Phylogenetic Context and Basal Metazoan Model Systems[1]

ALLEN G. COLLINS,[2],* PAULYN CARTWRIGHT,† CATHERINE S. MCFADDEN,‡ AND BERND SCHIERWATER*

*ITZ, Ecology & Evolution, TiHo Hannover, Bünteweg 17d, D-30559 Hannover, Germany
†Department of Ecology and Evolutionary Biology, 1200 Sunnyside Ave, University of Kansas, Lawrence, Kansas 66045
‡Biology and Department, 1250 N. Dartmouth Ave., Harvey Mudd College, Claremont, California 91711

SYNOPSIS.   In comparative studies using model organisms, extant taxa are often referred to as basal. The term suggests that such taxa are descendants of lineages that diverged early in the history of some larger taxon. By this usage, the basal metazoans comprise just four phyla (Placozoa, Porifera, Cnidaria, and Ctenophora) and the large clade Bilateria. We advise against this practice because basal refers to a region at the base or root of a phylogenetic tree. Thus, referring to an extant taxon or species as basal, or as more basal than another, can be misleading. While much progress has been made toward understanding some of the phylogenetic relationships within these groups, the relationships among them are still largely not known with certainty. Thus, sound inferences from comparative studies of model organisms demand continued illumination of phylogeny. Hypotheses about the mechanisms underlying metazoan evolution can be drawn from the study of model organisms in Cnidaria, Ctenophora, Placozoa, and Porifera, but it is clear that these model organisms are likely to be derived in many respects. Therefore, testing these hypotheses requires the study of yet additional model organisms. The most effective tests are those that investigate model organisms with phylogenetic positions among two sister groups comprising a larger clade of interest.

INTRODUCTION

As the rest of this volume attests, enormous insight can be gained through the study of model organisms belonging to the animal groups that diverged earliest in the history of Metazoa. Specifically, detailed knowledge of these so-called "basal metazoans" makes it possible to conceive of and evaluate hypotheses concerning early animal evolution, and these in turn provide clues about the origin of metazoan diversity. A key element of these comparative studies is that they are placed in a sound phylogenetic context. Thus, our aims in this review are threefold. First, we attempt to clarify how use of the term basal, as in the title of this symposium, can often lead to confusion. Second, we review current understanding of phylogenetic relationships between and within the early-diverging metazoan groups. Our discussion of Porifera is slight because a recent symposium (Integr. Comp. Biol., Vol. 45, 2) was dedicated entirely to this group, and Cnidaria is treated in greater depth because cnidarians outnumber the other groups in terms of model organisms. Finally, we reiterate and illustrate the importance of phylogenetic hypotheses for interpreting the evolutionary significance of observations made upon model organisms.

WHAT DOES BASAL MEAN?

In the context of a discussion of evolutionary relationships, basal refers to the region of the base or root of a phylogenetic tree. Often basal is used as an adjective to describe clades or taxa that branch near the root of a hypothesized phylogeny. For instance, as of 14 March 2005, a search of Google Scholar using the terms "basal Metazoa" OR "basal metazoan" OR "basal cnidarian" brings up 126 results, the vast majority representing papers dealing with model organisms published within the last six years. None of these papers define what a basal taxon is, but one can infer that the term is applied to taxa (often species) that derive from lineages that diverged early in the history of some larger clade. This usage can lead to conceptual problems (see also Krell and Cranston, 2004; Crisp and Cook, 2005). For example, in terms of metazoan phylogeny, there is little doubt that the lineages leading to extant members of the phyla Cnidaria, Ctenophora, Placozoa, and Porifera diverged relatively early in the history of Metazoa, and it is to these taxa (less Porifera) that "basal Metazoa" refers in the title of this symposium. However, Bilateria has a comparably ancient divergence within Metazoa because it is in all likelihood the sister group to one or some combination of these four phyla, and therefore is basal in the same sense.

Bilateria is probably not thought of as a basal metazoan group because bilaterians are relatively complex and likely derived in many respects, highlighting what might be a common misunderstanding about the word. Basal carries no explicit connotation about character states (see Crisp and Cook, 2005). Nevertheless, representatives of early-diverging taxa appear often to be chosen as subjects in comparative studies because of a priori expectations that these basal taxa possess character states that are more likely to be ancestral relative to those of later diverging taxa. Only in a comparative context can it be determined if these prior expectations are borne out. As a case in point, recent studies of gene expression have brought into question

