

Can we ever identify the Urmetazoan?

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Synopsis Unraveling the root of the metazoan tree of life has been a difficult task since the time of Haeckel and the invention of phylogenetics. Even considerable amounts of recent molecular data have not provided a generally accepted answer. Here, we review the major problems of this phylogenetic conundrum and provide some directions for solving it.

The ever lingering question: “What did the Urmetazoan look like?” has not lost its charm, appeal or elusiveness for one and a half centuries. A solid amount of organismal data give what some feel is a clear answer (e.g., Placozoa are at the base of the metazoan tree of life), a single “eye-catching” character gives an another appealing answer (e.g., Porifera at the base), and a diversity of molecular data gives almost as many answers as there are exemplars, and recent molecular analyses with large data sets even suggest that we may never be able to solve the question with molecular data (Collins et al. 2005; Hadrys et al. 2005; Rokas et al. 2005; Schierwater 2005; Rokas and Carroll 2006; see also Figs. 1–3). The problems involved in this phylogenetic conundrum encompass a wide array of roadblocks present in many deep phylogeny problems. This review examines these problems with an eye toward potential solutions.

The problem of “paucity of morphological characters”

The hypothetical “Urmetazoan” must have had an extremely simple morphology, perhaps just complex enough to pass the bridge between a protist and a metazoan (i.e., the possession of more than one somatic cell type). Intracellular differentiation became the motor for radiation of the metazoan *Bauplan*, which over time created *Bauplans* consisting of dozens of somatic cell types forming a diversity of organs and body structures. Thus, with more and more evolutionary divergence, the number of morphological characters has increased giving the

systematist more and more characters with which to work. At the base of the tree, however, morphology was “frozen” as very subtle and uninterpretable anatomical changes occurred, and hence we are left with very few anatomical characters and thus very few quantitative data. Placozoans meet these expectations; they possess the simplest *Bauplan* among extant metazoans. Only four or five somatic cell types (Jakob et al. 2004) have been recognized. The simplicity of placozoans is further highlighted by their lack of any kind of axis of symmetry, organs, nerve and muscle cells, basal lamina, and extracellular matrix (although cell-to-cell junctions of two types are known) (Grell and Ruthmann 1991). Hence, all these characters must be coded as “missing” in the Placozoa. In contrast to placozoans, Porifera usually possess more than a dozen somatic cell types and also an extracellular matrix (ECM). In Cnidaria and Ctenophora morphological complexity further increases. Comparative (and also functional) morphology allows for an interesting interpretation of metazoan phylogeny: of all extant metazoan groups Placozoa are the earliest diverging lineage (see Syed and Schierwater 2002a, 2002b for review). The number of characters is limited, however, and stretching the possible number of morphological characters present in basal (diploblastic) metazoans to the limit we achieved a maximum count of 17 morphological characters (Appendix 1), four of which are phylogenetically non-informative and two of which are coded as “missing” in Placozoa. While this analysis shows Placozoa to be basal, very little robustness can be ascertained in a phylogenetic analysis of these anatomical characters alone.

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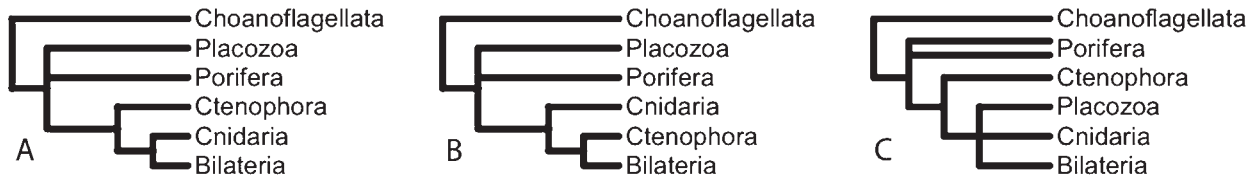


Fig. 1 Hypotheses of early animal phylogeny based on (A) the scenario by Salvini-Plawen (1978); (B) cladistic analyses by Schram (1991), Nielsen et al. (1996), Zrzavy' et al. (1998), and Peterson and Eernisse (2001); and (C) typical 18S rRNA analyses (Collins 1998; Kim et al. 1999). From Collins et al. 2005 ICB 45:585–594 with permission from SICB.

