# Cnidarian milestones in metazoan evolution

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**Synopsis** Cnidarians display most of the characters considered as milestones of metazoan evolution. Whereas a tissue-level organization was probably already present in the multicellular common ancestor of all animals, the Urmetazoa, the emergence of important animal features such as bilateral symmetry, triploblasty, a polarized nervous system, sense organs (eyes, statocysts), and a (chitinous or calcium-based) continuous skeleton can be traced back before the divergence between cnidarians and bilaterians. Modularity and metamery might be also regarded as two faces of the same medal, likely involving conserved molecular mechanisms ruling animal body architectures through regional specification of iterated units. Available evidence indicates that the common ancestor of cnidarians and bilaterians, the UrEumetazoa, was a surprisingly complex animal with nerve cell differentiation. We suggest that paedomorphic events in descendants of this ancestor led to the array of diversity seen in the main extant animal phyla. The use of molecular analyses and identifying the genetic determinants of anatomical organizations can provide an integrative test of hypotheses of homologies and independent evidence of the evolutionary relationships among extant taxa.

## Introduction

The history of life is reconstructed both by analysis of the fossil record and by identification of shared anatomical and genetic traits among extant representatives of the main lineages (usually phyla).

The distinction between structural similarities resulting either from shared ancestry (homology) or from functional convergence (analogy) is crucial for detecting meaningful evolutionary relationships among organisms. Homologous anatomical structures share the same structural plan and basic components, ontogeny, and topographical relationships with other body parts. In contrast, analogous structures are linked only by serving the same function (Minelli 2003).

For many years, embryology was used to test homology between similar structures. The development of molecular genetics, however, has suggested that the terms of homology and analogy, respectively based on similarities of developmental pathways or on the ultimate outcomes (i.e., biological functions), are not unequivocal. The new approach of testing homologies by molecular tools has shown unexpected results: that genetic novelties in the animal kingdom are rather limited, and that the re-elaboration of highly similar genes led to an array of forms that had been considered as analogous before genetic tests.

To define evolutionary relationships among genes, regardless of their functions and based on shared ancestry, molecular biologists introduced two additional descriptors: orthologs and paralogs (Fig. 1) (see also Sonnhammer and Koonin 2002).

It is acknowledged now that cooption of paralogous genes or changes in *cis*-regulatory control elements may explain analogies generated by similar developmental pathways, or that the extent of gene interaction networks can lead, by conservation of developmental constraints, to constancy of homologies through separate developmental pathways (True and Carroll 2002). Therefore, the similarity of developmental mechanisms cannot be blindly considered a proof of shared ancestry (e.g., see Fernald 2000; Gehring 2005 on contrasting views about eye development and evolution of photoreceptors) but, in many cases, it may represent a powerful tool supportive of hypotheses of evolutionary relationships between anatomical and embryological end-products. Combined sources of phylogenetic information must equally include data from classical morphology, developmental biology, simple sequences, and whole genomic organization.

Thus, the process of reconstructing phylogenies for higher taxa stems from the identification of key anatomical (including developmental) traits
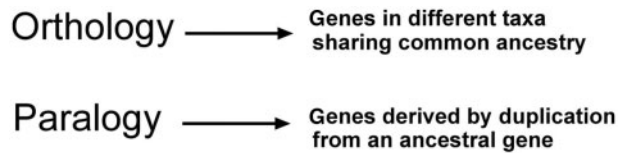
**Fig. 1** Orthologs are two genes in two different taxa that derive from a single gene in the last common ancestor of the taxa. Paralogs are found in the same genome and are genes derived from a single gene that was duplicated within an ancestral genome.

(Giribet 2002) whose genetic basis is then compared. The evolution of Metazoa is the most widely studied evolutionary pattern, due to the wide array of body plans that dominated the world in the past and that still characterize life in its most astonishing innovations.

The identification of relationships leading to a phylogeny is a powerful means of reconstructing steps of evolution. In this framework, phylogeny is mainly linked to pattern detection, whereas evolutionary biology must also study the processes (e.g., heterochrony) that led to the proposed phylogeny.