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[2] Present address of Allen G. Collins: NMFS, National Systematics Laboratory, National Museum of Natural History, MRC-153, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012; E-mail: CollinsA@SI.edu
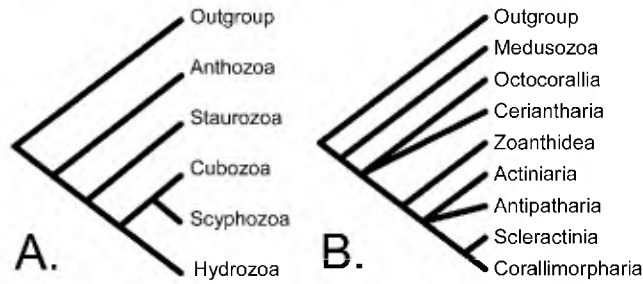
FIG. 1. Two equivalent phylogenetic hypotheses that show different levels of phylogenetic detail. On the left (A), the cnidarian classes, four of which make up the clade Medusozoa, are shown. On the right (B), Medusozoa is presented as a single taxon while the component taxa of Anthozoa are shown in greater detail. Anthozoa may be perceived as basal on the diagram to the left, whereas Medusozoa may appear to be basal on the diagram to the right. In either case, the underlying hypothesis that Anthozoa and Medusozoa are sister groups is unchanged.

whether radial symmetry and diploblasty, two characters often assumed to be ancestral for Cnidaria, are actually derived from bilateral symmetry and triploblasty inherited from the common ancestor of cnidarians and bilaterians (Finnerty *et al.*, 2004; Martindale *et al.*, 2004)

We concur with Krell and Cranston (2004) that taxa should not be identified as more (or less) basal than others, and that making explicit statements about relationships, especially sister group relationships, would lead to greater clarity in phylogenetic and evolutionary discussions. For example, Anthozoa, widely accepted as the sister group to all other cnidarians (Bridge *et al.*, 1992; and see discussion in Marques and Collins, 2004), is often referred to as basal within Cnidaria. The impression that Anthozoa is the basal cnidarian group may depend upon the taxa being discussed. In the context of relationships among cnidarian taxa assigned the Linnaean rank of class, Anthozoa may be perceived as basal (Fig. 1A). However, this tree can be redrawn with Anthozoa presented in greater phylogenetic detail, exposing much of the diversity present within it, and Medusozoa (comprising non-anthozoan cnidarians) in lesser detail (Fig. 1B). Relative to those leading to the orders within Anthozoa, the lineage leading to Medusozoa diverges earliest within Cnidaria. In this context, Medusozoa might therefore be perceived as the basal cnidarian group, even though the underlying hypothesized relationships are unchanged. Use of this example may appear as gamesmanship, but we contend that elements of both arbitrariness and subjectivity are often conveyed in statements about the basal taxon of a group (see also Crisp and Cook, 2005).

THE EARLY-DIVERGING METAZOAN PHYLA

Four phyla (Placozoa, Porifera, Cnidaria, and Ctenophora) are typically thought to have an ancient origin within Metazoa. The dicyemid mesozoans, once hypothesized to be non-bilaterian metazoans, have since been shown to possess central class Hox genes indi-

cating that these enigmatic parasites are secondarily simplified bilaterians (Kobayashi *et al.*, 1999; but see Noto and Endoh, 2004). Similarly, Myxozoa was for a long time considered to be a group of protists, later hypothesized to be aberrant cnidarians (*e.g.*, Siddall *et al.*, 1995), and still more recently claimed to be simplified bilaterians (Anderson *et al.*, 1998; Okamura *et al.*, 2002), or the sister group to bilaterians (Zrzavý and Hypša, 2003). While it remains possible that dicyemid mesozoans and myxozoans are non-bilaterian animal groups, the balance of available evidence suggests that these two groups are more likely to be derived and highly simplified bilaterians.

Placozoans possess the simplest bauplan among extant metazoans. Only four somatic cell types (see however Martinelli and Spring, [2003] and Jakob *et al.*, [2004] for possibly additional cell types) have been recognized. Gland and ciliated cylindrical cells are found in the lower digestive epithelium, whereas only ciliated cells are situated in the upper epithelium (Grell and Ruthmann, 1991). Between the two epithelia is a three-dimensional meshwork of syncytial fibre cells (Buchholz and Ruthmann, 1995). The simplicity of placozoans is further highlighted by their lack of any kind of symmetry axis, organs, nerve and muscle cells, basal lamina, and extracellular matrix, though cell-to-cell junctions of two types are known (Grell and Ruthmann, 1991). Since their discovery, these animals were often regarded as either the earliest diverging lineage of metazoans or as the earliest branch of Metazoa exclusive of sponges (see Syed and Schierwater [2002] for review). More recently, 18S analyses have challenged this view by suggesting that Placozoa forms a clade with Cnidaria and Bilateria (*e.g.*, Collins, 1998; Kim *et al.*, 1999).

