BASAL METAZOANS

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Summary

Basal metazoans comprise the four non-bilaterian animal phyla Porifera (sponges), Cnidaria (anthozoans and jellyfishes), Placozoa (*Trichoplax*) and Ctenophora (comb jellies). The phylogenetic position of these taxa in the animal tree is pivotal for our understanding of the last common metazoan ancestor and the character evolution all Metazoa, but is much debated. Morphological, evolutionary, internal and external phylogenetic aspects of the four phyla are highlighted and discussed.

1. Introduction on “Basal Metazoans”

In many textbooks the term “lower metazoans” still refers to an undefined assemblage of invertebrate phyla, whose phylogenetic relationships were rather undefined. This assemblage may contain both bilaterian and non-bilaterian taxa. Currently, “Basal Metazoa” refers to non-bilaterian animals only, four phyla that lack obvious bilateral symmetry, Porifera, Placozoa, Cnidaria and Ctenophora.

These four phyla have classically been known as "diploblastic" Metazoa. In diploblasts, the body wall is developed from only two embryonic germ layers, an exterior layer (e.g., pinacoderm, ectoderm) and an interior layer (e.g., choanoderm or endoderm). Between both layers we find a mostly non-cellular region (e.g. the mesohyle, mesoglea). This is opposed to triploblastic animals, in which a third germ layer is present, the mesoderm, that allows the development of connective- and other tissues. Several current studies discuss the tissue originating from the oral micromeres in Ctenophora as mesoderm, and consequently suggest a triploblastic nature of Ctenophora.

2. The Phylogenetic Relationships among Non-Bilaterian Metazoa

Higher-level non-bilaterian relationships have recently been discussed quite controversially and currently remain the most notable open questions in the higher-level relationships of the Metazoa. This controversy has been fueled by a number of phylogenomic studies that have resulted in conflicting hypotheses of relationships of the non-bilaterian taxa, including the origin of Porifera.

In recent years, DNA sequence data are generated in ever increasing amounts due to significant reductions in sequencing costs. Many animal genome and transcriptome sequencing projects are on their way or have been completed, and the data are now available to be included in "phylogenomic" analyses.

An early study that used 50 protein-coding genes was unable to resolve non-bilaterian relationships and attributed this to the fact that the cladogenetic events of interest occurred so fast (probably in less than 20 million years) and so long ago (more than 550 million years), before the so-called "Cambrian Explosion" that it is virtually impossible to resolve these relationships with sequence data from extant organisms.

Subsequent analyses applied much broader phylogenomic approaches by analyzing more than 100 genes at a time. One study focused on the relationships within Bilateria, where a large amount of new EST data for many taxa was added and significantly improved the resolution of this part of the tree. Only a few representatives of the Porifera, Cnidaria, and Ctenophora were included, i.e., the non-bilaterian taxa were not well sampled. However, although not the focus of the study, the result that received the most public attention was the position of the Ctenophora as the sister-group ("basal") to the remaining Metazoa, including sponges. This result was very controversial and a follow-up study, which significantly improved the non-bilaterian taxon sampling (they added EST data from 18 additional non-bilaterian species, including previously unsampled placozoans and sponges), suggested that the "basal" position of Ctenophora was due to the well-known artifact of long branch attraction, where taxa with long branches are artificially attracted to each other in phylogenetic analyses. In contrast to the earlier findings, a more classical topology was recovered, with monophyletic sponges branching off first, then the Ctenophora as the sister-group to the remaining eumetazoans (Cnidaria, Bilateria). These results were subsequently corroborated by a study that reanalyzed three phylogenomic datasets.

Another highly controversial study contained nuclear protein coding and mitochondrial genes and provided a combined analysis with morphological characters. Here, a clade of diploblastic (i.e., non-bilaterian) animals was recovered as the sister-group to the triploblastic Bilateria. Within the “Diploblastica”, Placozoa branched off first and a “modernized Urmetazoa hypothesis” was postulated, with far-reaching conclusions about the evolution of bilaterian key-traits, such as the nervous system. In the above mentioned study that reanalyzed three phylogenomic datasets it was convincingly shown that this “total evidence” supermatrix contained many errors, e.g., frameshift errors, other biological and *in silico* contaminations, and genes with questionable orthology. The analysis of a revised dataset cleaned of those errors did not support the diploblastic clade any longer.

