METAZOAN PHYLOGENY AS A TOOL IN EVOLUTIONARY BIOLOGY: CURRENT PROBLEMS AND DISCREPANCIES IN APPLICATION

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« Everything is what it is because it got that way»
D'Arcy Wentworth Thompson, 1917

Abstract. An ever increasing number of comparative studies try to shed light on various aspects of animal evolution. Particularly studies in comparative ultrastructure and evolutionary developmental biology propose remarkable hypotheses about the history of animal life. These studies must logically depend on an accurate and comprehensive knowledge of recent developments in phylogenetic methodology and hypotheses. Unfortunately, this requirement is often not met. I discuss some important recent investigations from various fields in order to illustrate the many pitfalls involved, and emphasize the necessity for sound insight into current phylogenetics as an essential prerequisite to studies of animal evolution.

Key words: Metazoa, phylogeny, comparative biology, evolutionary developmental biology, Urbilateria, tree pruning

INTRODUCTION

The study of higher-level animal relationships has a long pedigree, going back in its most familiar form to Haeckel's artistic trees. Enormous numbers of phylogenies have been published since then, making it very difficult to orient oneself in this expansive and quickly exploding literature. This has led a significant number of zoologists (especially those not directly involved in phylogenetic research) to fail to see the forest for the trees.

A useful way to organize the literature is to focus on the studies that employ cladistic principles for phylogeny reconstruction. A characteristic of many pre-cladistic studies is the absence of a rigorous and well-defined methodology of analysis. Intuitive methods that grouped species on the basis of general similarity or even common ascent (Janvier, 1996) prevailed in the past, and prevented any firm consensus about the phylogeny of the animal phyla. The advent of cladistics revolutionized the field, and a blossoming of higher-level

cladistic studies on the basis of both morphology and molecular sequence analyses has emerged in the last decade (*e.g.*, for molecular analyses see AGUINALDO *et al.*, 1997 ; FIELD *et al.*, 1988 ; LAKE, 1990 ; WINNEPENNINCKX *et al.*, 1995a ; for morphological analyses see AX, 1995 ; BRUSCA & BRUSCA, 1990 ; EERNISSE *et al.*, 1992 ; HASZPRUNAR, 1996 ; NIELSEN, 1995 ; NIELSEN, SCHARFF & EIBYE-JACOBSEN, 1996 ; SCHRAM, 1991). Some interesting results have arisen from this research. However, a detailed consensus is not yet apparent, both between and within the fields of molecular and morphological analyses. Upon examination of current cladistic analyses of metazoan morphology, we found that the lack of consensus is due to differences in fundamental methodology underlying the various cladistic analyses (JENNER & SCHRAM, in press). This study was a comprehensive attempt to explicitly introduce theoretical issues of cladistic methodology to explain the diversity of results of higher-level animal phylogenies. In order to construct a morphological reference framework, a more experimental approach toward higher-level animal phylogeny is needed. Increased attention to issues of character and taxon selection, character coding, scoring, weighting, and ground pattern reconstruction is of crucial importance (JENNER & SCHRAM, in press). Unfortunately, current authors frequently seem falsely convinced of the robustness of their phylogenies. The diversity of recently proposed phylogenetic schemes belies this misplaced confidence. It is time for a more constructive assessment of current conflicting hypotheses.

Apart from difficulties associated with the construction of a robust metazoan phylogeny in itself, there are also problems relating to the proper use of phylogenetic information in comparative biology. In this paper, I want to focus attention on how phylogenetic information should be used when studying animal evolution on the basis of comparative studies. Increasing numbers of researchers are trying to illuminate animal evolution by in-depth analysis of a small number of species, in particular by employing the model system organisms used in molecular and developmental biology. I suggest that conclusions drawn from the study of only a few model system organisms are likely to be meaningless when insufficient attention is paid to overall invertebrate phylogeny and modern phylogenetic methods.

USE AND MISUSE OF PHYLOGENIES :
CRITICAL REMARKS ON THE RECONSTRUCTION
OF THE « BILATERIAN ANCESTOR »

The need for a solid and well-resolved phylogeny of the Metazoa is now greater than ever. For a detailed understanding of metazoan evolution we need to compare and integrate the evidence from diverse fields, such as morphology, molecules, paleontology, and evolutionary developmental biology. Paraphrasing DOBZHANSKY, we could state, « Nothing in evolutionary biology makes sense, except in the light of phylogeny ». A phylogenetic framework is necessary for studying the evolution of any organismal feature (*e.g.*, a phenotypic trait, behavioral trait, life-history characteristic), and for reconstructing the ancestral features of a taxon or group of taxa. Researchers often rely, however, on « plausibility » or « common sense » approaches to argue for a particular evolutionary transformation. Unfortunately, such *ad hoc*, intuitive approaches lack any methodological

rigor and often lead to spurious results, as shown by various authors (*e.g.*, McHugh & Rouse, 1998; Packer, 1997; Sturmbauer *et al.*, 1996; Hart *et al.*, 1997).

