Macroevolution of Animal Body plans
Jenner, R.A.

Citation for published version (APA):
Chapter 7

Bilaterian Phylogeny and Uncritical Recycling of Morphological Data Sets

Ronald A. Jenner

Published in *Systematic Biology* 50: 730-742 (2001)
Bilaterian Phylogeny and Uncritical Recycling of Morphological Data Sets

RONALD A. JENNER

Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94766, 1090GT, Amsterdam, The Netherlands; E-mail: jenner@science.uva.nl

DATA QUALITY, DATA RECYCLING, AND DATA MATRIX COMPILATION

The vitality of systematic biology as a science depends on our invested efforts to ensure the quality of the input data of our analyses. When insufficient attention is directed towards the construction of the data matrix, proper interpretation of the results of cladistic analyses becomes impossible. Nevertheless, some systematists have argued that modern systematics is increasingly characterized by a shift of emphasis from observation as the source of the data matrix to what can be inferred from the data matrix after its construction (e.g., see Patterson and Johnson, 1997; Grande and Bemis, 1998; Rieppel and Zaher, 2000). The current availability of an unprecedented amount of comparative data and ever-increasing computing powers may have largely fostered such a shift.

It may therefore not be unexpected to discern a tendency among recent phylogenetic studies of higher-level animal relationships (both morphological and total evidence approaches) to use morphological characters garnered from character lists previously compiled by different authors as raw data for new phylogenetic studies. A partial list might include Eernisse et al. (1992), strongly relying on Brusca and Brusca (1990) and Meglitsch and Schram (1991); Kim et al. (1996), relying principally on data from Brusca and Brusca (1990), Eernisse et al. (1992), and Wheeler et al. (1993); Sundberg et al. (1998), relying on Brusca and Brusca (1990); Zrzavy et al. (1998), chiefly relying on data derived from Meigus and Schram (1991), Schram (1991), Eernisse et al. (1992), Backeljau et al. (1993), Schram and Ellis (1994), Nielsen (1995), Rouse and Fauchald (1995), Nielsen et al. (1996), Ax (1996), Haszprunar (1996a, b), Wallace et al. (1996), and Gilbert and Raunio (1997); Giribet (1999) and Giribet et al. (2000), which are exclusively based on the morphological data of Zrzavy et al. (1998); and Serensen et al. (2000), adopting a modified version of the data set of Nielsen et al. (1996). Indeed, observing that previously compiled data sets are recognized as valuable sources of phylogenetic information is encouraging and could signify the continual refinement and consolidation of an increasingly maturing morphological data set. However, several critical comments are in order.

Inevitably, any morphological phylogenetic analysis of higher-level animal taxa has to rely largely on information assembled from the published literature. One should be cautious, however, in extracting character information from the literature, especially from data sets previously compiled by different authors. Yet, the recent phylogenetic analyses listed above did not always attempt to explicitly and critically evaluate or reevaluate the recycled data matrices. Of course, a practical problem is apparent. The vastness of the ever-expanding field of invertebrate zoology makes it increasingly difficult and time consuming to obtain a complete first-hand overview and appreciation of comparative data for higher-level phylogenetic studies of the Metazoa. Nevertheless, insufficient attention to the quality of the data set may strongly impair the quality of the resulting phylogenies.

The morphological matrix of Zrzavy et al. (1998; ZEA) is especially important, comprising 276 characters and representing the most comprehensive morphological data matrix for the Metazoa compiled to date. Their morphological data set has been incorporated into several new phylogenetic studies of the Metazoa (Giribet, 1999; Giribet et al. 2000), and the phylogenetic results of this latter often-cited study have already been used as a framework for interpreting different aspects of animal evolution (de Queiroz, 1999; Vermeij and Lindberg, 2000). Determining
the quality of this matrix thus becomes critical. Below I will demonstrate legitimate reasons for questioning the quality of this matrix and show that its uncritical recycling seriously compromises several important results from the latest phylogenetic analysis of bilaterian relationships (Giribet et al., 2000; GEA), recently published in this journal. I will refer to particular characters from the matrix of ZEA as ZX, where X is the number of the character in the matrix of ZEA.