Another study, which contained 128 genes and the most comprehensive sampling of non-bilaterian taxa to date revived traditional views on deep animal relationships. This study recovered a highly supported monophyletic Porifera as a sister (“basal”) to the remaining Metazoa, and supported the classical “Coelenterata” concept with a clade uniting Ctenophora and Cnidaria as the sister to a Placozoa + Eumetazoa clade. The issue of sponge monophyly has also been a controversial issue since a number of previous studies based on smaller gene samplings suggested that sponges are a paraphyletic assemblage that shares a grade of construction. The topology of this study was the only one that withstood the recent re-analysis of the aforementioned phylogenomic studies.

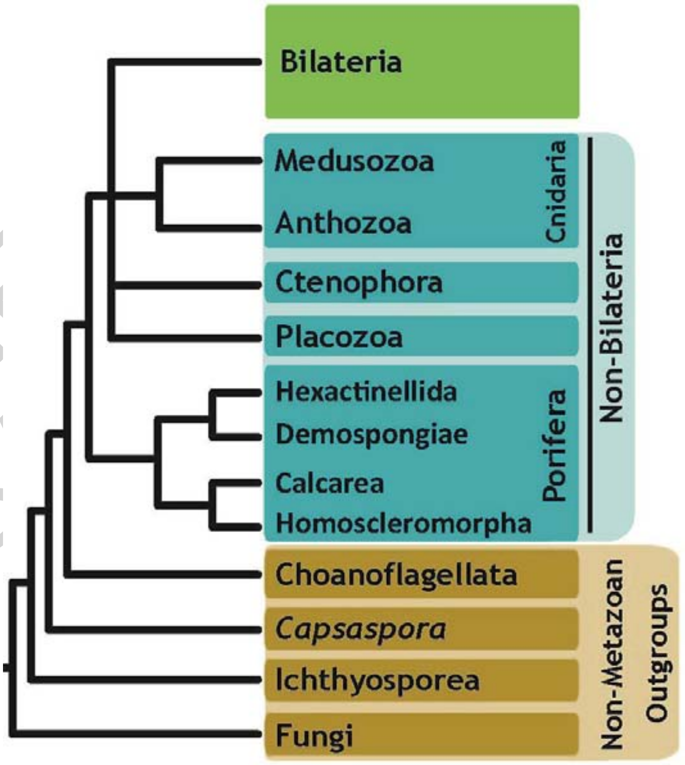

Figure 1. Best currently available working hypothesis of non-bilaterian metazoan relationships. See text for details. Please note that certain cnidarian clades are not included (Cubozoa, Staurozoa, Myxozoa) and taxon sampling for Ctenophora is low.

Recent genome sequencing of two ctenophore genomes provided additional data for phylogenomic analyses in the corresponding publications. Both again recovered the Ctenophora as the sister-group to the remaining Metazoa but again suffered from methodological flaws.

The topology displayed in Figure 1 is the best currently available working hypothesis of non-bilaterian relationships. Due to the still controversially discussed relationships of the Ctenophora, Cnidaria and Placozoa to the Bilateria, these relationships are drawn as unresolved.

3. Phylum Porifera

3.1. Introduction to sponges:

Porifera is probably the oldest extant multicellular phylum. Sponges are estimated to exist at least since the Late Proterozoic. Sponge-grade remains were reported from some 635 million years ago. This finding also supported earlier reports on the existence of sponge lineages in the Cryogenian based on molecular biomarkers. Sponges became the predominant reef builder in the Cambrian, but this habitat forming function decreased during the Mesozoic.

Recent sponges inhabit all marine and freshwater habitats from the tropics to polar seas, from the littoral to the abyss and in most freshwater habitats of all continents except Antarctica, where they play a vital role as potent filter feeders. There are currently about 8,500 sponge species described; however, about the same number is estimated yet undescribed or undiscovered. Currently Porifera are divided into four classes of which Demospongiae is the by far largest taxon with about 83% of all species. The classes Hexactinellida (glass sponges) and Calcarea (calcareous sponges) comprise about 7% of all sponge species each. Homoscleromorpha, the smallest sponge class, represent 1% of all species.