Recent advances in diverse fields of research (ultrastructure, molecular developmental biology, paleontology) have invited increased interest in the *Big Questions* about animal evolution. These include the origin, diversification, and stability of animal body plans, and trends of metazoan evolution such as changes in organismal complexity. A solid phylogenetic framework is the only valid background for such discussions. The choice of a particular phylogenetic framework is therefore a most crucial step during any study. An uncritical choice can easily render resultant hypotheses of evolutionary scenarios meaningless.

One problem that remains elusive to this day is the nature of the bilaterian ancestor. Striking similarities in the molecular developmental biology of insects and chordates (*e.g.*, formation of the dorso-ventral axis, development of « segments ») have particularly stimulated a resurgence of interest into the characteristics of their common ancestor (Arendt & Nübler-Jung, 1995; Holland *et al.*, 1997; Holley & Ferguson, 1997; Müller *et al.*, 1996). I will illustrate some recent approaches to the use of phylogenetic information in the reconstruction of the ancestor of the Bilateria. The first two studies I will discuss deal with evolutionary developmental biology. A common problem of these studies is that they routinely employ *pruned* phylogenies to depict the relationships of only a few model system species. These pruned phylogenies can either represent incomplete phylogenies due to paucity of data, or phylogenies from which taxa are deliberately removed. They are then used to reconstruct ancestral ground patterns and thus function as the foundation for evolutionary scenarios. I will first illustrate the dangers of this approach.

Pruned phylogenies : handle with care !

Arthur (1997) rightly argued for the importance of phylogeny for studying the origin and evolution of animal body plans. Some fundamental flaws in logic, however, underlie his discussion. Arthur argued that pruning a phylogeny to only those taxa of interest reduces the information content of the cladogram, but also reduces the probability of it being wrong. I agree with the first conclusion, but I strongly disagree with the second.

Why is pruning a problem? Let us examine a hypothetical phylogeny, and its pruned version (Fig. 1). Fig. 1a depicts the « real » evolutionary relationships of the taxa W, X, Y, and Z as inferred by a comprehensive phylogenetic analysis. « A » represents the last common ancestor of taxa W, X, Y, and Z. Characters 1 to 4 represent morphological synapomorphies at different levels in the tree. Character 5 evolved independently in taxa Y and Z, but appears very similar. Fig. 1b depicts a pruned version of this tree.

The first problem arises with the reconstruction of the ground pattern of the ancestor A. The pruning of the original tree removed the basal branches of the larger clade that includes Y and Z. Such basal taxa are essential, however, for a proper reconstruction of the ground pattern of ancestor A (Yeates, 1995). The anatomical variation present in the stripped taxa is not represented in the pruned tree, and will therefore not contribute to the reconstruction of ancestor A. The improper reconstruction of a segmented common ancestor of protostomes and deuterostomes by Holland *et al.* (1997) and De Robertis (1997) can be directly attributed to such a methodological oversight.
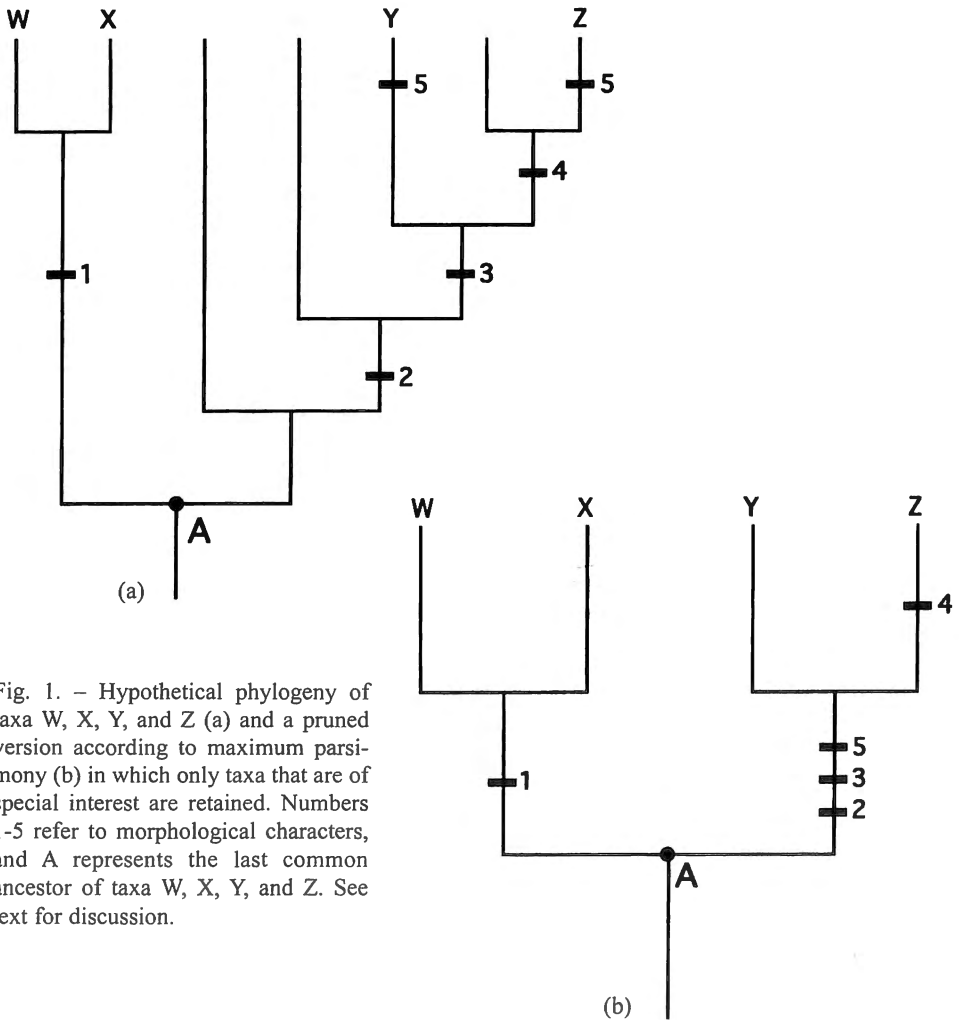
Fig. 1. – Hypothetical phylogeny of taxa W, X, Y, and Z (a) and a pruned version according to maximum parsimony (b) in which only taxa that are of special interest are retained. Numbers 1-5 refer to morphological characters, and A represents the last common ancestor of taxa W, X, Y, and Z. See text for discussion.