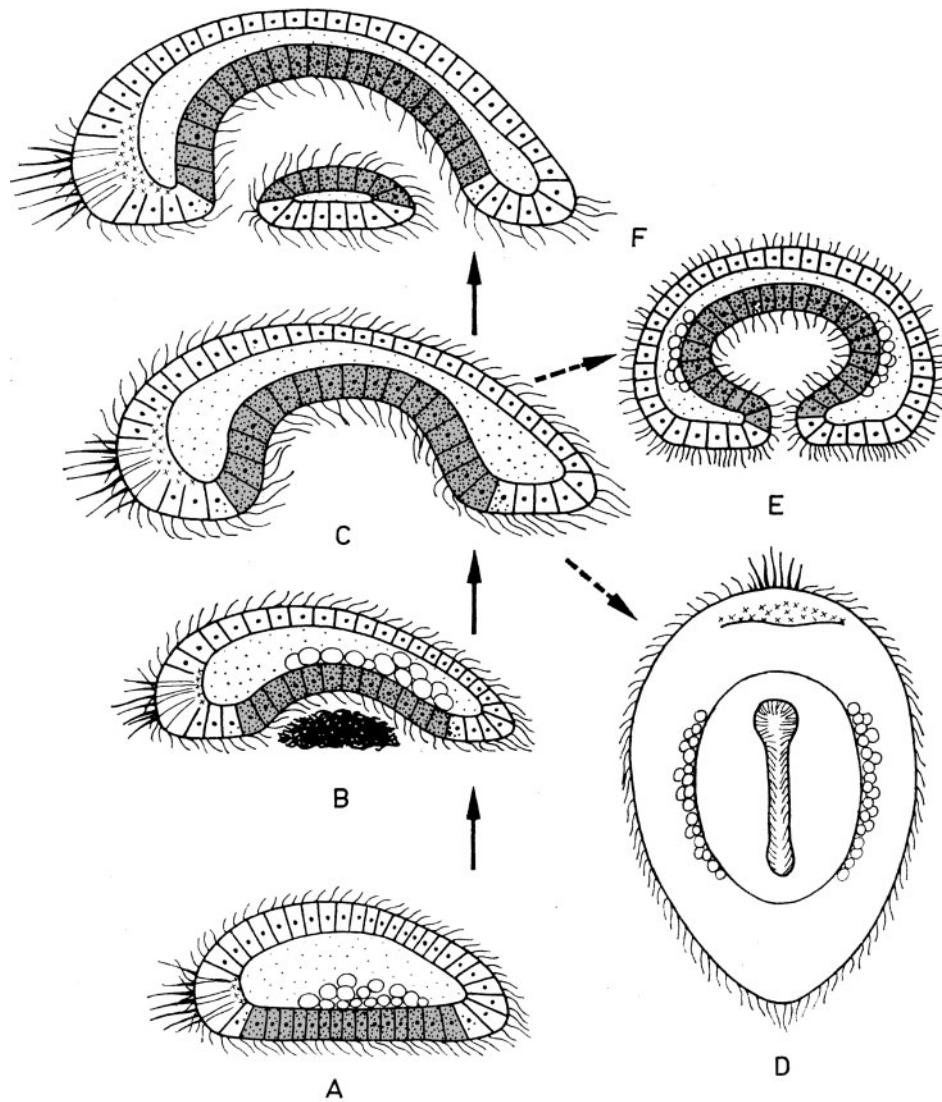


Fig. 2 Placula-hypothesis and Bilaterogastraea-hypothesis of metazoan evolution according to Jägersten (1955, 1959) and Bütschli (1884): a “benthoblastaea”-stage that looks similar to a placozoan *Bauplan* gives rise to the “bilaterogastraea” with a through-gut. Then polarity, specialized lower epithelium and an inner gastric cavity develop (D: transverse section showing “oral slit”). In this scenario, the vagile benthoblastaea has already developed an A/P-axis and bilateral symmetry and the presumed entoderm of the benthoblastaea (orange color) is homologous to the nutritive lower epithelium of *Trichoplax*. From Syed T, Schierwater B (2002a) with permission from Vie et Milieu.

The problem of “quality of characters”

Many researchers regard the quality of certain morphological characters that identify Placozoa as basal to all other diploblasts to be extremely reliable. For instance, the lack of an ECM could be considered of tantamount importance and relevance to the question. Others see Porifera as basal, and assess the reliability of a single morphological character as reliable—so highly reliable that all other characters become less important. The eye-catching character here is the morphological similarity between the choanocytes in the sponge gastrodermis and the single-celled choanoflagellates. In addition the basal placement of choanoflagellates as the outgroup to metazoan animals adds to the temptation to place prominence on this single character and judge it more important than any others (cf. Fig. 3). Both of these cases of reliance on a single preferred character are examples of single-character systematics, a practice whose validity as a systematic approach has been questioned for the past decade (Brower et al. 1996; Nixon and Carpenter 1996; Gatesy et al. 2002, 2003; Fitzhugh 2006; Reipel and Kearney 2006; Reipel et al. 2006).