In contrast to placozoans, poriferans possess a relatively large number of distinct cell types (usually more than a dozen) and possess characteristic molecules indicative of extracellular matrix, cell adhesion and apoptosis (Müller, 2003). Hexactinellids are quite unusual in possessing tissues that are largely syncytial (Mackie and Singla, 1983). Despite a high diversity of body organizations, many sponges are characterized by axial symmetry, particularly in the groups Hexactinellida and Calcispongia (=Calcarea), for which axial symmetry is inferred to be ancestral (Manuel *et al.*, 2003). Numerous molecular studies (starting with Wainright *et al.*, 1993) have buttressed the classical assertion (going back at least to Haeckel, 1874) that poriferans are likely to represent the earliest diverging metazoans, and many have further suggested that sponges form a paraphyletic grade at the base of Metazoa, with Calcispongia as the sister group to all non-sponge metazoans (Cavalier-Smith *et al.*, 1996; Collins, 1998; Kruse *et al.*, 1998; Zrzavý *et al.*, 1998; Borchiellini *et al.*, 2001; Medina *et al.*, 2001; but see Manuel *et al.*, 2003). More recent analyses with greater taxon sampling aimed primarily at understanding relationships within Demonspongiae, by far the most diverse group of sponges, have not shed light on the
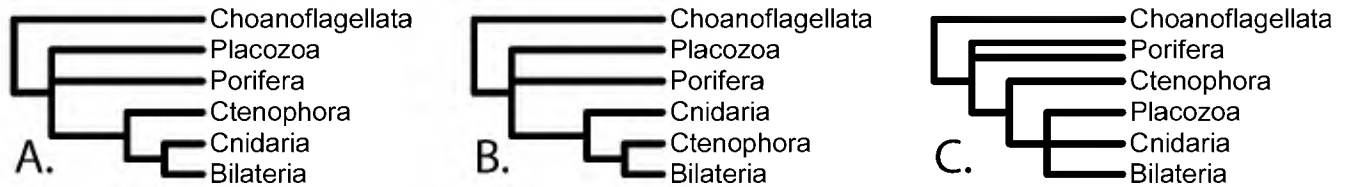
FIG. 2. Hypotheses of early animal phylogeny based on (A) the scenario by Salvini-Plawen (1978); (B) cladistic analyses by Schram (1991), Nielsen *et al.* (1996), Zrzavý *et al.* (1998), and Peterson and Eernisse (2001); and (C) typical 18S rRNA analyses (Collins, 1998; Kim *et al.*, 1999).

question of poriferan monophyly (Borchiellini *et al.*, 2004; Nichols, 2005).

The phylum Cnidaria is a diverse monophyletic group (Collins, 2002; but see Zrzavý and Hypša, [2003] regarding Myxozoa) of animals with two epithelia, an outer epidermis and inner digestive gastrodermis. The mesoglea sandwiched between the basal lamina of the epithelial layers is either acellular or contains mesenchymal cells (Hyman, 1940), and ranges from being nearly nonexistent to quite thick in medusae, giving them their characteristic gelatinous appearance. Cnidarians have many different cell types, including gametes and nematocytes, which originate in the adult form from an interstitial cell lineage (Hyman, 1940). Other cell types include epithelial muscular cells, gland cells, and nerve cells. Many cnidarians are characterized by a complex, metagenetic life cycle including a sexually produced planula larva that metamorphoses into a sessile polyp stage, which may in turn asexually produce morphologically distinct, free-swimming, sexual medusae. The phylum has traditionally been divided into four classes (Anthozoa, Scyphozoa, Hydrozoa and Cubozoa) distinguished by variation in these life cycle characteristics. Class Anthozoa lacks a medusoid stage entirely, and the polyps may be either solitary or colonial; in the other three classes, collectively termed Medusozoa, polyp and medusa stages alternate or the life cycle is predominated by one or the other stage. In Scyphozoa and Cubozoa the medusa stage usually predominates, while Hydrozoa typically alternate between sessile, colonial polyp and sexual medusa stages. Stauromedusans, which have traditionally been classified as an order of Scyphozoa but recently placed in the class Staurozoa (Marques and Collins, 2004), are benthic throughout their life cycles.