Secondly, the pruned tree misrepresents the topology of the original tree. The apparent symmetry of the pruned tree masks the asymmetry of the original cladogram. The exclusion of essential anatomical variation, and the misrepresentation of topology in the pruned tree do not allow the reconstruction of either the nature or the sequence of evolutionary changes on the tree. Moreover, ambiguity is introduced about the interpretation of the characters. Character 1 is a true synapomorphy of taxa W and X in both the original and pruned trees, but characters 2, 3, 4, and 5 introduce problems. Characters 2 and 3 are not synapomorphies of Y and Z as is implied by the pruned tree. Character 2 however, actually is a symplesiomorphy at the level of the last common ancestor of Y and Z, while character 3 does arise as an evolutionary novelty in this ancestor. The pruned topology also does not allow one to determine that character 2 evolved earlier than character 3.

Moreover, the pruned tree falsely suggests that character 5 was acquired by taxon Z earlier than character 4, where in fact the reverse is true. The pruned tree would also suggest that character 4 is an autapomorphy of Z, but in reality it arose in the last common ancestor of Z and its sister taxon. The pruned tree does not permit this resolution. Finally, character 5 evolved independently in taxa Y and Z. The pruned tree, however, would suggest it was a synapomorphy of these taxa.

Summarizing, it should be clear that in this case the pruned tree does not allow a proper reconstruction of ancestors. It does not allow one to distinguish between homoplasies, autapomorphies, synapomorphies or symplesiomorphies. The incorrect representation of phylogenetic information does not allow one to retrieve the true nature and sequence of evolutionary changes. All these problems contribute to the speculative nature of ground pattern reconstructions and evolutionary transformations in a significant number of studies in various fields of comparative research, notably evolutionary developmental biology (*e.g.*, DE ROBERTIS, 1997; GERHART & KIRSCHNER, 1997; HOLLAND *et al.*, 1997). A number of these important problems can also be recognized in phylogenetic analyses that deal with only a subset of the animal phyla (JENNER & SCHRAM, in press).

The roundish flatworm hypothesis

In their recent book on evolutionary developmental biology, GERHART & KIRSCHNER (1997) provided a hypothesis for the evolutionary origin of metazoan body plans. They focused on the diversification of the Nematoda, Arthropoda, Chordata, Mollusca, and Annelida from a common ancestor named the *roundish flatworm*. The roundish flatworm was first proposed as an appropriate ancestor for the protostomes and deuterostomes by VALENTINE (1994) on the basis of trace fossils, although his reconstruction differs from that of GERHART & KIRSCHNER. GERHART & KIRSCHNER reconstructed the body plan of the roundish flatworm and then proposed an evolutionary scenario deriving the body plans of the five modern phyla from this ancestor. There are, however, some fundamental flaws in their methodology that seriously undermine their hypothesis.

The first problem is the body plan reconstruction of the roundish flatworm. GERHART & KIRSCHNER (1997) derived this body plan by intuitively assembling some anatomical characters present in modern invertebrates, however no phylogenetic context was provided. Among the morphological features thought to be part of the roundish flatworm body plan were spiral cleavage, 4d-mesentoblast, blastopore becoming the mouth, pseudocoelom, and a complete gut. This assemblage of features is hardly more than speculation. A more rigorous method for reconstructing ancestral characters would have been to employ phylogenetic systematics with a maximum parsimony algorithm or maximum likelihood methods (CUNNINGHAM *et al.*, 1998; SWOFFORD & MADDISON, 1987). It then becomes clear that the phylogenetic distribution of anatomical features in Fig. 2 (fig. 7-28 in GERHART & KIRSCHNER) in fact does not support this body plan reconstruction! For example, spiral cleavage and a 4d-mesentoblast have only been convincingly demonstrated in molluscs and annelids. They are absent in chordates and nematodes and very debatable in arthropods. Blastopore fate has been overemphasized in traditional phylogenetic analyses, and the variation in blastopore fate in annelids, nematodes, arthropods, and