Compared to other Metazoa, sponge morphology is rather simple as they do not possess organs systems, and there are comparatively few differentiated cell types facilitating the vital functions. Sponge epithelia are relatively simple. The pinacoderm is a single cell layer covering the exterior of the sponge including the canals. The choanoderm is based on a single layer of flagelled choanocytes (Figure 2). Neither epithelia possesses the eumetazoan-typical junctions (*zonula adhaerens*), and a basal matrix is only found in sponges of the class Homoscleromorpha. Between pinacoderm and choanoderm is the mesohyl, filled with an extracellular matrix consisting of collagenous fibers, the sponge skeleton and various, mostly mobile, cell types. Among these are archaeocytes, omnipotent cells with a wide variety of functions in transport and reproduction, cells responsible for the production of organic (spongioblasts, lophocytes) or mineral (scleroblasts) skeletal elements, and secretory cells.

Sponges have a biphasic life cycle with a short lived, pelagic larvae followed by a sessile adult stage with a filter-feeding lifestyle, maintained by an elaborate water canal system. The water canal system dominates the bauplan of the adult sponge and is historically classified into different types, of which the leuconid is present in most sponge lineages, while the less complex asconid, syconoid or other forms are exclusively found in calcareous sponges. On the sponge surface, numerous mostly microscopic openings (ostia) facilitate the water influx, which is then led through a hierarchical system of cavities and channels to the choanocyte chambers in most sponges (Figure 2). The choanocyte chambers are the central unit of the filter-feeding system and consist of rows of choanocytes, whose coordinated flagella movement facilitates a continuous water current. The choanocytes also filter organic particles from the water, such as detritus or bacteria, which are subsequently phagocyted. Filtered water leaves the choanocyte chambers via a hierarchical system of exhalant canals and cavities before being released through oscula, which are large compared to the ostia, and occasionally chimney-like openings. Gas exchange is facilitated via the pinacoderm.

