is a ciliated gastrula, that feeds on plankton in the free water or on detritus at the bottom (Martin and Koss 2002). The cilia around the blastopore and in the archenteron are probably able to reverse their stroke, as those of many other ciliated epithelia, when transporting food particles in and out of the archenteron (Holley and Shelton 1984). The feeding biology of cnidarian larvae is poorly known, but *Porites* larvae become incompetent of settling if deprived of particulate food (Goreau et al. 1981). Larvae of *Caryophyllia* feed by ciliary currents or by ingesting particles caught in a mucous net (Tranter et al. 1982), and larvae of *Anthopleura* ingest both zooxanthellae and macerated *Artemia* (Schwartz et al. 2002). There is no observation of prey-capturing by use of the cnidae. The nervous system resembles that of the adults, but there is a concentration of nerves at the apical organ, which often has a long tuft of cilia (Chia and Koss 1979).

The apical organ degenerates after some time in the plankton. The larvae settle with the apical pole and the whole nervous system reorganizes with a new concentration of nervous cells around the mouth (Martin 2000).

FOURTH MAJOR STEP: THE ORIGIN OF MESODERM (TRIPLOBLASTICA)

The development of a third germ layer, the mesoderm, has often been seen as a very important step in metazoan evolution. Many authors, including many textbook authors, have interpreted mesoderm as an apomorphy of the Bilateria (Brusca and Brusca 2003; Ruppert et al. 2004), because the "mesenchymal" tissue between the ectoderm and the gut in ctenophores has been classified as nonmesodermal (Siewing 1977). However, newer studies interpret the tissues developing from the oral micromeres in ctenophores as the mesoderm, and it therefore seems appropriate to include the Ctenophora with its sister group Bilateria in a clade characterized by the possession of three germ layers. This is supported by the presence of acetylcholine in the chemical synapses of ctenophores and bilaterians. Also, the presence of an acrosome has been interpreted as a synapomorphy, and hence the alternative name Acrosomata (Ax 1995), although the validity of this character has been questioned (Scholtz 2004).

The molecular phylogenetic analyses (Table 1) show the ctenophores in many different phylogenetic positions.

CTENOPHORA

Most ctenophores are holopelagic, but a few genera, such as *Coeloplana* and *Tjalfiella*, have a creeping or sessile adult stage, respectively, which lacks the comb rows. However, they apparently all go through a pelagic "cydippid" stage, resembling the juveniles of more usual comb jellies (Mortensen 1912; Dawydoff 1933).

The ctenophores are strictly biradial, having a body plan of the gastraea type with the blastopore remaining as the mouth–anus. However, many authors interpret the muscles and other cells situated between the ectoderm and the endoderm and derived from the oral micromeres as the mesoderm (Nielsen 2001; Byrum and Martindale 2004; Ruppert et al. 2004; Martindale 2005), whereas this is questioned by others (e.g., Scholtz 2004). The cydippids have anucleate striated muscle units in the tentacles, but they are supposed to function only once and their structure indicates that they are not homologous with the bilaterian striated muscles (Burton 2008). Several epithelial zones are multiciliate, with the ciliary combs representing a unique type of organization with compound cilia formed by cilia from a number of multiciliate cells (Hernandez-Nicaise 1991). The epithelial cells are joined by spot desmosomes, zonula adherens, and special apical zonular junctions; septate junctions have not been found, but their function may be served by a series of punctate contacts, which resemble the vertebrate zonula occludens (Hernandez-Nicaise 1991; Tyler 2003). The nervous system consists of a complicated apical organ and rather diffuse nerve nets with concentrations below the comb rows and in the mouth region. There are both chemical synapses with FRMFamides and acetylcholine and gap junctions (Hernandez-Nicaise 1991).