Insufficient attention to data matrix construction and uncritical use of data matrices of other authors is especially problematic for phylogenetic analyses of higher-level taxa. Brower (2000:15) writes about “characters as observations” to highlight the link between data matrix entry and its empirical support in phylogenetic analyses. However, for phylogenetic studies of higher-level taxa, to equate characters with observations is an oversimplification. Observations on characters are filtered through multiple layers, often implicit, of interpretation in their transformation to a definitive data matrix entry. The scoring of a single 0 or 1 for a given supraspecific terminal taxon in the matrix may encapsulate a variety of information and interpretation, including synopses of the extent and importance of character variation within terminal taxa, the internal phylogenetic relationships within the terminals along with their bearing on the reconstructed ground patterns that are scored in the matrix, the density and reliability of original observations for the terminal units (e.g., recent or old literature), the adopted strategy for character selection, and the many difficult decisions of character coding of complex features that may be functionally and structurally linked. Recycling of any morphological data matrix for use in a new study would supposedly logically imply a full agreement with all the decisions that fed into construction of the matrix. When these issues are not explicitly confronted, however, the data matrix is reduced to a black box. If our goal is to deepen our understanding of the evolution of metazoan morphology and phylogeny, we need to scrutinize the many facets of data matrix construction (Jenner and Schram, 1999).

PROBLEMS WITH THE MORPHOLOGICAL DATA MATRIX OF ZEA

Although ZEA’s data matrix unites disparate information, the lack of any attempt at integration or quality assessment of primary homologies hinders the discrimination of valuable and problematic data. The matrix is chiefly based on syntheses, especially other phylogenetic analyses and textbooks (see listing above). It is impossible to trace the exact source of character information in the matrix, and none of the character definitions are discussed. ZEA (p. 251) write, “The original data were not reanalyzed prior to analysis. . . .” This is a problem because various authors have shown that several of the sources of the matrix suffer from a range of difficulties (e.g., see Rouse and Fauchald, 1995; Nielsen et al., 1996; Jenner and Schram, 1999). Nevertheless, ZEA’s matrix was adopted without change by Giribet (1999) and GEA.

Given the impossibility of assessing the quality of a morphological matrix by appealing to general principles, I will offer a broad range of examples for diverse taxa and characters. Nevertheless, I will propose several general and related categories of problems associated with this large morphological data matrix, the recognition of which will be essential for a proper evaluation of the phylogenetic results of ZEA and GEA. I will specifically illustrate the problems involved by briefly discussing several of the major conclusions from the recent study of GEA.

Theory-Driven Scoring Without Empirical Support

These problems refer to data matrix entries that are largely or wholly determined by a priori assumptions of character evolution. For example, among the taxa scored present for Z93 (tripartite body and coelom) are the chordates. The trimeric, archimeric, or oligomeric organization of the ancestral deuterostome has played an important role in hypotheses about the evolution of the chordate body plan (e.g., Nielsen, 1995; Presley et al., 1996). However, the scoring of chordates for possession of a trimeric organization is not rooted in observations, but represents an evolutionary inference contingent upon the nesting of chordates within a clade of arguably trimeric nonchordate invertebrates: Brachiopoda, Phoronida, Pterobranchia, Enteropneusta, and Echinoidea. Neither ontogeny nor morphology illustrates the trimeric nature of the
body in Urochordata, Cephalochordata, and Vertebrata. Rather, cephalochordates and vertebrates elaborate a metameric body plan, whereas even the presence of coeloms in urochordates awaits confirmation (Welsch, 1995; Presley et al., 1996; Burighel and Cloney, 1997). The scoring of trimergy in the matrix of ZEA in these taxa is therefore without any empirical support. Consequently, the optimization of this character as the only potentially unique synapomorphy for a clade of (Phoronida Brachiopoda Hemichordata Echinodermata Chordata) (Bryozoa is scored "?") in the morphological and total evidence analyses of ZEA should be reevaluated. Other recent studies exemplify similar errors. The characters are often easily diagnosed by the adjective "modified," or something similar, and such identifying statements of "modified spiral cleavage" for crustaceans (Valentine, 1997:8001), cleavage of the "distorted spiral type" in acanthocephalans (Crompton, 1989:254), or "modified enterocoely" in phoronids (Williams et al., 1996) should instill caution in the interpretation of the evolutionary significance of these features.

Data Matrix Inconsistency

Morphological data matrices may include conflicting characters for two distinct reasons: (1) homoplasy, that is, meaningful and properly scored potential synapomorphies refuted by character congruence, and (2) poorly identified and scored characters. Therefore, the most potent method for identifying careless data matrix construction is to scan it for characters that actively contradict each other. Here I discuss three examples of this.