Members of the phylum Ctenophora bear some general similarities to cnidarian medusae including a gelatinous mesenchymal layer and a symmetrical organization around an aboral/oral axis (Hyman, 1940). However, unlike cnidarians, ctenophores possess rows of cilia and a unique, complex sensory organ and lack cnidae and metagenesis. It is therefore largely appreciated that the resemblance between ctenophores and cnidarians is superficial and does not reflect a particularly close evolutionary relationship (Harbison, 1985). Ctenophora appears to be a monophyletic group (Podar *et al.*, 2001), separate from other non-bilaterian phyla, and is often regarded as the sister group to Bi-

lateria based on morphological considerations (Schram, 1991; Nielsen *et al.*, 1996; Zrzavý *et al.*, 1998; Peterson and Eernisse, 2001). In contrast, 18S rDNA data suggest that Ctenophora is less closely related to Bilateria than is Cnidaria (Collins, 1998; Kim *et al.*, 1999).

RELATIONSHIPS AMONG EARLY-DIVERGING METAZOAN LINEAGES

Over the past century and more, a number of different approaches have been taken to provide a historical context for understanding early animal character evolution. For most of this period, a scenario-based approach has predominated. The hallmark of the scenario approach is the synthesis of biological observations into a plausible historical narrative containing explicit descriptions of hypothetical ancestors. Indeed, hypothetical ancestors (*e.g.,* gastrea, planuloid, plakula, etc.) figure so prominently in these elaborate scenarios that competing accounts of early metazoan evolution came to be known by their names. Alternative scenario-based views are reviewed by a number of researchers (*e.g.,* Salvini-Plawen, 1978; Willmer, 1990; Nielsen, 2001) and are sometimes criticized for having led to little consensus about early character evolution in Metazoa. However, it is likely that the most widespread views of the early phylogenetic history of Metazoa have not dramatically changed over the past 100 years. Indeed, many early scenarios remain viable today because great uncertainty about the relationships among early-diverging metazoan lineages continues to persist.

During the past decade or so, a number of analyses based upon more explicit phylogenetic methods involving large matrices of characters have been conducted. Despite these attempts, relationships among Bilateria, Cnidaria, Ctenophora, Placozoa, and Porifera remain obscure. Cladistic analyses of morphological and developmental characters have consistently suggested that Cnidaria is the sister group to Ctenophora plus Bilateria and that both Porifera and Placozoa diverged earlier in metazoan history (Schram, 1991; Nielsen *et al.*, 1996; Zrzavý *et al.*, 1998; Peterson and Eernisse, 2001) (Fig. 2B). Such a hypothesis conforms to one element of a scenario offered by Salvini-Plawen (1978) that has Placozoa and Porifera diverging early in metazoan history, but contradicts a second element that has Cnidaria sharing a closer relationship with Bilateria than does Ctenophora (Fig. 2A). The latter

view, which stems from observed similarities between the bilateral planulae of cnidarians and the structure of bilaterian acoel flatworms (Salvini-Plawen, 1978) gains some support from 18S molecular studies (*e.g.,* Collins, 1998; Kim *et al.*, 1999), which suggest that Cnidaria is more closely related to Bilateria than is Ctenophora. However, these same data suggest that Placozoa may also be more closely related to Bilateria than is Ctenophora, a hypothesis that is at odds with most, if not all, analyses of morphology (Fig. 2C).

Recent studies using sequences from the 28S rDNA gene have shown that this marker can be helpful in assessing relationships among and within various metazoan groups (*e.g.,* Mallatt and Winchell, 2002; Winchell *et al.*, 2002; Telford *et al.*, 2003; Mallatt *et al.*, 2004). Unfortunately, 28S evidence appears to be inconclusive concerning relationships among Porifera, Cnidaria, Ctenophora, and Bilateria (Medina *et al.*, 2001). Not surprisingly, adding 28S data from placozoans (Voigt *et al.*, 2004) does little to further our understanding of metazoan relationships other than to quash the tenuous conclusion of Cavalier-Smith and Chao (2003), who recently revived the old view of placozoans as degenerate cnidarians (Krumbach, 1907). Still other nuclear markers have been shown to have little or no ability to discriminate among competing hypotheses of early animal phylogeny (Rokas *et al.*, 2003). Thus, present knowledge of basal metazoan phylogeny is surprisingly limited, given the relatively small number of taxa involved. Answering these outstanding questions is of obvious importance for inferring the ancestral states from which the living animal phyla were derived.

RELATIONSHIPS WITHIN CTENOPHORA AND PLACOZOA

Very little work has been conducted on the internal relationships of ctenophores and placozoans. The two most important works on ctenophoran relationships are those of Harbison (1985) and Podar *et al.* (2001). Fortunately, these two studies reinforce each other in many respects despite the fact that their methods of analysis and underlying data differ greatly. One of the two plausible scenarios for ctenophore relationships laid out by Harbison (1985), when re-rooted, is very closely approximated by the 18S-based hypothesis (Podar *et al.*, 2001). These analyses show that the lobate, beroid, and platyctenid groups are all likely descended from cydippid ctenophores. Placozoan phylogeny was not addressed until very recently (Voigt *et al.*, 2004), which is not surprising since the phylum was believed to contain just a single species. In contrast to its monotypic taxonomy, Placozoa contains several deep phylogenetic divergences (Voigt *et al.*, 2004), and it would appear that its true diversity is largely unexplored.