The problem of the “diploblast-triploblast paradigm”

Traditionally it was considered that Placozoa, Porifera, Cnidaria, and Ctenophora were clearly built upon two cell layers only, the ectoderm and endoderm. Attempts to question this paradigm, however, have had more exposure in recent publications (see Boero et al. 2007, this volume, for review). These attempts try to assign certain cells in Cnidaria to a third epithelial layer, thus “transforming” them into triploblastic animals. Similar attempts have been made to question the ancestral nature of radial symmetry in Cnidaria and Porifera, and subsequently force the emergence of bilateral symmetry and the Bilateria (triploblasts) to be much earlier in the metazoan tree of life than traditionally thought (see Miller et al. 2007, this volume, for references). Although the above attempts hardly question the existence of diploblasts and triploblasts each as a monophyletic group, they nonetheless force a critical change in direction for the way we think about the order of important evolutionary events. In this case, the split of the Bilateria from the diploblasts. Increasing evidence suggests that this split occurred rather early (Collins et al. 2005; Dellaporta et al. 2006). A scenario that fits well with some traditional ideas, e.g., Bütschli’s placula hypothesis (Bütschli 1884), in which Bilateria emerge

as a sister group to Cnidaria or to Cnidaria/Ctenophora/Porifera as a monophyletic group (Syed and Schierwater 2002a, 2002b; Matus et al. 2006). Unfortunately these “long-branch” scenarios do not make molecular phylogenetic analyses any easier.

The “Rosetta Stone” problem

Choosing single characters as having pre-eminence over others (“cherry picking” of characters) can directionally affect the way we view phylogenetic relationships. Using “Rosetta Stones” (important discoveries of developmental and molecular processes that underlay major anatomical changes) (Slack 1984) can also have huge effects on our thinking about important evolutionary relationships. While cherry picking can lead to a multitude of potential inferences about relationships, Rosetta Stones lead to conservation of ideas about relationships. In the context of the radiation of early metazoans and particularly of the diploblast triploblast dichotomy—we find a good example of Rosetta-Stone fixation. The Hox cluster has been used as the Rosetta Stone of comparative developmental biology since the discovery of the widespread phylogenetic distribution of this cluster (McGinnis 1994; Slack 1984). The origin of the Hox cluster has been approached by means of attractive hypotheses uniting all metazoan animals by the possession of a canonical Hox system (Martindale and Kourakis 1999; Ferrier and Holland 2001). While this paradigm has been extremely valuable in the study at particular phylogenetic levels, indiscriminate application of the paradigm can limit interpretation of phylogenetic relationships. To best explain how this might happen we ask the question: “What happens when empirical evidence questions the existing hypotheses on the origin of the Hox system?” (Schierwater and DeSalle 2001; Amemiya and Wagner 2006; Chourrout et al. 2006; Kamm et al. 2006; Kamm and Schierwater 2006; Ryan et al. 2007). We observe that there are three categories of responses to this particular conflict. The first category of responses stick to the old view despite the new evidence (make the Rosetta Stone even more difficult to crack). Responses in the second category consider that the Rosetta Stone does not matter much anyway because the predominant method of selecting characters is “cherry picking”. The final category of scientists incorporate the new knowledge into their system of testing hypotheses and attempt to work within the structures of the new evidence. In the first category, authors may defend the old view by using the following *ad hoc* weapons: (1) redefine terms (e.g., the definition