The first involves two characters and at least three independently incorrectly scored taxa. Z38 codes the presence of a heart with a coelomic pericardium, and Z42 codes the presence of a hemal system with an axial complex. A hemal system with an axial complex refers to a blood vascular system that is structurally and functionally linked to a set of coelomic spaces, together termed the axial complex (Nielsen, 1995). One of the fundamental components of this complex structure is a heart surrounded by a coelomic pericardium, and the axial complex is uniquely present in enteropneusts, pterobranchs, and echinoderms. ZEA correctly score this complex character as present in these three taxa. However, when we consider the scoring of Z38, a striking incongruence is revealed. The broad scoring of Z38 in ZEA across Protostomia (molluscs, annelids, onychophorans, arthropods) and Deuterostomia (urochordates, vertebrates) justifies a broad character definition—a definition that would encompass both anatomically restricted hearts with a specialized pericardium, such as in molluscs, and muscular longitudinal blood vessels referred to as hearts with a relatively nonspecialized "pericardium," that is, composed of the lining of the body coelom, such as found in polychaetes. In view of this broad definition, the scoring for various other taxa remains unexplained, especially echinoderms, pterobranchs, and enteropneusts, which are incorrectly scored in ZEA as lacking a heart with a coelomic pericardium.

A second instance of data matrix inconsistency concerns characters Z43 (podocytes) and Z57 (ultrafiltration through podocytes), incidentally involving the same terminal taxa. Pterobranchs and echinoderms are both scored as possessing ultrafiltration through podocytes and also as lacking podocytes.

Third, urochordates are scored as possessing longitudinal muscles along the chorda and undulatory movement of the finned tail (Z83), but scored as "?" for a chorda (Z78).

Imprecise Character Definitions and Insufficient Attention to Primary Homology

An important flaw of the morphological matrix of ZEA is the absence of explicit character definitions. The only information is the name given to the character. This makes it virtually impossible to assess the merit of the primary homology assessments that constitute the data matrix. Although some characters are not a problem, for example, protonephridia (Z48), the precise meaning of many other characters cannot be evaluated. Two particularly clear examples of lack of attention to primary homology are as follows.

Even the scoring of a character with an ostensibly straightforward definition such as position of the anus as anterior/dorsal or posterior (Z73) can mask many difficulties of interpretation. Although explicit specification is lacking, the scoring indicates that the character refers to adult morphology. Such broad-scale comparison of adult anus
position across the Bilateria is complicated by the changing body axis orientation during the ontogeny of many of the scored taxa (or even the complete loss of the larval anus during metamorphosis). This can be illustrated by addressing the difficulty of substantiating the primary homology of an anterior/dorsal anus even for the most closely related taxa that share this feature, the two sister group pairings in the morphological analysis of ZEA: (Brachiopoda Phoronida) and (Cyclophora Entoprocta).

Nielsen (1991) drew attention to the fact that the anterior/dorsal anus of Brachiopoda (Inarticulata) and Phoronida arrive at their adult position through very different ontogenetic pathways, providing little basis for proposing primary homology of adult anus position. Brachiopod metamorphosis (exemplified by Crania annonata) involves the shortening of the ventral side of the body, whereas in phoronds the dorsal side is extremely shortened. The scoring in ZEA totally ignores the change in position of the anus with respect to the larval body axes in phoronds and brachiopods. Second, scoring of the sister taxa Cyclophora and Entoprocta for anterior/dorsal anus may seem at first glance more defensible, based on the superficial morphologies of the adults, but complex problems remain. The anterior/dorsal anus of the entoproct adult directly corresponds to the posterior position of the anus in the larva before metamorphosis (Nielsen, 1971), but the anteroposterior axis of the cyclophoran feeding stage (adult) is developed diametrically opposite to the anteroposterior axis of the pandora larva in which it develops in the asexual part of the life cycle (Funch and Kristensen, 1997). Any relationship of body axes between the adult feeding stage and the chordoid larva from which it develops in the sexual part of the life cycle is obscured by the degeneration of the chordoid larva after it settles on a host. Moreover, none of the cyclophoran free-living stages develops a digestive system. A meaningful comparison of adult anus position without attention to ontogenetic changes is obviously not straightforward. Furthermore, relating the presence of broadly U-shaped guts with considerations of ecology and functional morphology in different taxa would only lessen the probability for scoring anterior/dorsal anus as a primary homology across Bilateria.