A number of species show dissogony, i.e., sexual maturity in both the early larval and in the adult stages separated by a period with reduced gonads. Eggs of juvenile *Eucharis* are only half the size (in diameter) of those of the adults (Chun 1880, as *Leucothea*). Juveniles of *Pleurobrachia* of only 0.5–1.5 mm in diameter are sexually mature (Remane 1956). Juveniles of *Mnemiopsis* about 1.8 mm in diameter had three to four eggs per gonad; they were spawned in the normal way, could be fertilized by sperm from other juveniles, and developed normally (Martindale 1987). The sperm shows a typical acrosome (Hernandez-Nicaise 1991).

The first cleavage stages resemble those of the cnidarians, and the polar bodies are situated at the blastoporal pole (Freeman 1977). Early embryology shows a biradial cleavage pattern with separation of very small cells at the apical pole, large equatorial cells, and very small cells at the oral-blastoporal pole. The apical micromeres become the ectoderm, the macromeres become the endoderm, and the oral micromeres differentiate into a number of mesodermal elements, including muscles of tentacles, pharynx, and body wall (Martindale and Henry 1999; Byrum and Martindale 2004).

Hox genes have not been found (Lee et al. 2003). An extensive study of 18S rRNA sequences strongly pointed to the ctenophores being the sister group of cnidarians+bilaterians (Wallberg et al. 2004), and this result was also obtained in a number of other studies using 18S rRNA (see Table 1); however, morphology and gene expression indicate that they are closer to the bilaterians (Henry and Martindale 2004; Martindale 2005).

I have here put emphasis on the interpretation of the oral micromeres and their progeny as mesoderm and on the presence of acetylcholine in synapses, and accordingly placed the ctenophores in the Triploblastica. Further studies are needed before a more firm conclusion can be reached.

FIFTH MAJOR STEP: THE ORIGIN OF BILATERALITY (BILATERIA)

As mentioned above, the Bilateria is a monophyletic group characterized by a long series of apomorphies. They will be treated only briefly here, to complete the phylogeny proposed in Fig. 3.

The ancestral form, urbilateria (De Robertis and Sasai 1996), developed bilateral symmetry with a secondary (anterior–posterior) body axis and an anterior brain. The primary, apical–blastoporal axis was apparently retained, but the polar bodies are situated at the apical pole, both in acoelomorphs and eubilaterians (Henry et al. 2000), as opposed to the blastoporal position in cnidarians and ctenophores. The same "opposite" orientation is indicated through expression of bilaterian "brain" genes at the blastoporal pole in a ctenophore (Yamada and Martindale 2002). This remains unexplained (Martindale and Finnerty 2005; Rieger et al. 2005).

The Bilateria has traditionally been divided into Protostomia and Deuterostomia, but new information from both morphology and molecules indicate that the Acoela (and probably the Nemertodermatida, together called Acoelomorpha) is the sister group of the remaining bilaterians (Nielsen 2005), which have been called Eubilateria by Baguñà and Riutort (2004) and Nephrozoa by Jondelius et al. (2002).

The Acoelomorpha look like ordinary "turbellarians," but their brains are somewhat different from those of the other bilaterians (Reuter and Halton 2001). Their extracellular matrix is incomplete and they lack striated muscles (Rieger 1985; Rieger et al. 1991). The gut has only one opening, and there is no indication that this is due to a loss. The cleavage is a biradial duet cleavage quite distinct from that of other eumetazoans (Henry et al. 2000). The Hox cluster is very short (Baguñà and Riutort 2004; Cook et al. 2004) and this, together with the unusual nervous system, indicates that the acoelomorphs are bilateral but that they have not developed a through gut and the associated regionation of the body related to the long Hox cluster characteristic of the eubilaterians. Only a subset of the miRNAs characteristic of the eubilaterians has been found (Sempere et al. 2006). The living acoelomorphs are holobenthic, but the cnidarians are ancestrally pelago-benthic, and the eubilaterian ancestor may well have been pelago-benthic too, so the acoelomorphs may have lost the free-swimming stage.