Life is expressed through an amazing diversity of body plans. In a parsimony-oriented science, ancestry is usually related to simplicity. Eukaryotes have more genes than do the simpler prokaryotes (Vellai and Vida 1999; Ball and Cherry 2001). The increase in genetic complexity over the course of evolution generally provides for the radiation of developmental and metabolic patterns, and biodiversity (Carroll 2001). The fossil record, up to a certain point, supports this assumption, since very simple organisms appear as first signs of life, and its corollary is that life became more and more complex. This is not to say that all phylogenies will show the simplest character states at the ancestral nodes. The reverse may be the case if, at smaller scale, reconstruction of phylogenies by comparative analysis of selected traits includes homoplasies (by polarity reversal and parallelism) and secondary adaptations. The diversity of several gene families of deuterostomes is reduced by repeated loss of genes in model ecdysozoans even though they are largely represented in the lower branches of the metazoan tree (Technau et al. 2005).

A useful exercise to reconstruct a phylogeny, thus, is to single out milestones of evolution and, from them, infer the pattern of radiation of phyla. A milestone may be defined as a major evolutionary innovation that is introduced in a given position of metazoan phylogeny and is then conserved

(at least in its genetic specification) throughout the emerging clades.

Metazoan phylogeny, in this framework, can be properly reconstructed under the eye-glass of evolution.

The way animals are represented in textbooks of zoology is based on the emergence of novelties. In other words, the evolution of the Metazoa seems to be characterized by a steady increase in complexity ("anagenetic evolution"). Nevertheless, the extant phyla appear in the fossil record in a relatively short period, soon after the Precambrian. This sudden radiation of almost all metazoan *Bauplans* led paleontologists to describe their emergence as an "explosion." In order to have an explosion, an explosive is needed. Because one of the extant metazoan phyla already present at that time is the Cnidaria, we consider the hypothesis that cnidarians were the explosives (or among the available ones) of the Cambrian explosion.

## Milestones in metazoan evolution

In the following we seek to identify key innovations in body architecture, and argue about what might have been the ancestral organism in which these can be recognized as real novelties. This exercise leads to a series of hypotheses of homology based on comparative anatomy.

## Animal multicellularity, triploblasty, and bilaterality

The simplest known multicellular animals are the Placozoa. Possessing only four somatic cell types, and lacking any specialized nerve or sensory or muscle cells and any kind of extracellular matrix, including basal lamina (Grell and Ruthmann 1991), these microscopic animals are by far simpler than any sponge or cnidarian (Schierwater 2005). Whereas phylogenetic reconstructions based on 18S rRNA sequences (e.g., Collins 1998; Collins et al. 2005) suggest that the simplicity of placozoan body architecture is secondarily derived, mitochondrial genome analysis interprets their simple features as ancestral rather than derived (Dellaporta et al. 2006). In this latter view, placozoans are considered the earliest divergent metazoans in which the ancestral state of animal multicellularity is conserved. Nevertheless, these contrasting outcomes of different analyses maintain the uncertainty surrounding these views (cf. Schierwater and DeSalle; this volume).

In an alternative and widely accepted scenario, sponges are usually regarded as animals without true tissues and thus representing the earliest stage in the evolution of animal multicellularity, a view

traditionally supported by the remarkable similarity between sponge collar cells and choanoflagellates (Brusca and Brusca 2002), but also by molecular and morphological analyses (Borchiellini et al. 2001; Maldonado, 2004). It is known that at least the members of the small sponge subclass Homoscleromorpha already possess typical eumetazoan features, including the tissue grade of organization (see Boury-Esnault et al. 2003; Maldonado 2004; Wang and Lavrov 2007 and references therein). Furthermore, their mitochondrial genomic organization is supportive of closer affinities with other metazoans than with other demosponges. In general, the mt genome in sponges is far more complex than previously appreciated (Erpenbeck et al. 2007) and the diversification of some of the major developmental classes of bilaterian transcription factors took place before sponges diverged from the rest of the Metazoa (Larroux et al. 2006). As already suggested (Müller 2001), the simplicity of most extant sponges must be considered as derived and Eumetazoa must be considered to include Porifera.