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chordates should be carefully assessed. Furthermore, possession of a pseudocoelom in the roundish flatworm, based as it is on nematode morphology, is highly questionable considering the range of anatomical variation present in nematodes, and the inappropriateness of a pseudocoelom as a well-defined anatomical feature (*e.g.*, see RUPPERT, 1991). A complete gut is the only character likely to be present in the roundish flatworm ancestor. Note that, even if they were used at all, the out-groups are singularly unhelpful for establishing the body plan of the roundish flatworm.
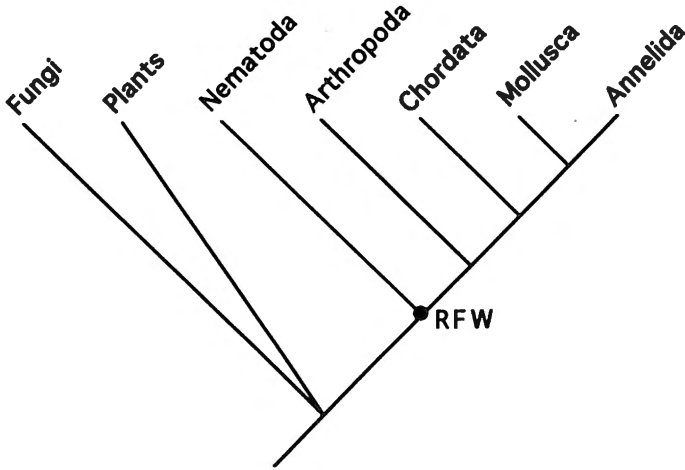

Fig. 2. – Phylogenetic relationships of Nematoda, Arthropoda, Mollusca, Annelida, and Chordata as depicted in GERHART & KIRSCHNER (1997) (see their figs 7-28). RFW represents the roundish flatworm proposed by GERHART & KIRSCHNER (1997) as the last common ancestor of these five phyla.

The second problem is the supposed phylogeny on which GERHART & KIRSCHNER (1997) based their scenario. They quote a number of phylogenetic studies primarily based on molecular data, to provide a branching sequence for the nematodes, arthropods, chordates, annelids, and molluscs. Although the data allowed for a number of different branching sequences, they exhibited one consistent feature : the chordates are derived from within the protostomes, making the protostomes paraphyletic. This particular hypothesis, however, is likely to be the result of undersampling of the chordates, and works published both before (TELFORD & HOLLAND, 1993 ; TURBEVILLE *et al.*, 1992 ; WINNEPENNINCKX *et al.*, 1996) and after (AGUINALDO *et al.*, 1997 ; GIRIBET & RIBERA, 1998) the publication of GERHART & KIRSCHNER's book indicate a monophyletic Protostomia and Deuterostomia (excluding the lophophorate phyla). Another consistent feature of GERHART & KIRSCHNER's phylogeny is the position of the nematodes, basal to the molluscs, annelids, arthropods, and chordates. This is likely to be an artifact that results from the fast rates of molecular evolution of the sampled nematodes. Substitution rates are known to be 2-3 times greater for some nematodes (such as the widely studied *Caenorhabditis elegans*) than for most other Metazoa (AGUINALDO *et al.*, 1997). This may result in long-branch attraction and forcing the nema-

todes to a basal position in the metazoan tree (*e.g.*, WINNEPENNINCKX *et al.*, 1995b). It would seem that these critical problems of GERHART & KIRSCHNER's phylogeny are sufficient to raise serious doubts about the value of their evolutionary scenario.

A third problem concerns the re-introduction of morphology into the molecular phylogeny to provide an evolutionary scenario of body plan changes. The principal difficulty arises with the introduction of morphology when the phylogeny is pruned down to only those few phyla under consideration. As discussed above, the use of such pruned trees to visualize relationships is methodologically flawed, and is likely to lead to untrustworthy results. For example, GERHART & KIRSCHNER hypothesize teloblastic segmentation to have arisen somewhere before the split of the chordates from the protostomes. The segmented mesoderm in chordates, and the segments of arthropods and annelids, may thus have been derived from a common ancestor as suggested by GERHART & KIRSCHNER. However, when one considers the diversity present in other phyla not included in their phylogeny, the picture changes drastically (*e.g.*, AGUINALDO *et al.*, 1997; NIELSEN *et al.*, 1996). These more comprehensive morphological and molecular phylogenetic studies indicate that the most parsimonious solution is the independent evolution of segmentation in chordates and protostomes. If segmentation is derived from a common ancestor, it must have been lost several times independently in a large number of phyla. I believe that if GERHART & KIRSCHNER would have paid more attention to the comparative anatomy of the phyla within a rigorous phylogenetic context, the improbability of deriving the chordates from deep within the protostome clade would have been apparent (*e.g.*, SCHAEFFER, 1987; NIELSEN, 1995).

We can thus identify some very serious shortcomings of GERHART & KIRSCHNER's roundish flatworm hypothesis. Explicit incorporation of animal phylogeny, rigorous use of phylogenetic systematics, and increased attention to comparative anatomy is needed for a more robust hypothesis to emerge that transcends the anecdotal realm (LEROI, 1998). Although G & K admit that theirs is « but a hypothesis », it is not supported by available data.