SIXTH MAJOR STEP: THE ESTABLISHMENT OF A TUBULAR GUT (EUBILATERIA)

Almost all eubilaterians have a tubular gut with a mouth and an anus. The lack of an anus in platyhelminths, ophiuroids, and articulate brachiopods must be interpreted as specializations (discussed in Nielsen 2005). Eubilaterians mostly have a centralized nervous system with a well-developed brain. There is a long Hox cluster, with anterior, group 3, central, and posterior Hox genes, which is organized colinearly with the antero-posterior axis (Lemons and McGinnis 2006; Ryan et al. 2007); some organisms have all the genes but in an "exploded" pattern (Seo et al. 2004). A long series of miRNAs has been found (Sempere et al. 2006).

The organization with an anterior brain and a through gut must have enabled the evolution of larger organisms with more complicated behavior. The excretory organs are of various types, but most of the "lower" forms have protonephridia (Bartolomaeus and Ax 1992). Striated muscles are the main effectors in rapid movements.

The ancestral protostome was probably a neurogastraea with a periblastoporal ring of compound cilia functioning as a downstream-collecting system for particle collection; this has been called a trochaea (Nielsen 2001) (Fig. 1K). From this holopelagic ancestor, the pelago-benthic life cycle with a trochophora larva and a creeping benthic adult evolved.

The early evolution of the ancestral deuterostome is more difficult to envisage, but the development of the nonchordate deuterostomes indicates that the ancestor had a pelagic, planktotrophic dipleurula larva, and a benthic adult (Nielsen 2001).

DISCUSSION

The philosophy behind the scenario presented here (Fig. 3) is that every proposed ancestral stage and every transitional stage should have been viable, i.e., able to feed and reproduce. Where possible, adaptive advantages of the evolutionary steps should be sought for and explained. This ought to be evident, but such functional speculations have been absent from very many previous scenarios.

Haeckel's gastraea theory emphasized the occurrence of blastula and gastrula stages in the embryology of almost all animal groups from sponges to vertebrates. It proposed that

animal evolution passed through similar evolutionary stages called blastaea and gastraea, with the blastaea envisaged as a sphere of monociliate cells, without any discussion of its feeding mechanism (Haeckel 1874). The present scenario emphasizes the origin of the metazoans from a colonial choanoflagellate through the establishment of the choanoblastaea consisting of feeding choanocytes. The cells were connected so that nutrients could be shared, enabling the evolution of nonfeeding cell types (Step 1). The advanced choanoblastaea gave rise to the sponges, which retained the choanocytes as the feeding structures, whereas the planktonic larval stage became lecithotrophic. This is in agreement with a number of classical papers (Ivanov 1971). The three apparently monophyletic sponge groups Silicea, Calcarea, and Homoscleromorpha do not constitute a monophyletic group, and the "phylum Porifera" thus has to be abandoned, but it is still possible to speak about the sponge grade of organization. This is now recognized by a number of zoologists, especially those working on molecular phylogeny (see also Table 1).

The following evolutionary stage coincides with Haeckel's gastraea, but it is here proposed that the gastraea evolved from a homoscleromorph-like larva that became sexually mature, possibly through dissogony, and that the adult stage was subsequently lost through a case of extreme progenesis. This gastraea had sealed epithelia, which made extracellular digestion in the isolated space of the archenteron possible, and this marks the origin of the Eumetazoa (Step 2). The idea of a neotenic homoscleromorph larva as the ancestor of the eumetazoans was considered by Maldonado (2004) but was found less probable. However, the existence of dissogony, i.e., sexual maturity in both larval and adult stages separated by a stage with reduced gonads, in living ctenophores makes it probable that a similar evolution could have given rise to sexually mature homoscleromorph-like larva, and that the "sponge-stage" could then become abandoned.