Regardless of whether Placozoa or Porifera was the inventor of animal multicellularity, the two views (and all related molecular findings) do not go against each other, but certainly additional data will be needed to integrate them and to resolve the uncertainty still surrounding the early origin of animals. Moreover, the position of Ctenophora is still far from resolved, and ctenophores may be the earliest diverging extant lineage (Collins et al. 2005). Genomics data consistently show that lower organisms, including cnidarians, retained ancestral but complex genomes (Technau et al. 2005). Cnidarians were long considered as diploblastic animals due to prolonged supremacy of the *Hydra*-based model system in experimental studies. Recent studies and a critical revaluation of old knowledge disclosed the growing view that the origin of triploblasty predated the cnidarian-bilaterian divergence (Boero et al. 1998; Martindale et al. 2004; Boero et al. 2005; Seipel and Schmid 2005, 2006) (Fig. 2). Histological and experimental data corroborate the existence of mesodermal differentiation in all cnidarian classes. In Anthozoa, Scyphozoa, Staurozoa, bundles of subepidermal polyp retractor muscles (smooth or striated fibers) are embedded in the extracellular matrix and are not part of the myo-epithelial muscle compartment; instead, they derive from differentiation of mesenchymal cells migrating from ectoderm. In jellyfishes, cross-sections of tentacles from cubozoan and scyphozoan medusae show tubular bundles of smooth muscles and nerve cells largely isolated from ectodermal and endodermal layers (reviewed by Seipel and Schmid 2006, and references therein). More strikingly, in the hydrozoan medusa bud, the entocodon (i.e., the proliferative blastema derived by delamination of the ectoderm) develops into a three-dimensional, nonmesenchymal mesothelial layer lining a secondary cavity (the subumbrellar compartment) (Boero et al. 1998; Ball et al. 2004) and differentiating into the striated muscle layer. Even in the more restrictive definition of mesoderm (see Hyman 1951; Pantin 1960; Chapman 1966) the hydrozoan entocodon can be classified as a true mesoderm (Seipel and Schmid 2006). The finding of bilaterian mesoderm orthologs in cnidarians and their expression studies (Spring et al. 2000; Spring et al. 2002; Seipel and Schmid 2005) offers further molecular support to this conclusion (reviewed in Ball et al. 2004).

According to these data, the last common ancestor of cnidarians and bilaterians, in the Neoproterozoic era, already possessed the genetic toolkit for a triploblastic *Bauplan* and the diploblastic organization of hydrozoan polyps may be interpreted as a derived state or as a ''larval'' diploblastic state preceding the fully triploblastic adult (the medusa). Furthermore, cnidarians are not completely radial, since at least at the start of their life they show more or less evident signs of bilaterality (Piraino et al. 2003) and the expression patterns of dorso-ventralizing genes in anthozoan embryos (Hayward et al. 2002; Finnerty 2003; Finnerty et al. 2004; Matus et al. 2006) provide compelling evidence that the origin of certain fundamentals of bilaterality pre-dated the cnidarian-bilaterian divergence. In this framework, the discovery of cellular and molecular antecedents of the bilaterian central nervous system in cnidarian development (see subsequently) is no longer an unexpected event (cf. Miller et al.; this volume).