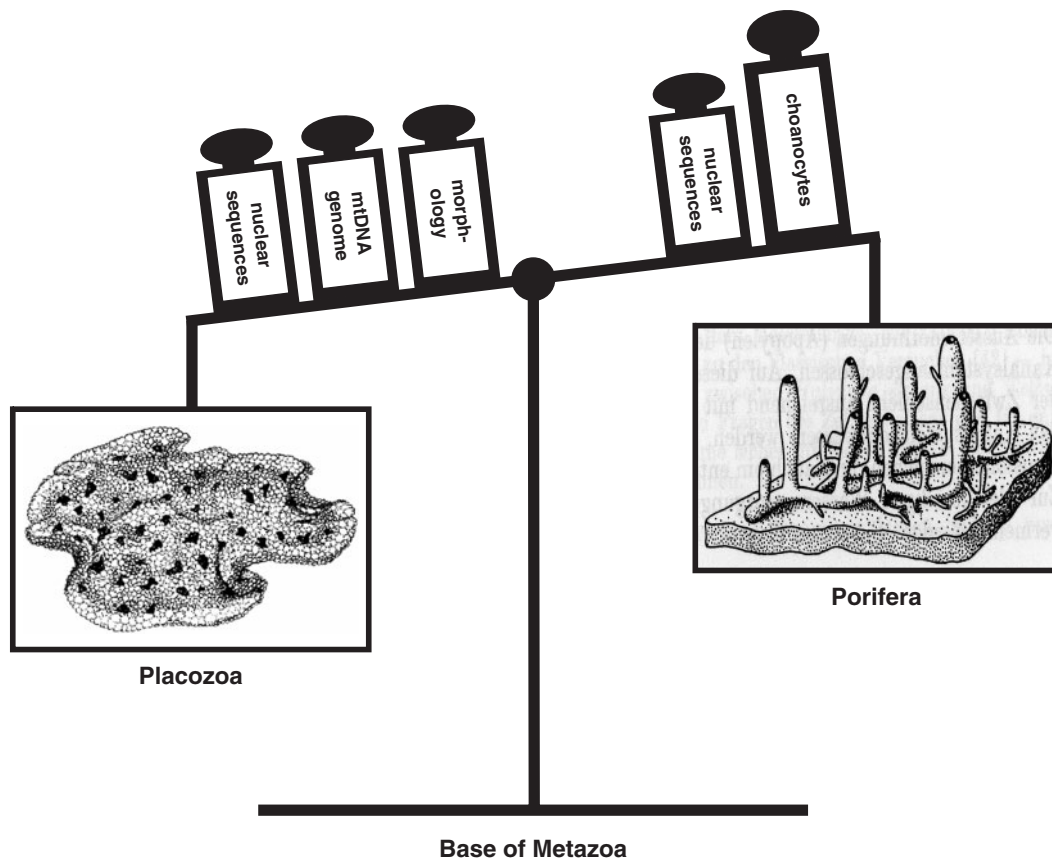


Fig. 3 The balance can never be objective. The researcher decides the weights. Researchers on Porifera seem to have a tendency to feel the weights on the right as heavy, while placozoan researchers might feel the opposite. The load of the weights seems to be influenced by one or more of the problems discussed in the text.

of a Hox system), (2) pick a tree-building algorithm that gives the “right” answer, (3) lower thresholds of statistical support, or (4) merely claim that observations that do not fit are secondarily derived traits. The second category of workers will simply smile about the degree of personal freedom in scientific interpretation. The final category of scientists will stick to normal scientific standards and attempt to continue their hypothesis testing with all potential evidence. It is clearly much harder to change a Rosetta Stone than to pick and eat a cherry, and there are several good reasons for this preference. Perhaps neither the preventing of cracks in a Rosetta Stone nor the picking of a cherry are the best ways to approach these problems.

The problem of the “right number of characters”

The development of the ability to sequence whole genomes has led to the promise of a veritable “bottomless cup” of systematic characters. This

ability leads naturally to the questions “do I really need whole genome sequences to resolve a phylogenetic question? How many characters do I really need to establish a reliable and robust phylogeny?” Attempts to determine the limits of systematic analysis in this context have been both promising and depressing. The genomic perspective on this problem began with an analysis of eight whole yeast genomes (Rokas et al. 2003) in a phylogenetic context. Rokas et al. (2003) suggested that a potential bottom line for reasonable and robust resolution of a phylogeny might be on the order of 15–20 genes and 15,000 characters for the specific phylogenetic question they had examined. Subsequent analysis of the deep metazoan phylogeny problem, however, by the same group resulted in the depressing result that “despite the amount of data and breadth of taxa analyzed, relationships among most metazoan phyla remained unresolved” (Rokas et al. 2005, p 1933). Furthermore, Rokas and Carroll (2006) suggested that the branching of taxa at the base of the metazoa might be the result of closely spaced series of

cladogenetic events that cause a severe phylogenetic “compression”, producing a bush in the tree of life. They even suggested that hundreds of genes “might not suffice” to resolve the problem (Rokas and Carroll 2006). To date at least 20 publications have addressed this important issue using the data set of Rokas et al. (2003). These studies have recently been summarized in Baurain et al. (2007) and Gatesy et al. (2007) challenging the conclusions of the original paper. While the issues raised about the base of the metazoan tree are important, we suggest that the best approach to the problem is not to give up, but rather to attempt to find better ways to incorporate all information in analyses.