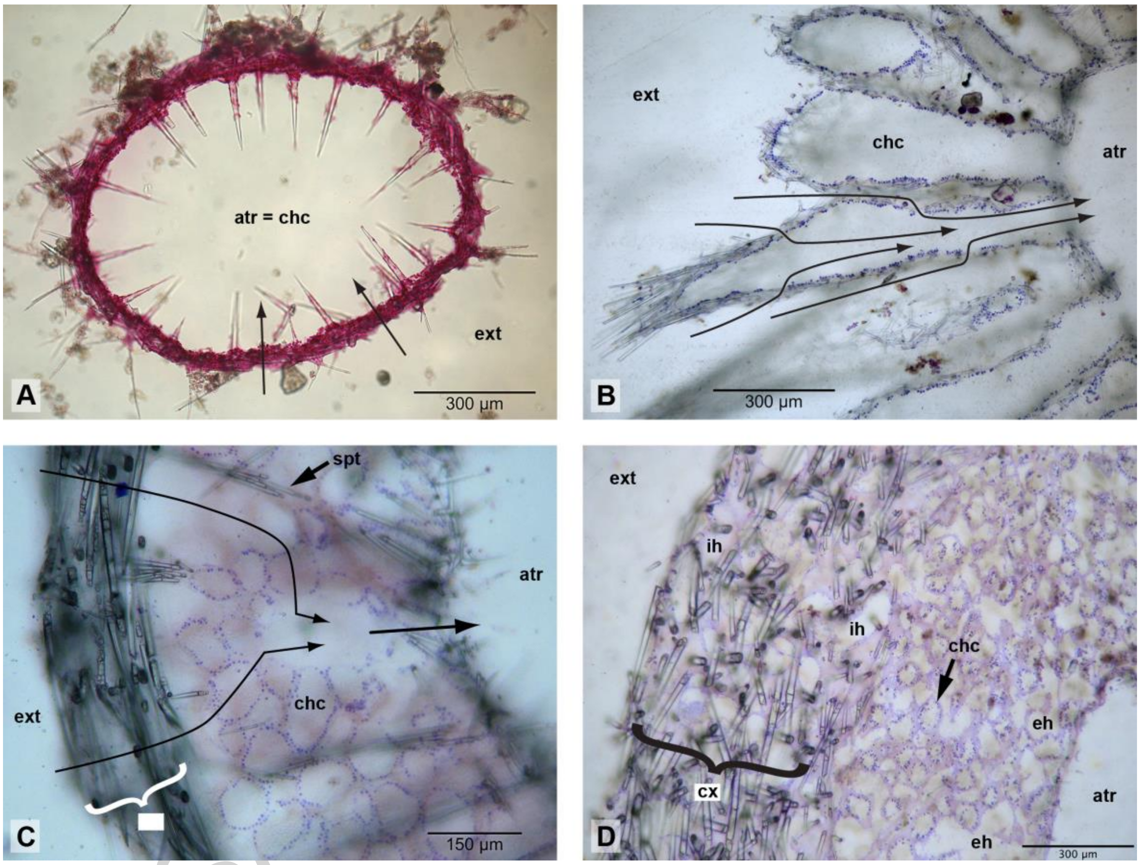

Figure 2. Different organizations of the aquiferous system in Calcarea, transversial sections through sponge walls. A: asconoid (*Soleneiscus radovani*); B: syconoid (*Sycon coronatum*,); C: sylleibid (*Grantiopsis cylindrica*); D: leuconoid (*Leucettusa* sp.). Thin arrows illustrate the direction of water flow in A, B and C. atr= atrium; chc=choanocyte chambers; cx=cortex; eh= exhalant channel; ext: exterior of the sponge; ih= inhalant channel; spt= spicule tract of modified triactines. Modified from Voigt, et al. 2012.