Because the analysis of GEA is specifically aimed at resolving the phylogenetic placement of acelomates and aschelminth taxa, including the recently discovered phylum Cyclophora, the exertion of special care in the scoring of characters for these taxa is critical, especially those features pertaining to body cavity organization. Yet, a pseudo-coelom (Z34) is still coded as a diagnosable character. The definitional problems associated with this character do not justify its coding as a separate character (see discussions in Ruppert, 1991a; Ahlrichs, 1995; Ax, 1995). Ultrastructurally, there is no sharp distinction between the acelomate and pseudocoelomate organizations, which constitute different points along a continuum of noncoelomate organization. Both can be designated as primary body cavities lined by extracellular matrix. A primary body cavity may range from a virtual absence of any cavity (so-called acelomate organization), such as the very narrow interstitial spaces in gnathostomulids, to a more spacious cavity such as found in priapulids (pseudo-coelom). Even according to these graded distinctions, cyclophorans and kinorhynchs are misscored in ZEA as possessing a pseudo-coel (see Kristensen and Higgins [1991] for kinorhynchs; Funch and Kristensen [1995, 1997] for cyclophorans). These considerations necessitate the rescoring of other noncoelomates in the matrix of ZEA; moreover, in view of larval body cavities in coelomates (see below) and the presence of a spacious primary body cavity in various adult coelomates such as the molluscan hemocoel (Salvini-Plawen and Bartolomaeus, 1995), most of the Bilateria would have to be rescoring.

Other characters with very unclear definitions include terminal differentiation, Z2: regular metagenesis, Z130 (coding urachordates and cyclophorans despite the lack of any developmental and morphological similarities); prevalence of the left body side, Z84; specific type of cnidocyte morphogenesis, Z213; eutely, Z10 (organs or organisms?); bipartite body, Z86 (scored for such distinctly differently organized animals as the noncoelomate kinorhynchs and coelomate chaetognaths); mono- and biphasic life cycles, Z131 (character states in the literature have been variously defined on the basis of ecological, morphological, or functional criteria, creating substantial confusion in
studies of metazoan life cycle evolution (McEdward and Janies, 1993); primary larvae Z133 (see Jenner, 2000; and below); and dipleurula larvae, Z143 (known as a hypothetical ancestral deuterostome larval type that is misscored for Pterobranchia if an explicit character definition is adopted [Nielsen, 1995; Salvini-Plawen, 1998]).

**Life Cycle Stages and Character Coding**

This subject deserves more attention by workers on metazoan phylogeny. It relates to how the phylogenetic significance of morphological characters depends on which part of the life cycle is used for comparison. An ingrained concept of phylogenetic systematics is that only "comparable semaphoronts" (defining a semaphoront as an organism at a particular stage in the life cycle) should be compared (Wiley, 1981:119). Although this idea has a pedigree going back to Hennig's original conceptualizations of phylogenetic systematics, that does not make it well-founded, at least not for all phylogenetic levels. A consideration of the empirical evidence marshalled in support of this premise by Hennig (1966) makes strikingly clear that the relevance of this concept for higher-level animal phylogenetics is far from obvious. The concept is usually illustrated by examples from animals with relatively simple life cycles (traditionally characterized as direct development), especially arthropods (insects) and vertebrates (Hennig, 1966). These examples deal with relatively closely related species in which the correspondence of life cycle stages (larvae, juveniles, adults) is relatively clear-cut. The situation is much more complicated for higher-level animal taxa, there being no generally accepted theory of life cycle evolution. Such a theory is necessary for a proper understanding of the correspondence of life cycle stages across the Metazoa. Until we develop such a theory, evaluating the value of the prescription that "larvae can only be compared with larvae and adults with adults" (Bartolomaeus and Ruhberg, 1999:172) will remain very difficult when comparing animals with diverse life cycles, such as the directly developing arthropods and the indirectly developing polychaetes. What is clear, however, is that different decisions about comparability of life cycle stages will have different effects on the outcome of a phylogenetic analysis. The prevailing tendency is to score adults and larvae as separate life cycle stages. When no life cycle stage is specified, one has to guess from the character scoring what life cycle stages are being compared.