## Nervous system, sensory cells, and cnidocytes

Cnidarians are among the lower animals with sensory specializations such as mechanoreceptors (cnidocytes), photoreceptors and chemoreceptors, and motor and gangliar neurons, all differentiating from a common stem cell line. Expression of homologous regulatory genes has been documented in the formation of cnidarian and bilaterian nervous systems (Miljkovic-Licina et al. 2004). Polyps have nerve nets with a subset of neurons forming a more complex structure, the nerve ring, at the bases of tentacles. Jellyfishes integrate a relatively simple nervous system with complex sense organs
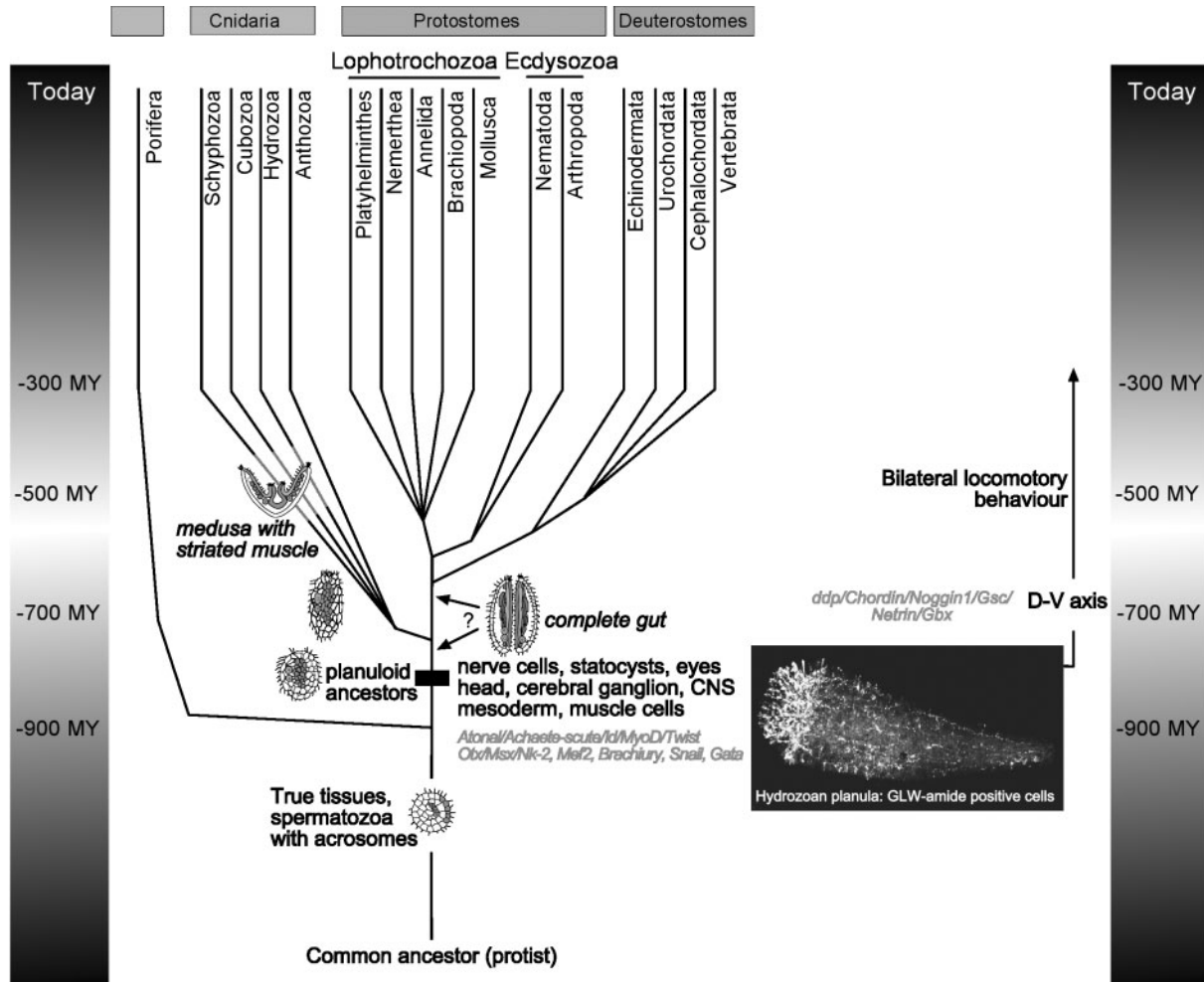
**Fig. 2** A hypothetical cnidarian-focused model of animal evolution (modified from Galliot and Miller 2000; Seipel and Schmid 2005). The placement of Ctenophora and Placozoa is omitted (see text). At the end of the Neoproterozoic period, a common ancestor of cnidarians and bilaterians (the UrEumetazoa) already possessed a central nervous system with complex visual organs and a triploblastic, bilateral organization. The appearance of a functional complete gut may or may not also pre-date the divergence between cnidarians and bilaterians. This model might also incorporate pedomorphic processes, such as the loss of cnidocysts from the UrMetazoan ancestor. The lack of true tissues in most sponges is recognized now as a derived feature; therefore, the UrEumetazoa is now defined as the ancestral animal with neuronal differentiation (see text).

(statocysts, ocelli, rhopalia) formed by different neuronal types. Indeed, jellyfishes have a simple or (when the velum is present) double nerve ring, with peripheral concentrations of neurons in connection with tentacles and sense organs. Hydrozoan planulae exhibiting a bilateral crawling pattern reveal a dramatic A–P polarization of the nervous system (Piraino et al. unpublished). These larvae show a dense concentration of different types of sensory nerve cells in their anterior pole, a commissural neural network circumferentially embracing the anterior third of the larval body, and a subset of neurons (FMRF-amide and GLW-amide positive cells) giving rise to longitudinal fibers directed toward the posterior pole. Contrary to current

views of metazoan nervous system evolution, by which cnidarians are thought to be radial animals with simple, diffuse nerve nets (Willmer 1990), the foremost nerve cell concentrations in these planula larvae (Fig. 2, small box) can be clearly defined as a cephalic ganglion, i.e., the first brain-like structure within the Metazoa (Piraino et al. unpublished).