RELATIONSHIPS WITHIN CNIDARIA

As with Metazoa generally, cnidarian phylogeny and diversification have been analyzed for more than a century, continuing to the present day (Marques and Collins, 2004). While remaining agnostic about whether an actinula or polyp might have been the ancestral cnidarian body plan, Brooks (1886) favored the idea that this ancestor was pelagic and directly gave rise to the trachyline hydrozoans at an early stage, implying that even anthozoans were derived from pelagic ancestors. This view was presented in several textbooks (*e.g.,* Hyman, 1940), but was far from universally held among cnidarian workers. Instead, many used a scenario approach to conclude that solitary sessile polyps represent the ancestral cnidarian form (Haeckel, 1879; Hadzi, 1953; Werner, 1973; Salvini-Plawen, 1978). This latter hypothesis has emerged as the consensus view among cnidarian workers due to new data, and suggests that an optimistic outlook about the tractability of this and other questions concerning cnidarian phylogeny is warranted. It now appears clear that Cnidaria comprises two major clades, Anthozoa and Medusozoa (Werner, 1973; Salvini-Plawen, 1978; Schuchert, 1993; Bridge *et al.*, 1995; Kim *et al.*, 1999; Collins, 2002), the latter being characterized by a linear mitochondrial genome (Bridge *et al.*, 1992) as well as by medusae. Even within these two diverse clades, many major relationships have become clarified.

Within Anthozoa, monophyly of the two sub-classes, Hexacorallia and Octocorallia, is supported by 16S (France *et al.*, 1996) and 18S (Song and Won, 1997; Berntson *et al.*, 1999) sequence data and by a combined analysis of rDNA with morphological characters (Daly *et al.*, 2003). Ordinal relationships within each of these distinct sub-classes have, however, been more difficult to ascertain. Several recent analyses of both morphological and molecular data support Ceriantharia (tube anemones) as the sister group to all other members of Hexacorallia (France *et al.*, 1996; Daly *et al.*, 2003), although a study based on partial 28S rDNA sequences placed Ceriantharia as the sister group to all other anthozoans (Chen *et al.*, 1995). There is an emerging consensus that order Actiniaria (sea anemones) forms a monophyletic sister group to a clade comprising orders Scleractinia, Corallimorpharia, and Antipatharia, each of which appears to be monophyletic (Berntson *et al.*, 1999; Won *et al.*, 2001; Daly *et al.*, 2002, 2003). Relationships of families within the Scleractinia, however, appear to be highly polyphyletic (Romano and Palumbi, 1996; Romano and Cairns, 2000; Fukami *et al.*, 2004).

Ordinal relationships within Octocorallia are much less well resolved than those of Hexacorallia. Data from 18S rDNA (Berntson *et al.*, 2001), rRNA secondary structure (Sánchez *et al.*, 2003a), and several mitochondrial genes (France *et al.*, 1996; C.S.M. *et al.*, unpublished data) support two distinct clades of Octocorallia that do not conform to the current taxonomic division of this group into orders Pennatulacea (sea pens), Helioporacea (blue coral) and Alcyonacea (soft corals and gorgonian sea fans). Although pennatulaceans and *Heliopora* are confined to one of these two clades, both clades include a taxonomically heterogeneous mix of alcyonaceans. For example, gorgonians

of sub-order Calcaxonia (Grasshoff, 1999) appear to be more closely related to pennatulaceans than to holaxonian gorgonians with which they were formerly united as order Gorgonacea. Members of other recognized subordinal groups, such as Alcyoniina, Scleraxonia and Stolonifera, also appear in both clades, as do members of the soft coral family Alcyoniidae. Within each of these two distinct octocoral clades, nuclear rDNA and mitochondrial genes provide little taxonomic resolution among sub-ordinal groups (Berntson et al., 2001; Sánchez et al., 2003b; C.S.M. et al., unpublished data).