For example, some characters apparently code only the adult stage. Z270 codes the life habit (free-living or ecto- or endoparasitic) of the taxa, scoring exclusively ectoparasitism for Myzostomida, which nevertheless possess free swimming larvae. Z247 scores the presence of a basi-epithelial nervous system, mainly for deuterostomes and lophophorates. First, there are misscorings of the adult stage, such as Chaetognatha (Salvini-Plawen, 1988; Shinn, 1997), Loricifera (Kristensen, 1991), and Priapulida (Storch, 1991). Moreover, the scoring of Z247 ignores the observations of the basi-epithelial position of many regions of the central nervous system in many protocestomes, particularly during earlier stages of ontogeny (Nielsen, 1995). Z34 codes the presence of a pseudocoel and scores a restricted set of no-coelomate taxa such as kinorhynchs, loriciferans, and rotifers. Ignoring the problems of definition of this character for the moment (see above), when entire life cycles are being considered, this feature should also be scored as present in coelomates, where it is observed in the larvae before they develop their coeloms.

For these three characters the implicit assumption is to score adults only, although a viable alternative would call for scoring across the entire life cycle. The choice should be explicitly justified, because it directly determines the phylogenetic significance of the characters involved: Scoring the character across all life cycle stages would result in potential synapomorphies for a more encompassing set of taxa than that obtained when only adults are scored. Other characters apparently do score across the entire life cycle, such as Z48, which records the presence of protonephridia. The current scoring records both taxa where protonephridia can be argued to be present in the adult ground pattern (e.g., Priapulida, Kinorhyncha, Entoprocta) and taxa where published reports provide support only for their presence in the larval ground pattern (e.g., actinotroch larva of phoronida, trochopora of
Mollusca and Echiura, and chordoid larva of Cyclophora).

A satisfactory hypothesis of the relationship between the nature of metazoan life cycles and cladistic character coding for higher-level phylogenetic analyses has not yet been developed. Such a hypothesis, however, is necessary to justify our choice of semaphoronts for coding characters for cladistic analyses. For example, if we follow the recent hypothesis that all ecdyszoans (moulting protostomes) have secondarily lost their larval forms (Peterson et al., 2000), than the recent claim justifying the comparison of “larval” arthropods and annelid larvae (Bartolomaus and Ruhberg, 1999) has to be questioned. Therefore, it would be instructive to study whether the choice of semaphoronts in recent phylogenetic analyses of the Metazoa is consistent with our current understanding of metazoan life cycles.

Ground Patterns of Higher Taxa and Character Variation

One of the most important but difficult aspects of using higher-level taxa as terminals in a phylogenetic analysis is related to deciding to code either ground patterns or exemplar species (e.g., see Yeates, 1995; Bininda-Emond et al., 1998; Wiens, 2000; see also Dayrat and Tillier, 2000, for an especially striking case study). The ground pattern is the set of characters primitively present in a clade, irrespective of its phylogenetic level. These characters may include both ancestrally inherited plesiomorphies and newly evolved apomorphies for that clade. When coding ground patterns, these have to be deduced or assumed for all characters for all terminals before performing phylogenetic analysis. Interestingly, inattention to methods of ground pattern reconstruction may lie at the heart of conflicting phylogenetic hypotheses (Jenner and Schram, 1999). The matrix of ZEA exhibits strictly unambiguous data entries, that is, no scored polymorphisms (distinct from the ?'s), and ZEA (p. 252) explain that character information is extrapolated to “whole 'phyla' if counter-evidence is absent.” However, using the unambiguous scorings to instill confidence in the robustness of the data entries would be misleading. A few examples will illustrate this.

ZEAs scored Nemertea for two characters associated with the presence of statocysts (Z232, Z253). This necessarily implies the presence of statocysts in the ground pattern of Nemertea, which proves to be a very questionable assumption. Traditionally, Nemertea is divided into two groups, the Anopla and Enopla. However, we currently lack a cladistically framed phylogeny of the Nemertea, which makes it very difficult to deduce the presence of statocysts in the nemertean ground pattern from their occurrence in only two interstitial genera of hoplonemertean (Enopla) (Otonemertes and Olotyphlonemertes) (Turbeville, 1996). Although it is not uncommon to extrapolate characters present in any of the members of a higher taxon to be part of the ground pattern of that higher taxon, that strategy can be justified only in the absence of conflicting data. Here, the choice for two hoplonemertean as representatives of the nemertean ground pattern is at the very least completely arbitrary. Scoring nemertean as absent (accepting the evolution of statocysts within the Nemertea) or polymorphic (reflecting absence of statocysts in virtually all nemertean) are viable alternative character scorings.