The gastraea consisted of ectoderm and digestive endoderm, and the enigmatic *Trichoplax* has this type of organization, although the "endoderm" is a flat underside. *Trichoplax* can be interpreted in two ways, either as an expanded gastraea (gastraea theory) or as an evolutionary stage, which later gave rise to the gastraea (plakula theory). The embryology of *Trichoplax* is unknown, which makes it difficult to choose between the two theories, but the widespread occurrence of a gastrula stage in most eumetazoan groups supports the gastraea theory.

The establishment of a nervous system, with sensory cells, cells conducting electrical impulses to cells in other regions of the animal, and a coordinating centre, and the organization of electrical and chemical synapses, constitutes a major evolutionary step (Step 3). The name Neuralia is therefore coined for animals with a nervous system, and the ancestor is called neurogastraea. Hexactinellids show conduction of electrical impulses along the syncytial tissue, but no special sensory cells or synapses between cells have been found (Leys and Mackie 1997). Thus, the eumetazoan nervous system is a highly complex synapomorphy. The neuralian ancestor was probably a gastrula with a nervous system, and this is called the neurogastraea.

The cnidarians are organized as a neurogastraea, but with an added sessile adult stage, and an additional pelagic adult stage in the medusozoans. The presence in certain anthozoans of genes used in specification of the bilaterian body plan does not indicate that these genes have the same functions as in the bilaterians, because it seems impossible to relate the bilaterian body axes to any orientation of a cnidarian.

Both morphological and some molecular studies now support the interpretation of ctenophores as triploblastic, with the mesoderm originating from the oral micromeres (Step 4). Their phylogenetic position is still controversial (see also Table 1), though it can hardly be questioned that they belong to the Neuralia, and the presence of mesoderm and acetylcholine in the chemical synapses links them with the Bilateria. However, their organization is that of a gastraea with a blastopore functioning as a mouth and an anus, and they are biradial with no trace of bilaterality.

Bilateria, characterized by their bilaterality and the presence of a short Hox cluster, is a clade that is recognized in almost all morphological and molecular studies (Step 5).

The acoelomorphs have traditionally been regarded as "primitive turbellarians," but especially the molecular studies and the short Hox cluster indicate that they must be regarded as the sister group of the remaining bilaterians, here called the Eubilateria.

The eubilaterians are characterized by the presence of a tubular gut with separate mouth and anus and by the presence of a long Hox cluster (Step 6). Their origin and radiation with the sister groups Protostomia and Deuterostomia have been discussed earlier (Nielsen 2001).

The most commonly adopted alternatives to the present scenario are variations over the "planuloid–acoeloid theory" advocated so forcefully by Hyman (1951). It proposes that the bilaterians should have evolved from a compact planula-like ancestor, which developed a gut like that of a turbellarian. However, as discussed in detail elsewhere (Nielsen 2001), the compact planula is lecithotrophic and completely unable to feed, except perhaps through osmotrophy, so it must depend on another stage in a life cycle that is able to feed. This alone makes the various planula theories very improbable.

A general pattern of the molecular evolution behind the establishment of new body plans emerges clearly from a number of the observations discussed above: A number of genes (and their proteins), generally considered to be characteristic of organs or tissues of a certain group, are found in its sister group, and are therefore presumably evolved in their common ancestor, where they must have been involved in

other processes. At the origin of the new body plan, such genes become co-opted into a new network, which specifies the new structure. An illuminating example is the presence of most of the genes of the postsynaptic scaffold in sponges, which lack a nervous system and therefore synapse (Sakarya et al. 2007). Their function in the sponge is unknown, but it appears that only very few genes are needed for completing the network characteristic of the synapse of the sea-anemone *Nematostella* and further of the bilaterians.

Finally, it should be emphasized that the present interpretation of early metazoan evolution implies that all eumetazoans, including man, are descendants of a derived sponge larva or, more specifically, a larva of a homoscleromorph-like ancestor.

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