Correspondingly, asymmetric expressions of some genes along the directive axis of the *Nematostella* embryo indicate an early molecular origin of the pathway to formation of the CNS (Matus et al. 2006). From invertebrates to vertebrates *Noggin1* is a gene promoting neural differentiation as one of the inhibitors of TGF-β signaling. The *Nematostella*

ortholog NV-*Noggin1* in the amphibian ectopic dorsalization assay has the same BMP antagonist function as does the corresponding vertebrate gene (Matus et al. 2006). Cloning of *bmp*2/4-Am from *Acropora millepora* and rescue experiments of fly mutants showed functional conservation between orthologs of *dpp*/BMP 2/4 proteins, which are known to play a key role in the specification pathway of dorsal structures (including the nervous system) in Bilateria (Hayward et al. 2002).

The formation of visual organs in Bilateria is regulated by conserved gene interactions with a central role of members of the Pax and Six families (Gehring 2005). Members of these gene families have been cloned from cnidarians, showing a remarkable similarity of structure and expression patterns with developmental pathways of higher metazoans. Therefore, there appears to be a continuity of genetic information that regulates similar, but not necessarily homologous, eyes (Fernald 2006; Gehring 2005). Even if not all eyes are homologous, and their monophyly is still uncertain, an upstream regulatory network for the development of visual structures was present in the ancestor of Cnidaria and Bilateria (Stierwald et al. 2004).

Bonner (1988) argued that the evolution of complexity is seen in the increasing number of cell types throughout metazoan evolution. Lower animals have few cell types, higher animals have many cell types. The Cnidaria, as the explosive of the Cambrian explosion, remained faithful to their extreme cell specialization, the cnidocyst, the fastest-acting known biological structure (Tardent 1995) and unparalleled apex of organelle specialization. Their presence is probably the secret of the success of such an ancient phylum. No other metazoans can differentiate cnidocysts, but distant taxa such as ctenophores, turbellarians, and molluscs are able to incorporate unexploded and functional cnidocysts from cnidarian prey tissues into their own bodies (Pearse et al. 1987). Based on current (poor) knowledge, one can only speculate on whether such widespread cnidocyst-handling ability is monophyletic, i.e., based on cell recognition systems and surface molecules already available at the dawn of Eumetazoa, or polyphyletically derived from succeeding, independent coevolutionary events.

Based on the shared ancestry of Cnidaria and Bilateria, one can argue that functional specialization of early mechanoreceptors into firing cnidocysts occurred after the emergence of the cnidarian lineage from the main stem of Metazoa. The early sensory cells of the common ancestor diversified their functionalities, in the course of evolution,

in different taxa (Fritzsch and Piatigorsky 2005; Tessmar-Raible et al. 2005). This is supported by the observation that the finding of several types of sensory cells (photoreceptors, mechanoreceptors, chemoreceptors) in a wide range of metazoan taxa (from *Drosophila* to vertebrates) depends on the activity of a shared combination of well-conserved transcription factors (*Pax6 - Pax2/5/8*, *atonal* and *POU IV* class orthologs). A striking example is seen in the basic mechanisms for specification of several mechanoreceptors which depends on the activity of orthologs of the basic helix-loop-helix (bHLH) transcription factors *Atonal* and *Achaete-scute*, a conserved pathway from cnidarians (Seipel et al. 2004) to vertebrates (Hassan and Bellen 2000).

Alternatively, a potential milestone of metazoan evolution might have been the loss of cnidocysts from a shared ancestor. Cnidocysts are not present in cnidarian embryos before gastrulation, except in species whose cnidocytes are also maternally inherited. They are formed in the planula stage, when differentiation of stem cells into nerve cells and cnidocyte precursors starts. The development of cnidocytes is one of the milestones of present-day cnidarian ontogeny. Because they are formed early in development, the chance of losing them should have been small, but it might have happened. In this framework, paedomorphosis can provide an appropriate lens to analyze evolutionary patterns. Because cnidocysts appear in the early ontogeny of cnidarians, the loss of cnidocysts would have occurred by an early mutation in the life cycle of the Urmetazoa. This hypothetical animal without cnidocysts would have been harmless, a condition that would have promoted the evolution of new distinctive features, such as the definitive establishment of the bilateral bauplan, giving origin to the Urbilateria. The absence of such an advantageous structure might have triggered specialization in other focused directions, leading to the Cambrian explosion. The bulk of available morphological and molecular data suggestive of the monophyly of Bilateria does not contradict this hypothesis.