In contrast, the scoring of various larval characters for nemertean such as prototroch developed as ciliated lobes (Z139) is based on controversial information from heteronemertean pilidium larvae (Anopla; see, e.g., Nielsen, 1998; Rouse, 1999). Another example is the scoring of compound cilia for Nemertea (Z186). So far, compound cilia have been described only from pilidium larvae (Nielsen, 1987), which are not considered to be present in the nemertean ground pattern (Ax, 1995; Nielsen, 1995).

A final illustration of arbitrary character scoring relates to the cytology of muscles, either smooth or striated (Z261). This is a highly variable character both within and between higher-level taxa and is difficult to score this character without polymorphisms in the absence of explicit justification. For example, the unambiguous scoring of striated muscle for polychaetes in the matrix of ZEA ignores the common presence of smooth muscles in polychaetes (Gardiner, 1992).

Miscellaneous Misscorings

Although a variety of dubious or incorrect data matrix entries can be understood
as manifestations of a few general classes of problems, a hotchpotch of problematic character codings and scorings remains. A selection of examples from diverse taxa and characters will provide final illustrations of the many pitfalls involved.

Radial cleavage in ctenophores, Z5 (ctenophore cleavage is very distinct from radial cleavage [Martindale and Henry, 1997]); presence of a mixocoel in molluscs, Z36 (during mollusc ontogeny there is no confluence of coelomic and primary body cavity spaces [Raven, 1966; Salvini-Plawen and Bartolomaeus, 1995]); presence of a postanal tail in enteropneusts, Z85 (the anus is located at the posterior tip of the body [Benito and Pardos, 1997]); gametes passing through coelom and metanephridia in gastrotrichs and Lobatocerebromorpha, Z109 (both taxa lack coeloms and metanephridia [Rieger, 1980; Ruppert, 1991b]); lack of podocytes in various taxa such as onychophorans, arthropods, echinoderms, pterobranchs, and vertebrates, Z43 (Ruppert and Smith, 1988; Storch and Ruberg, 1993; Hessler and Elofsson, 1995; Nielsen, 1995; Benito and Pardos, 1997); serially repeated nephridiopores in kinorhynchs, Z51 (kinorhynchs possess a single pair of protonephridia [Kristensen and Higgins, 1991]); larval planktrotrophy in cyclophorans, Z135 (cyclophoran chordoid larvae completely lack endodermal cells, let alone a mouth and anus [Funch, 1996; Funch and Kristensen, 1997]); absence of epidermal microvilli in bryozoans and "?" in entoprocts and cyclophorans, Z184 (these taxa do possess epidermal microvilli, and ZEA do not address why taxa such as arthropods, onychophorans, and chaetognaths are scored as lacking epidermal microvilli; the microvilli are present and play an important role during cuticle formation [Minelli, 1993; Storch and Ruberg, 1993; Funch and Kristensen, 1997; Mukai et al., 1997; Nielsen and Jespersen, 1997; Shinn, 1997; Schmidt-Rhaesa et al., 1998]); presence of epidermis with intracellular skeletal lamina in urochordates, Z189 (Burighel and Cloney, 1997); and locomotion in adult ctenophores muscular rather than ciliary, Z258 (Hernandez-Nicaisse, 1991).

The above examples reveal only part of the problems inherent in the morphological matrix of ZEA. More generally, the above listing presents a cross-section of the intricate difficulties associated with character interpretation in recent phylogenetic studies of the Metazoa (Jenner and Schram, 1999; and in prep.). Many of these issues are not unique to the matrix of ZEA, but it is essential to make them explicit if we want to use morphological data to produce robust and reliable phylogenetic analyses. Admittedly, publishing a matrix that is completely free of accidental mistakes seems hardly possible; however, the above discussion shows that the information in the data matrix of ZEA should be treated with appropriate caution.