Reconstructing Urbilateria : insects, chordates, and segmentation

Recently, there have been tantalizing claims in the literature for the existence of a common segmented ancestor for the protostomes and deuterostomes (HOLLAND *et al.*, 1997; KIMMEL, 1996). This hypothesis is based on exciting new discoveries of the molecular developmental biology underlying segment formation in insects and chordates. Unfortunately, the authors paid insufficient attention to the comparative context of their work, which resulted in their advancing a hypothesis prematurely. The early introduction of an explicit phylogenetic framework is a necessary but missing step in these analyses. A comprehensive and detailed discussion of the nature, developmental control, and evolution of segmentation in the Metazoa is not my purpose here. In 1996, a European symposium was largely devoted to this subject (MINELLI, 1998). Instead, my goal is to point out how a phylogenetic framework is a necessary and powerful tool for understanding the true evolutionary meaning of these findings from molecular developmental biology.

MÜLLER *et al.* (1996) discovered that the zebrafish expression pattern of *her-1*, a vertebrate homolog of the insect pair-rule gene *hairy*, was strikingly similar to that of its insect homolog in developing short germ band insects. This led KIMMEL (1996) to suggest the possibility of a common segmented ancestor of protostomes and deuterostomes, named Urbilateria by DE ROBERTIS & SASAI (1996). Subsequently, HOLLAND *et al.* (1997) found a surprising resemblance in the expression pattern of the *Drosophila* segment-polarity gene *engrailed* and its chordate homolog *AmphiEn* in *Branchiostoma* (amphioxus). Both *engrailed* and *AmphiEn* are expressed in the developing segments before these become morphologically distinct. This suggests that these genes may play homologous roles in segment development in protostomes and deuterostomes.

To assess the evolutionary meaning of these findings, two steps need to be undertaken. First, we have to establish whether the gene expression patterns under investigation show detailed similarities that would allow a primary hypothesis of homology to be proposed. Second, this information should be assessed in a phylogenetic context, either by performing a character congruence study that allows the findings to be evaluated against all other informative characters, or by mapping the character onto a phylogeny in order to assess the initial homology determination. Unfortunately, it is all too common in recent studies in evolutionary developmental biology to completely neglect the second step in the procedure.

In this example, the expression patterns of the insect pair-rule gene *hairy* and its vertebrate homolog *her-1* do indeed show remarkable resemblances in both the pattern and dynamics of gene expression (MÜLLER *et al.*, 1996 ; KIMMEL, 1996). Likewise, the expression patterns of the insect segment-polarity gene *engrailed* and its homolog *AmphiEn* in amphioxus appear similar (HOLLAND *et al.*, 1997 ; DE ROBERTIS, 1997). These similarities can serve as a basis to suggest the homology of the expression patterns and, by extrapolation, the resultant phenotypes. However, this is precisely the point where the studies terminate the analytical process. DE ROBERTIS (1997, p.25) simply stated that « The fact that *engrailed* is expressed in both *Drosophila* and chordate metameres tells us that segmentation was present in the common ancestor from which the insect and chordate lineages diverged 500 million years ago, the Urbilateria ». HOLLAND *et al.* (1997, p.1731) go so far as to suggest that this information « favors phylogenetic scenarios deriving vertebrates from annelid-like or arthropod-like body plans ». However, these are extremely premature conclusions not supported by available information.

Consideration of the phylogenetic relationships of insects and chordates in the context of all associated phyla would provide a powerful opportunity to test the proposed homology, and to uncover any alternative perspectives. When the phylogenetic relationships of insects and chordates are considered on the basis of presently available molecular or morphological information from all phyla, it becomes clear that segmentation has evolved independently in these lineages. Indeed, this alternative is currently by far the most parsimonious interpretation (AGUINALDO *et al.*, 1997 ; NIELSEN *et al.*, 1996). If insects and chordates really did diverge from a common segmented ancestor, then the multiple independent losses of segmentation in other protostomes and deuterostomes have to be explained. HOLLAND *et al.* (1997) only briefly mention the possible loss of segmentation in echinoderms, hemichordates, and urochordates, but they never even take up the pro-

blem of explaining the supposed loss of segmentation in various protostome phyla (possibly including various acoelomate and pseudocoelomate groups). With the possible exception of the molluscs, there is precious little evidence for this scenario of multiple losses of body segmentation. Explicit attention to a total invertebrate phylogeny might suggest a different interpretation of the data, one connected to the hierarchical nature of homology (*e.g.*, ABOUHEIF, 1997; BOLKER & RAFF, 1996).