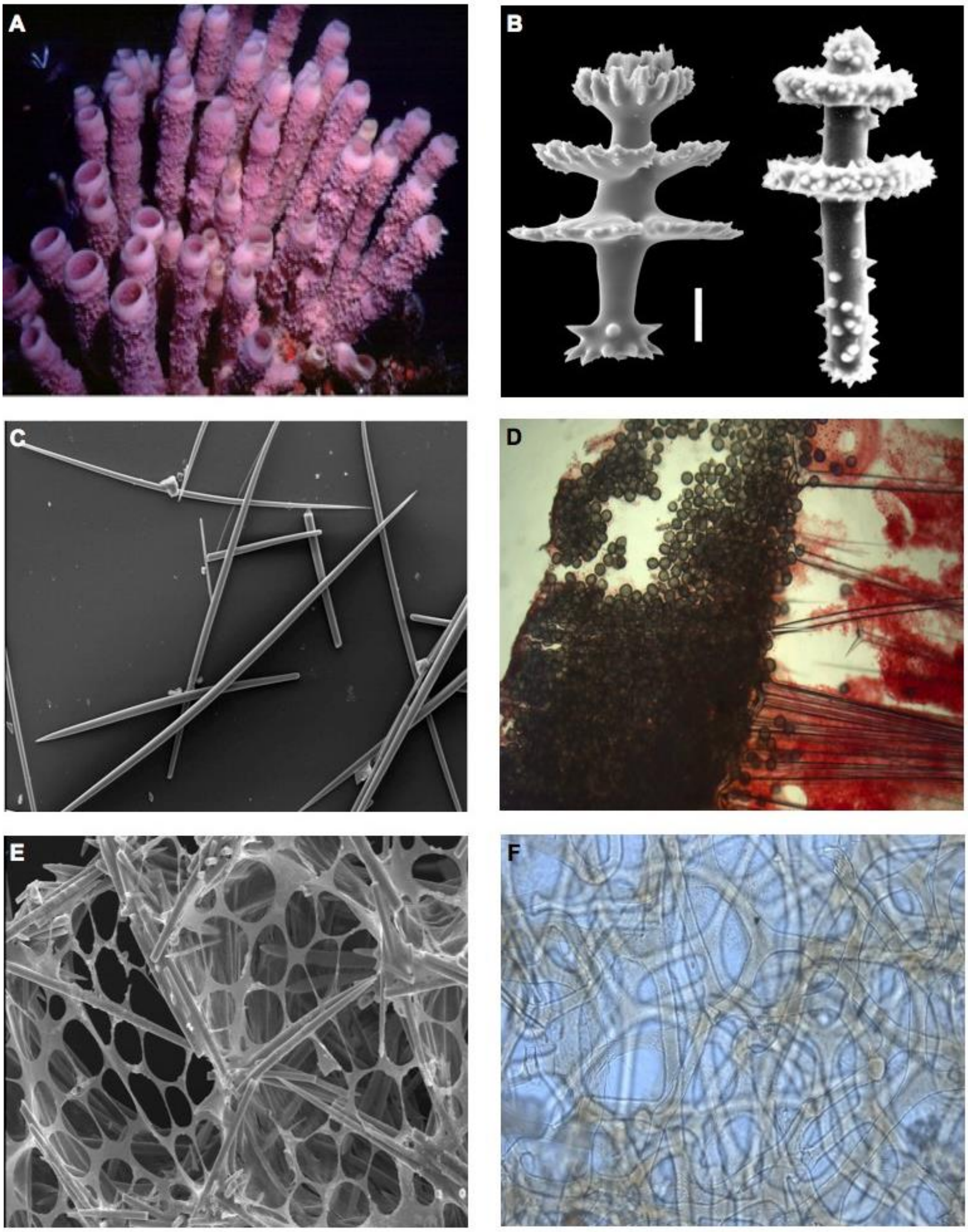

Figure 3. Demosponges and skeletal types. A: The marine haplosclerid *Callyspongia* sp.; B: Microscleres of comparatively similar shape but different evolutionary origin: discorhabd from *Latrunculia brevis* (left, scale bar = 10µm), didiscorhabd from *Didiscus aceratus* (right). C: Monaxonic megascleres of *Hymeniacidon perlevis*; D: Ectosomal skeleton of asters supported by triaene choanosomal skeleton in *Geodia* sp. E: Arrangements of megascleres to the skeleton of *Halichondria panicea*; F: Spongin fibers constitute the skeleton of *Spongia* sp.

The growth form is species- specific and comprises encrusting, globular, cup-shape, tubular (e.g. Figure 3A), fistulose and many different other shapes. Its final form, however, is largely influenced by environmental and ecological factors. Sponge growth is theoretically unlimited but always followed by the extension of its water canal system. Shape and growth forms of most sponges are facilitated by a mineral skeleton (Figure 3 B-E). Only a few demosponge groups lack any skeletal elements but possess a complex arrangement of collagenous fibers instead. Several demosponge groups do not possess a mineral skeleton, but have a framework of spongin fibers instead, which likewise can be arranged reticulated of dendritic shapes (e.g. Figure 3 F). Mineral components such as sand grains or foreign spicules may be incorporated into the spongin fibers, providing them with additional rigidity. Most sponges possess a mineral skeleton of calcareous and/or siliceous elements, whose taxonomic distribution is highly distinct. Siliceous spicules (e.g. Figure 3 B-E) are present in three classes, the Hexactinellida, Homoscleromorpha and Demospongiae. The siliceous spicules can have various sizes, shapes and functions and are distinguished into the larger megascleres with mostly structural importance, and microscleres, that may possess highly specific functions in the skeleton. Calcareous spicules do not possess the wide shape varieties of siliceous spicules and are found in Calcarea only. Calcarea and Demospongiae may also possess a massive calcareous basal skeleton, which evolved several times independently in the Porifera and is built by different pathways.

Calcareous and siliceous sponges synthetize their spicules differently. Siliceous spicules are produced intercellularly in single scleroblasts by adding silica around an organic (axial) filament. Calcareous spicules are produced extracellularly from several scleroblasts and possess no axial filament.

The connection between the spicule elements differs among the sponge classes. In demosponges, these spicules are mostly cemented with spongin to a skeletal network of different shapes, such as reticulated, radial, confused or rectangular meshed shapes. Hexactinellida do not produce spongin, their skeletal elements may be fused and form a solid skeletal grid. The sporadic siliceous spicules of Homoscleromorpha never form a coherent skeleton, but are loosely distributed in the sponge body. Calcareous sponge spicules are generally loose.

Despite earlier beliefs, a prominent defensive function of spicules could not yet be shown. Protection against predators, parasites or competitors for space is mostly facilitated with chemical defense mechanisms based on secondary metabolites, which presumably played a role in ensuring their survival for hundreds of million years. Sponges are not necessarily the producers of all secondary metabolites. A bewildering variety of symbiotic microorganisms is associated with sponges and was repeatedly identified as a source for several bioactive compounds extracted from sponges. Many symbioses with bacteria may be obligate, and their complexity is a major factor why sponges are relatively difficult to grow in aquaria. Symbioses with micro-organisms are facilitated by the absence of tight cell connections, which provides relatively easy access to the interstitial spaces compared to the epithelia of Eumetazoa. Transfer of symbionts into the next generation is mediated with sponge larvae.

Reproduction of sponges can be sexual or asexual. Asexual reproduction is mostly facilitated by the formation of sponge buds that already contain a complete set of cells. Freshwater sponges (Spongillina) display a specialized form of asexual reproduction (see below).

Gametes for oviparous or viviparous sexual reproduction are derived from archaeocytes (eggs) and choanocytes (spermatozoa). Gametes are either released into the water or, in the case of vivipary, eggs are retained and fertilized after released spermatozoa are ingested. Among the different sponge lineages we find a wide range of larval forms and types, and they may also differ drastically in their development. We can distinguish between many different types of larva, of which the parencheymella is the most common among the different taxa.

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