The monophyly of the Medusozoa is nearly beyond dispute given that medusozoans possess the only linear mitochondrial genomes known in Metazoa (Bridge et al., 1992). Monophyly of cubozoans is widely assumed based upon morphology, particularly the structural complexity of the eye (e.g., Berger, 1898; Martin, 2002), and molecular analyses that include multiple representatives of Cubozoa support this hypothesis (Kim et al., 1999; Collins, 2002). Sequence data also provide strong support for the monophyly of Hydrozoa, the most diverse group of medusozoans (Bridge et al., 1995; Kim et al., 1999; Collins, 2002). In contrast, no study has found a monophyletic Class Scyphozoa, as traditionally defined, supporting efforts to split the group into two classes (Marques and Collins, 2004; Collins and Daly, 2005). Stauromedusae, which lacks a medusoid stage, has been placed into the class Staurozoa, whereas the three orders where a polyp generates medusae through strobilation (Coronatae, Rhizostomeae, and Semaeostomeae) now constitute Scyphozoa (Marques and Collins, 2004). No published molecular studies have provided convincing evidence of the relationships among Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa, though 16S data indicate that Staurozoa is the sister group to a clade containing the other groups (Collins and Daly, 2005). Data from the 28S gene reinforce the assertion that Stauromedusae is the sister group to all other medusozoans and show great promise in working out many of the other troublesome medusozoan nodes (A.G.C., unpublished data).

PHYLOGENETIC JUSTIFICATIONS FOR PICKING MODEL ORGANISMS

Model organisms are essential for providing in-depth information about a particular taxon, and the justifications for choosing a model organism are varied. Among the more important considerations are the ability to culture the organism (ideally over the entire life cycle) and the amenability of the organism to experimental manipulation. Another important factor is its phylogenetic position. Presently, many species are highlighted for study because of their membership in Cnidaria, Ctenophora, Placozoa, or Porifera. For example, as noted above, the phrase "basal metazoan" is often applied to species from these phyla when they are used in descriptive studies with a comparative slant. However, being part of an early-diverging clade

does not necessarily mean that the species exhibits features that are representative of the group. For instance, two very important model organisms, Hydra and Nematostella, are derived cnidarians in many respects. Hydra lives in freshwater, lacks a ciliated planula, and has clearly lost a medusa stage during its ancestry. Nematostella lives in brackish water, burrows in soft sediment, and lacks a pedal disk and other adult characters. Their phylogenetic positions, within Medusozoa and Anthozoa respectively, do not provide any information about whether these animals retain ancestral states for other characters under study.

Understanding how model organisms are useful in a comparative context could be advanced by more strongly emphasizing specific ancestral nodes that might be illuminated from study rather than on the taxon to which a species belongs. For example, studies on the model organism Hydra have revealed detailed information about mechanisms that control its development. The extent to which these results can be generalized can only be determined by investigating the mechanisms that control development in other cnidarians. The most effective test is to investigate one of the many cnidarians that is most distantly related to Hydra because such a comparison is most likely to reveal information about the most recent common ancestor of Cnidaria. Recent and ongoing effort to develop Nematostella as a new model organism (e.g., Wikramanayake et al., 2003; Darling et al., 2005) is phylogenetically justified because distantly related cnidarians, especially Hydra, are established model systems. Nematostella's study is not warranted simply because it is a member of "the basal cnidarian class" Anthozoa. Even if Nematostella had been developed as a model organism prior to Hydra, studies of medusozoan taxa would have been necessary to determine the generality of the results derived from Nematostella. Similarly, when differences arise between results obtained from Hydra and those from Nematostella, there can be little justification for assuming one set of results is more likely to represent ancestral states in Cnidaria (in the absence of relevant information obtained from outgroup taxa). Instead, other model organisms—ones that are most likely to reveal ancestral character states for Anthozoa and Medusozoa—will have to be developed in order to resolve the differences.

THE IMPORTANCE OF A PHYLOGENETIC CONTEXT

The origination and elaboration of animal development and bauplan organization evolved during the early history of Metazoa. Therefore, the elucidation of developmental processes in taxa the diverged early in metazoan history is key for understanding animal diversification. For example, the study of regulatory genes involved in setting up bauplan organization can provide insight into the mechanisms involved in the evolution of new bauplans (e.g., Schierwater and DeSalle, 2001; Valentine, 2003; Ronshaugen et al., 2002). Model organisms play an essential role in this

understanding because in-depth developmental information from model systems can be used to generate hypotheses about mechanisms underlying character evolution (Kellogg and Shaffer, 1993).

The model organism *Hydra* is one of the most intensely studied "basal metazoans." Studies of *Hydra* have implicated many regulatory genes in its development (*e.g.,* Steele, 2002; Broun and Bode, 2002) and have shown that the expression patterns of these genes are restricted to specific regions and/or structures of the polyp (*e.g.,* Shenk *et al.,* 1993; Grens *et al.,* 1996; Steele *et al.,* 1996; Martinez *et al.,* 1997; Hassel *et al.,* 1998; Braun *et al.,* 1999; Smith *et al.,* 1999; Bridge *et al.,* 2000; Hobmayer *et al.,* 2000; Smith *et al.,* 2000). The evolution of many cnidarians has been accompanied by axial patterning changes in the very same regions and/or structures of the polyp that have been implicated to be under the control of the developmental regulatory genes mentioned above. Thus, developmental information from *Hydra* can be used to generate hypotheses about the mechanisms underlying cnidarian evolution (Cartwright, 2004).