It can be dangerous to use shared patterns of regulatory gene expression to determine morphological homology. There is mounting evidence that the genotype-phenotype map may be very fluid. This means that developmental regulatory genes can function in very different contexts within a single organism and between different organisms (*e.g.*, LOWE & WRAY, 1997; PANGANIBAN *et al.*, 1997; RAFF, 1996; WU & ANDERSON, 1997). These genes are not rigidly restricted to the development of a certain morphological character. Therefore, the determination of morphological homologies as indicated by regulatory gene expression patterns may in fact not be straightforward (*e.g.*, ABOUHEIF, 1997; DICKINSON, 1995; MÜLLER & WAGNER, 1996). This is clearly indicated by current debates on the supposed homology of insect and vertebrate eyes as revealed by expression of insect *eyeless* and vertebrate *Pax-6* genes, and the evolution of animal appendages as revealed by expression of *Distal-less* in body wall outgrowths of various animal phyla (PANGANIBAN *et al.*, 1997). In such cases, the use of phylogenetic information will prove to be especially valuable.

The actual distribution of segmentation among all the phyla, and the lack of detailed anatomical correspondence between insect and chordate segmentation do not support real homology. The information does indicate, however, that the last common ancestor of insects and chordates possessed homologs of the pair-rule gene *hairy* and the segment-polarity gene *engrailed*. This indicates the possibility of a deeper homology. For example, we might suspect that *engrailed* may have originally functioned in regional patterning in general, and later became independently co-opted into the formation of insect and chordate metameres. However, HOLLAND & HOLLAND (1998) do not explore this alternative perspective. They simply state (p.656) that « it is important to stress that we are comparing body parts and not deeper homologies ». However, consideration of a real phylogeny would immediately suggest the value of this different, and useful hypothesis. Various authors have pointed out the value of explicitly incorporating phylogenetic information into the study of the evolution of developmental processes in more or less closely related species (*e.g.*, ABOUHEIF, 1997; MEYER, 1996; RAFF & POPODI, 1996). Furthermore, a phylogenetic framework is also absolutely necessary when one wants to compare distantly related organisms, such as the widely used model systems of molecular and developmental biology.

Evolving bilateral symmetry : insights from the scleractinians?

EZAKI (1998) argued that scleractinian corals may have evolved as early as the Paleozoic and could thus constitute an early anthozoan radiation. EZAKI argued that understanding the evolution of the scleractinian body plan may help to understand the evolutionary origin of the Bilateria, and in particular the evolution of a bilaterally symmetrical

body plan. Ezaki's argument is as follows. Anthozoa (including scleractinians) share a bilaterally symmetrical body plan that is traditionally considered as derived from other radially symmetrical cnidarians. Traditionally, the Bilateria are thought to have been derived from the Radiata, and the bilaterally symmetrical Anthozoa are the most likely candidates. The early origin of the scleractinian body plan may thus help to elucidate the anthozoan radiation within the Bilateria and so shed light on the early evolution of body plans within the Bilateria. He presented a phylogeny of the Cnidaria (Fig. 3a; Fig. 5 in Ezaki, 1998) to support his arguments. Unfortunately, insufficient attention to the phylogenetic basis of his arguments resulted in a conclusion that is virtually devoid of evolutionary significance.
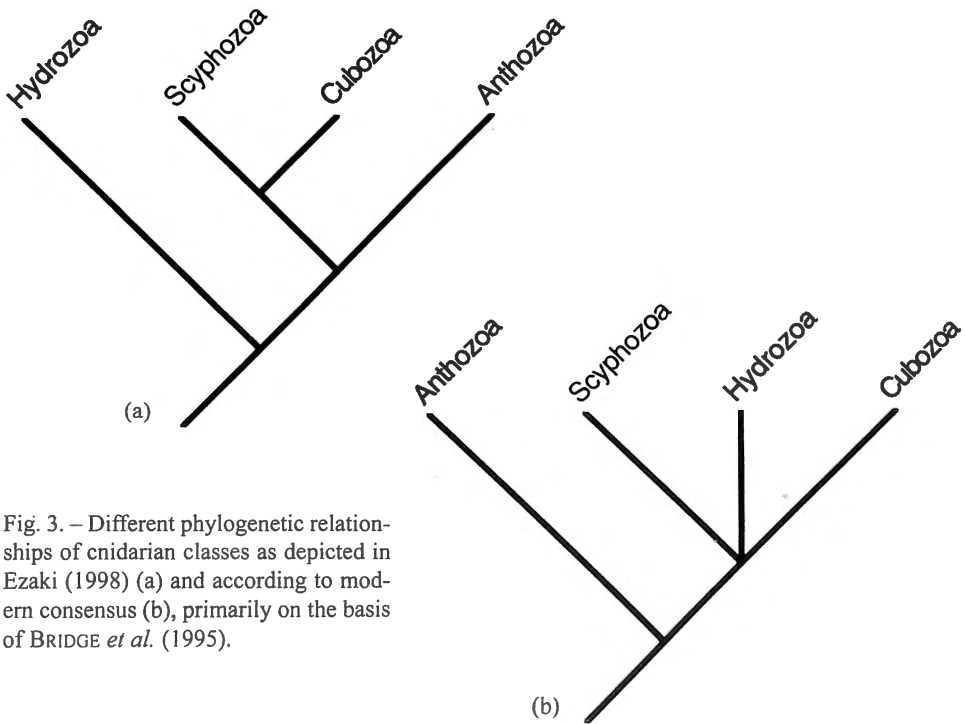

Fig. 3. – Different phylogenetic relationships of cnidarian classes as depicted in Ezaki (1998) (a) and according to modern consensus (b), primarily on the basis of Bridge et al. (1995).