Other pedomorphic events might have occurred, such as the onset of sexuality at the larval stage. In fact, a sexual triploblastic planuloid animal, comparable to an acoel flatworm, would have started a lineage that we currently identify as the Acoelomata (Salvini-Plawen 1978). Similarly, by pedomorphic processes, in many species development of a medusa bud can be interrupted by anticipation of gonadal development. In this case, medusae (medusoids) are not released and become sexually competent, retaining a closed subumbrellar cavity as happens in a few

meiofaunal hydrozoans (e.g., *Otohydra vagans*, Swedmark and Teissier 1958). The closed cavity is formed through a process of schizocoely, similar to coelom formation in Sipunculida (Salvini-Plawen and Bartholomaeus 1995), and such a derived medusa might be suggestive of a schizocoelomate *Bauplan* (discussed by Boero et al. 1998). Finally, it has been argued that enterocoelomates might have originated from the closure of gastral septa in some anthozoan polyps (Sedgwick 1884). It is widely accepted that the origin of coelomic cavities is polyphyletic. Therefore, we are intrigued by the hypothesis that ancestors of the main lineages that make up the array of the Metazoa (acoelomates, schizocoelomates, enterocoelomates) might have sprung from separate heterochronic events.

## Building blocks: modules and skeletons

Animals are largely composed of many similar parts. The evolution of animal forms reflects the extent of variation of recurrent architectural motifs (serial homologies) at different dimensional scales, from cells to organs and systems, and can be recognized from lower invertebrates to vertebrates (Carroll 2001).

The architecture of cnidarians contains many more arguments in favor of a shared ancestry with bilaterians than previously thought, since it became apparent that basic dogmas about cnidarian features arose from the highly derived *Bauplan* of *Hydra* spp. polyps (e.g., lacking medusa and planula stages, entocodon formation, several orthologous genes of well-conserved transcription factors from cnidarians to vertebrates). Modularity, common in many cnidarian representatives, might have been derived by the evolution of molecular clues for regional specification of body architectures, including the polarization and iteration of modular units. Homeotic selector genes repeatedly would allow specifying groups of modular units as it happens for the regulation of segmentation in metameric bilaterians. The question is: are there shared molecular mechanisms leading to homologous processes of unit iteration in distantly related modular taxa ranging from sponges and cnidarians to chordates? The evolution of homeotic genes would have represented another milestone for metazoan diversification: increasing positional and structural specification pathways among phyla (e.g., Jakob and Schierwater 2007, Kamm et al. 2006, Ryan et al. 2007). It will be worth exploring the genetic devices adopted by modular animal taxa

to learn more about the evolutionary background available to the UrMetazoa.

Furthermore, the skeletal structures of various cnidarians feature organizations and components can be found throughout all the Metazoa, from internal or external carbonate elements (e.g., spicules of octocorals, corallites of hexacorals), to chitin-based exoskeletons (e.g., perisarc of hydrozoans, scyphozoans, or some creeping octocorals). Cnidarians generally do not moult as they can grow through modular additions of polyps and not by increments in individual polyp size. Several chitin-encased species, however, undergo a partial moult and skeletal renovation (Bouillon 1994). Are modularity and skeletons additional examples of convergent evolution or do they connect the Cnidaria with the milestones of metazoan evolution? A possible contribution to this argument will hopefully derive from future investigations on the developmental molecular pathways underlying these features among different taxa. The identification of genes that specify these features and the structural and functional comparison of these genes in representatives of different phyla are a first approach.

## Coda (*in cauda venenum*)

The present analysis of cnidarian features is mostly based on very old knowledge. Much evidence, e.g., regarding cnidarian mesodermal anatomies (reviewed by Seipel and Schmid 2006), has been known for almost a century. We are acquiring new powerful tools to test hypotheses but, paradoxically, we are losing the ability to build them. For many, morphology is seen as a dead discipline; comparative anatomy survives in paleontology, but it is considered as dead as morphology: a fossil. The dismissal of functional morphology and comparative anatomy was also linked to the excessively reductive attitude that prevailed after the invention of the electron microscope. Tiny details were at hand, but we did not look deep enough to understand the heuristic potential in the development of the entocodon within the medusa bud! Molecular fingerprints of developmental pathways and sequence data from lower Metazoa will shed light on the genetic basis of animal diversity only if we look at the whole picture with perceptive eyes, through the old, but sharp, lenses of morphology and comparative anatomy. If we can embrace such a fully integrative mode, the Cnidaria will most likely no longer appear as a dead branch of animal evolution.

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