Although the majority of developmental studies of cnidarians have concentrated on *Hydra,* developmental regulatory genes have also been characterized in other species including *Hydractinia* (Cartwright *et al.,* 1999; Mokady *et al.,* 1998), *Podocoryna*[3] (Aerne *et al.,* 1995; Muller *et al.,* 1999; Masuda-Nakagawa *et al.,* 2000; Yanze *et al.,* 2001; Spring *et al.,* 2002; Bridge *et al.,* 2004), *Nematostella* (Technau and Bode, 1999; Scholz and Technau, 2003; Finnerty *et al.,* 2003; Wikramanayake *et al.,* 2003) and *Acropora* (Hayward *et al.,* 2001; Hayward *et al.,* 2002; Hayward *et al.,* 2004). As more information becomes available about the role of developmental regulatory genes in these and other cnidarians, it is of critical importance that these data are integrated into a phylogenetic context. Phylogenies provide the framework from which developmental hypotheses about character evolution can be generated (*e.g.,* Wake *et al.,* 1991; Raff, 1996; Mabee, 2000; Swalla and Collazo, 2000; Santini and Stellwag, 2002). Interpretation of developmental data in a phylogenetic context is essential for proposing explicit hypotheses about the role of these genes/gene pathways in character evolution. Morphological and developmental characters can either be mapped on the phylogeny or inferred using more sophisticated methods (see review of Crisp and Cook, 2005). Moreover, tests for correlation of the gene expression pattern and the morphological character the gene is purported to control can be performed in a phylogenetic context (Mabee, 2000).

Although evolutionary developmental studies in model organisms representing clades that diverged ear-

ly in metazoan history are in their infancy, some comparative data are available. The most well studied regulatory gene in these taxa is the homeobox gene Cnox-2 (Schierwater *et al.,* 1991). This gene, along with the *Trichoplax* gene Trox-2, has been proposed to be either a gsx parahox homolog (Finnerty and Martindale, 1999; Finnerty *et al.,* 2003) or a protohox gene belonging to the Diplox-2 gene family, which also contains the *Trichoplax* gene Trox-2 (Schierwater and Kuhn, 1998; Jakob *et al.,* 2004). This dispute highlights the need for more reliable methods for determining homology, though character-based algorithms appear to show some promise (Sarkar *et al.,* 2002). Curiously, no Cnox-2 homolog has been identified in sponges or ctenophores.

Expression of Cnox-2 has been characterized in the polyps of the anthozoans, *Acropora* (Hayward *et al.,* 2001) and *Nematostella* (Finnerty *et al.,* 2003), and the hydrozoans, *Hydra* (Shenk *et al.,* 1993), *Hydractinia* (Cartwright *et al.,* 1999) and *Podocoryna* (Yanze *et al.,* 2001). Mapping Cnox-2 expression patterns onto a phylogeny and reconstructing the ancestral patterns of expression can lead to explicit hypotheses about the role of Cnox-2 in cnidarian evolution. Expression of Cnox-2 among cnidarian taxa is highly variable. Shenk *et al.* (1993) reported Cnox-2 expression in the aboral regions of the adult body column in *Hydra.* A similar aboral expression was also found in *Hydractinia* (Cartwright *et al.,* 1999). In *Podocoryna,* Cnox-2 is expressed in the aboral region in the developing polyp, and expression is undetectable in the adult polyp (Yanze *et al.,* 2001). By contrast, Anthox-2 (the Cnox-2 anthozoan homolog) is expressed in the oral region of the anthozoan polyps *Acropora* (Hayward *et al.,* 2001) and *Nematostella* (Finnerty *et al.,* 2003).

In *Trichoplax* the expression of the Cnox-2 homolog, Trox-2, is restricted to the periphery of the organism, at the boundary between the upper and lower epithelia (Jakob *et al.,* 2004). *Trichoplax* lacks a defined axis yet the spatially restricted pattern of Trox-2 expression suggests a role in defining positional boundaries. Given that *Trichoplax* is an outgroup to the investigated cnidarians (Ender and Schierwater, 2003), the spatially restricted pattern of expression of Cnox-2 is likely to be ancestral for cnidarians and *Trichoplax.* However, because this gene has an oral expression pattern in the sampled anthozoans and an aboral expression in the sampled hydrozoans (Fig. 3), the likely state of this character at the basal cnidarian node cannot be inferred. Comparative information about *Cnox-2* expression in other relevant groups, especially ctenophores and scyphozoans, are necessary to resolve the ambiguous ancestral states. Note that even within Anthozoa and Hydrozoa, major clades, *e.g.,* Octocorallia and Trachylina respectively, have yet to be sampled and could provide key information about the likelihood of various ancestral states of *Cnox-2* expression.