Ezaki presented a « traditional » phylogeny of the Cnidaria in which the Hydrozoa is a sister group to the other cnidarians. Anthozoa is the sister group to the Scyphozoa + Cubozoa. This phylogeny is hardly a reflection of our current understanding of cnidarian phylogeny. In fact, Ezaki did not mention any source for this phylogeny, nor did he specify what kinds of data it is based on (molecular or morphological), nor what the supportive characters for this phylogeny are. The close relationship between Scyphozoa and Anthozoa has traditionally been based upon the shared possession of a cellular mesoglea, gastrodermal gonads, and gastrodermal nematocytes (e.g., Barnes & Harrison, 1991; Meglitsch & Schram, 1991; Ruppert & Barnes, 1994). It was not until recently, how-

ever, that this hypothesis was tested by a comprehensive character congruence study. BRIDGE *et al.* (1995) showed that these characters do not effectively support a close relationship between Anthozoa and Scyphozoa (and Cubozoa) (Fig. 3b). This clearly shows the danger of proposing sister group relationships on the basis of single characters outside the context of all pertinent information. The current consensus on cnidarian relationships (based on morphology and 18S rDNA data) now indicates a basal position of the Anthozoa and the existence of a clade of cnidarians with medusae (Scyphozoa, Cubozoa, Hydrozoa) (AX, 1995; BRIDGE *et al.*, 1995; NIELSEN, 1995; SCHUCHERT, 1993). Furthermore, there is no firm evidence for a phylogenetic link between Anthozoa and Bilateria. In this scheme, it is likely that the supposedly bilateral symmetry of anthozoans and Bilateria is convergent, and the evolution of the scleractinian body plan within the Anthozoa is unrelated to the origin of the Bilateria. Consequently, the anthozoan condition may be more accurately described as biradial. EZAKI's study clearly indicates the danger of relying on outdated, and weakly supported textbook trees.

Evolution of muscle and body cavities: reconstructing the bilaterian ground pattern

Based on ultrastructural studies of muscle systems and body cavities in various invertebrate groups, RIEGER and BARTOLOMAEUS advanced opposing hypotheses of the nature of the bilaterian ancestor (BARTOLOMAEUS, 1994; RIEGER, 1986; 1988; RIEGER & LOMBARDI, 1987). RIEGER suggested a myoepithelial organization of the coelomic lining as the ground pattern of the Bilateria. The acoelomates and pseudocoelomates would have been derived from this bilaterian stem species by repeated events of progenesis. In contrast, BARTOLOMAEUS (1994) argued for a compact bilaterian ancestor without internal body cavities. The pseudocoelomate and coelomate organizations would have been derived from this ancestor. The detailed arguments these authors use are not important here. What is important is that both authors make only minimal use of both phylogenetic methods as well as currently available information about invertebrate phylogeny.

RIEGER only inserts various intuitive phylogenetic arguments into his work. His conclusions are based chiefly upon extrapolation from echinoderms and annelids to the whole of the coelomate Bilateria. This implies that the coelomate Bilateria (protostomes and deuterostomes) are monophyletic. In this regard, however, it is crucial to understand the phylogenetic position of the pseudocoelomate and acoelomate phyla. While recent phylogenetic analyses of the animal phyla do not agree in detail, both molecular (AGUINALDO *et al.*, 1997; WINNEPENNINCKX, 1995a, b), and morphological analyses (EERNISSE *et al.*, 1992; NIELSEN *et al.*, 1996) suggest that the pseudocoelomates and acoelomates may be distributed among coelomate bilaterians. Irrespective of whether or not they form coherent clades, this possibility indicates that a coelomate bilaterian common ancestor of all coelomate phyla might not even have existed. Even if the coelomate bilaterians form a coherent clade with the acoelomates and pseudocoelomates outside it, RIEGER's extrapolations at the very best are only able to reconstruct the ground pattern of the coelomate Bilateria. Bilateria and coelomate Bilateria are, however, used interchangeably in RIEGER & LOMBARDI (1987). Only explicit consideration of phylogenetic relationships of all the invertebrate phyla will resolve this ambiguity.

BARTOLOMAEUS' (1994) scenario suffers from similar problems. He reconstructed the bilaterian ground pattern by reference to Ctenophora and acoelomate Bilateria (*e.g.*, Platyhelminthes, Nemertinea, Entoprocta, Gastrotricha). This only makes sense, however, if these phyla are in fact primitive branches within the Bilateria. The basal branches of a clade are the ones most likely to provide relevant information concerning the ground pattern of the clade (YEATES, 1995). Here again, a comprehensive phylogenetic framework is needed for an accurate re-evaluation of this hypothesis.