The problem of the “right algorithm”

There are many potential ways to align any two sequences, not to mention alignment of multiple sequences. These different alignments almost assuredly will result in different phylogenetic hypotheses (Fitch and Smith 1983; Gatesy et al. 1993). Add to this the fact that there are at least 20 different ways of analyzing a morphological data matrix, given different methods of weighting, character optimization, and character ordering. If we just consider information on DNA sequences in a likelihood framework the number of ways to analyze a matrix goes up exponentially. Currently, there are 201 different models that can be tested in a likelihood framework (ModelTest; Posada and Crandall 1998; Posada 2006). Add to these methods the potential for analyzing a matrix using phenetic approaches, parsimony approaches, likelihood approaches or Bayesian approaches, the number of ways to analyze a matrix can result in a veritable forest of phylogenetic trees. A part of this problem can be alleviated by testing for the appropriateness of models in likelihood approaches (Posada and Crandall 1998), but still the potential for *ad hoc* manipulation of results abounds. In order to best approach this problem and avoid making inferences influenced by *ad hoc* input, a better understanding of the models used, of the uses of pluralistic approaches (Giribet et al. 2002) and of robustness of phylogenetic inference is essential.

The problem of “grant money”

Finally, we discuss a financial/sociological problem that may cause a solution to a robust phylogeny to be difficult. Consider the following statement: “Researchers who work on organism A have an easier time finding funding if A can be shown to be basal in a phylogeny and possibly a good model

system for higher Metazoa. Researchers who work on organism B find it harder to get funding for their work if B is a derived ‘dead end’ or off-shoot (an evolutionary *cul-de-sac*) and a bad model.” While we have crafted this statement to demonstrate an extreme, we feel it close to the way funding decisions are made. In this context, if the phylogenetic position of an organism is assumed to be derived, then it has no chance of becoming a model organism nor of obtaining funding for its analysis. We suggest that in adopting this attitude, current funding policy is “painting the basal metazoan tree of life into a phylogenetic corner”. By not allowing for the possibility that a supposed evolutionary *cul-de-sac* may actually not be a dead end, the potential discovery that the best explained topology for the basal metazoan tree of life may never be attained.

How do we proceed?

We have raised some serious issues concerning the obtaining of a well-corroborated basal metazoan tree of life. We could end our review here with the depressing suggestion that we will never find the best explanation. None of the problems listed earlier, however, are insurmountable if we take a strict scientific approach to the problem. If we crack our Rosetta Stones, stop picking cherries, and focus on the best explanations of the available data, we will come closer to a better scientific explanation for this important problem.

In addition, we most likely need to think about the problem in more creative and phylogenetically sound ways. While we disagree with the depressing suggestion of Rokas et al. (2005) and Rokas and Carroll (2006) that the best explanation is a “bush” at the base of the metazoan tree of life, we applaud their creative and novel approach to attempt to understand the problem using simulations. As a result of the need for novel objective approaches to address this conundrum, we point to the solution of one of the most difficult problems in the construction of the tree of life. This problem concerns rooting the tree of life amongst the three major domains of life—Bacteria, Archaea, and Eukarya. This problem is impossible to solve with an out-group because currently there is no known living (or extinct for that matter) out-group. The problem has been approached, however, using the creative and objective method of paralog rooting (Gogarten et al. 1989, 1996; Brown and Doolittle 1995). We are certain that other, equally clever, objective approaches are possible for resolving

the important phylogenetic conundrum that is the subject of this review—we just need to think of them.

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Appendix

The following 17 morphological characters can be scored: soma-germ-line differentiation (0 = exceptionally; 1 = always), intrasomatic differentiation (0 = absent, 1 = 2–5 somatic cell types), contractile cells (0 = absent, 1 = epithelio-muscle cells, 2 = muscle cells), excitation (conducting) cells (0, 1 = in non-specialized cells, 2 = nerve cells), totipotent cell lineages (0, 1), cell re-differentiation (0, 1), collagen (0, 1), extracellular matrix (0, 1), basal lamina (0, 1), digestive cavity (0, 1), multicellular symmetry (0 = absent, 1 = radial, 2 = biradial), defined body axis (0, 1), mouth and/or anus (0, 1), sensory organs (0, 1), ectoderm (0, 1), entoderm (0, 1), mesogloea or mesoderm (0, 1).

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