Studies of *forkhead* gene expression patterns in *Hy-*

[3] Present taxonomists of Cnidaria consider the correct spelling of the genus to be *Podocoryna* (Calder, 1988), the experimental animal called *Podocoryne carnea* to be in either *Podocoryna* or *Hydractinia,* and the species may actually be *Hydractinia exigua* (Haeckel, 1879) (P. Schuchert, personal communication).
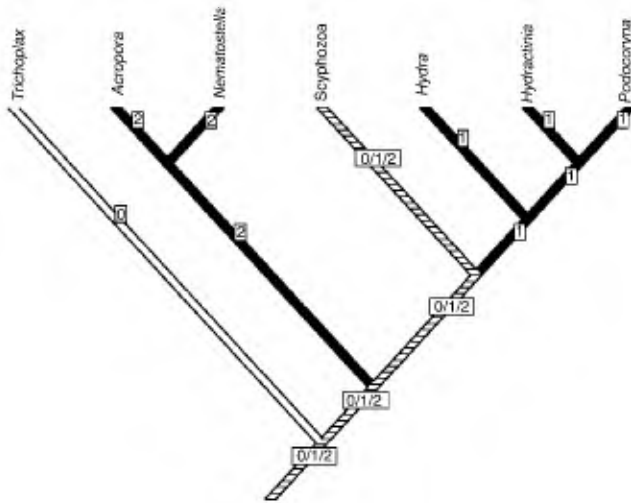
FIG. 3. Phylogenetic relationships among model organisms often referred to as basal metazoans showing the character states of Cnox-2 expression for terminal taxa, and the most parsimonious state at ancestral nodes. 0 = spatially restricted expression; 1 = aboral expression; 2 = oral expression. Character reconstruction was performed in MacClade vs. 4.06 (Maddison and Maddison, 2001).

dra and *Nematostella* serve as an additional example of interpretations of ancestral and derived conditions in a phylogenetic context. In *Hydra* the forkhead gene *budhead* is expressed in a distinct band on the hypostome, a region just proximal to the mouth (Martínez et al., 1997). In *Nematostella*, the *forkhead* homolog is expressed in the pharynx and pharyngeal mesenteries (Fritzenwanker et al., 2004). Anthozoans lack a hypostome and instead, the mouth is located on a flat oral disk that folds inwardly to the pharynx. These studies demonstrate that the expression of *forkhead* in proximity to the mouth is an ancestral condition and it has been suggested that these expression patterns provide evidence that the pharynx in anthozoans and the hypostome in hydrozoans are homologous structures (Martindale et al., 2004).

Comparisons between *Hydra* and *Nematostella* at the genomic level have also been shown to inform the reconstruction of ancestral states. The Wnt family of genes has undergone extensive diversification in vertebrates, which have twelve members and *Drosophila* and *C. elegans*, which have six (Prud'homme et al., 2002). Only one *Hydra* Wnt gene has been identified (Hobmayer et al., 2000). If it is assumed that the *Hydra* condition is ancestral, then the Wnt family must have diversified after the cnidarian lineage diverged from bilaterians. An unexpected recent finding however has shown that *Nematostella* has eleven of the twelve orthologs found in vertebrates (Kusserow et al., 2005), demonstrating that the Wnt family likely diversified prior to the cnidarian/bilaterian divergence and that genes were subsequently lost in the lineages leading to *Hydra* and Ecdysozoa.

FUTURE DIRECTIONS

Understanding the early phylogenetic history of Metazoa is gradually becoming clearer, especially through the continued application of those approaches that rely on rigorous analytical methods and the utilization of different types of data including molecular sequences. A consensus is beginning to emerge regarding many key nodes in basal metazoan and cnidarian phylogeny, but many important questions remain. For instance, what is the earliest diverging lineage of Metazoa, and what is the sister group of Bilateria? Increased taxon sampling with the inclusion of fossil taxa, in conjunction with the use of novel molecular markers and new analytical methods may help resolve many of these nodes. Most importantly, it should not be long until the complete genome is known for one or more species belonging to each of the major clades that diverged early in metazoan history. These developments should lead to a rapid solution to longstanding questions about early animal phylogeny, as well as an accumulation of new hypotheses about the mechanisms involved in the evolution of metazoan bodyplans and genomes.

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