Although RIEGER'S and BARTOLOMAEUS' hypotheses about the bilaterian stem-forms are presented as alternative reconstructions, a more rigorous use of phylogenetic methods would have alerted them to the pointlessness of the debate. In fact, RIEGER and BARTOLOMAEUS reconstructed different ancestors. Fig. 4 illustrates this. By extrapolating from studies on echinoderms and annelids to the whole coelomate Bilateria, RIEGER reconstructed an ancestral ground pattern at the in-group node (node I) of the phylogeny. In contrast, by focusing on the presumptive sister group of the Bilateria (Ctenophora), Bartolomaeus reconstructed the ground pattern of the out-group node (node O) of the phylogeny. This means that in principle both hypotheses could be vindicated by the data, because character transformations may occur on the internode connecting the in-group and out-group nodes. The relative merits of the contrasting hypotheses proposed by RIEGER and by BARTOLOMAEUS need to be re-assessed, but that can only be done with reference to cladistically-framed hypotheses about the phylogenetic relationships of all the invertebrate phyla, and by employing parsimony algorithms for ground pattern reconstructions.
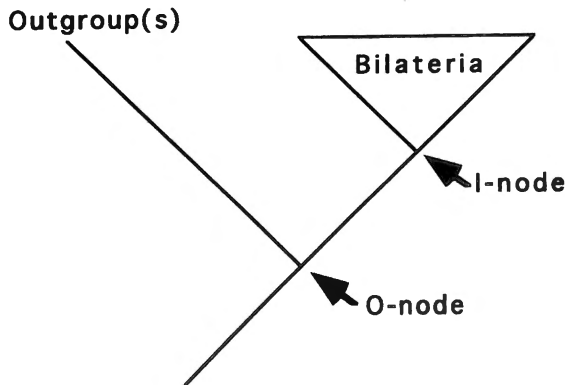

Fig. 4. – Locations of the in-group node (I-node) and out-group node (O-node) with regard to the different hypotheses for the bilaterian ancestor proposed by BARTOLOMAEUS (1994) and RIEGER & LOMBARDI (1987). See text for discussion.

CONCLUSION AND RECOMMENDATION

This is a time in which many new research programs are established in a variety of biological disciplines. These developments are associated with the transformation of com-

parative biology by phylogenetic systematics. Although the comparative method has existed at least since CUVIER, and with a particular focus on phylogenetic relationships since HAECKEL, the elaboration of explicit phylogenetic methods greatly facilitated the rigorous use of phylogenetic information in comparative studies. The modern meaning of a comparative, historical, or evolutionary perspective therefore refers to the phylogenetic relationships of organisms. This phylogenetic perspective was a major force for the establishment of, for example, historical ecology and ethology, various parts of evolutionary paleobiology, and evolutionary developmental biology (*e.g.*, ARTHUR, 1997; BROOKS & McLENNAN, 1991; HARVEY & PAGEL, 1991; JABLONSKI *et al.*, 1996).

Evolutionary biology principally derives its strength and merit from extrapolation from case studies to more comprehensive contexts (GRANDCOLAS *et al.*, 1997). Phylogenetic methods and information provide a robust and testable means for such extrapolation. It should be clear, however, that posing a question in an evolutionary context is not the same as incorporating evolutionary information into the answer. This pinpoints the problem with a variety of modern comparative studies, especially in the field of evolutionary developmental biology. Frequently, the central importance of primary homology assessments is over-emphasized, while the assimilation of phylogenetic information is ignored (*e.g.*, GILBERT *et al.*, 1996; HOLLAND & HOLLAND, 1998; HOLLAND *et al.*, 1996). In addition, studies of this kind often put disproportionate emphasis on only one or very few characters. Such a monothetic approach fundamentally violates the principles of phylogenetic systematics, which I believe is the only rigorous method currently available for reconstructing phylogeny. Taking up only one or a few characters is often misleading and very unlikely to increase our understanding of evolution. In other cases, phylogenetic data are only used in an intuitive fashion, with little attention to selection of a particular phylogeny (*e.g.*, EZAKI, 1998) or proper methods of phylogenetic inference (*e.g.*, BARTOLOMAEUS, 1994; GERHART & KIRSCHNER, 1997; RIEGER & LOMBARDI, 1987). We are on the right track, however. A more intense dialogue between phylogeneticists and other biologists is necessary for a proper understanding of macroevolutionary change, and for the development of a more robust and unified evolutionary theory (GILBERT *et al.*, 1996; GRANDCOLAS *et al.*, 1997; LARSEN *et al.*, 1997).

The literature on all aspects of animal evolution is expanding at an ever increasing rate. Indeed, a high rate of research may lead to rapid turnover of phylogenies. Continual re-evaluation and additions to the character sets, however, should result in increasingly robust phylogenetic hypotheses. Nevertheless, the dynamics of the field cannot be used as an argument for ignoring phylogenetic information in comparative studies. Clearly, there are phylogenies (both molecular and morphological) available, and despite differences in their topology there is much to be gained from incorporating them into comparative studies.

If we do not want to be swamped by the growing forest of trees and evolutionary scenarios we need to be conscious of our methods of analysis (JENNER & SCHRAM, in press). Our own future efforts will concern a comprehensive cladistic analysis of comparative anatomy, embryology, and developmental genetics to shed light on the higher-level phylogeny of invertebrates.

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