**MORPHOLOGY AND TOTAL EVIDENCE IN GEA**

Although the study of GEA is a total evidence approach to determining bilaterian relationships, the treatment of morphological data is rather limited and minimally transparent. It provides neither an explicit list of included characters nor a data matrix illustrating the distribution of characters among taxa (although a reference to the paper of ZEA is included). This makes it virtually impossible to evaluate GEA's morphological and total evidence trees. Although GEA claim to use 276 morphological characters for their analysis, 69 characters are uniform for Bilateria and therefore uninformative for sorting their relationships (this excludes variable characters that are autapomorphies, at least in the morphological analysis, for single terminals), and 27 of these are exclusively scored for cnidarians, which were not included in the analysis. In addition to uncritically recycling the problematic matrix of ZEA, the phylogenetic significance of none of the morphological characters is discussed explicitly. GEA merely provided a few simple lists of synapomorphies for some of the major clades found by their analysis. Nevertheless, we may still be tempted to conclude that the poor quality of the morphological matrix did not directly affect the main phylogenetic conclusions of GEA's total evidence analysis. Unfortunately, ZEA's problems are directly manifested in scoring errors for the morphological synapomorphies proposed to support the major bilaterian clades in GEA. I will illustrate this for the clades Trochozoa and Platyzoa, for which none of the proposed synapomorphies of GEA is free of flaws of character coding and scoring.
The total evidence analysis of GEA found (largely molecular) support for a monophyletic Platyzoa, a clade of noncoelomates defined by Cavalier-Smith (1998) on the basis of a largely intuitive narrative assessment of phylogenetic data. GEA write (p. 551), “The absence of coelom (as defined histologically) is the only morphological synapomorphy that might define Platyzoa, although its optimization is ambiguous (this character state is also present in Entoprocta, Nematodermatida, Kinorhyncha, Nematomata, and Nematomorpha; it is coded as unknown for Syndermata).” Three problems are apparent with this.

First, ZEA scored the presence of a coelom in Priapulida. Although the nature of the priapulid body cavity has been the subject of some debate over the past few decades, application of electron microscopy indicates that a spacious primary body cavity represents the likely priapulid ground pattern (Storch, 1991; Neuhaus, 1994; Ahlrichs, 1995; Schmidt-Rhaesa, 1996) and GEA claim to score ground pattern features for the morphological terminals. A genuine coelomic cavity that answers to a histological definition occurs in only 1 of the 18 currently described extant species of priapulids (Shirley and Storch, 1999). The small coelomic cavities surrounding the mouth cone (in addition to the spacious noncoelomic major body cavity) of the meio- benthic priapulid Meiopriapulus fijiensis represent a peculiarity within priapulids (Storch, 1991). In addition, Meiopriapulus may be one of the most evolutionally derived extant priapulids (Adrianov and Malakhov, 1996; but see also Wills, 1998). This suggests the absence of a coelom is likely to be primitive for Priapulida, which would necessitate rescoring this feature.

Second, the scoring of syndermates (Rotifera, Acanthocephala, and Seison) as “unknown” for presence of a coelom is inaccurate. All syndermates unambiguously lack a histologically defined coelom (Clement and Wurdak, 1991; Dunagan and Miller, 1991; Ahlrichs, 1995).

Third, on a more interpretational level, the absence of a coelom would be scored for a much broader range of bilaterians if entire life cycles, including larvae, were considered (see discussion above). This would mean that absence of a coelom is a synaplesiomorphy rather than a synapomorphy in GEA’s analysis. Although the exclusion of nonbilaterians is understandable as a method to avoid potential problems of rooting with distant outgroups, including the noncoelomate nonbilaterians into the analysis would be necessary for a proper optimization of coelom characters (and other characters that can in principle be scored for nonbilaterians) at the base of the Bilateria.

The scoring of all three proposed synapomorphies for Trochozoa (respiratory pigments, hemal system, and primary larvae) in GEA is likewise fraught with problems. First, Z44 codes for the presence of respiratory pigments. The broad scoring of taxa indicates that this character at least unites hemoglobin and hemerythrins. However, that does not explain the scoring noted for several taxa, such as echinoderms, gastrotrichs, and entoprocts. Hemoglobin has been found in some echinoderms and gastrotrichs (Ruppert, 1991b; Byrne, 1994; Smiley, 1994; Terwilliger, 1998), but respiratory pigments have not been found in any entoprocts (Terwilliger, 1998; C. Nielsen, pers. comm.). More importantly, there is no molecular support for the purported homology of the different types of respiratory proteins, hemoglobins, hemerythrins, and hemocyanins that would validate their inclusion within a single character.

Second, five taxa are misscored for the hemal system (Z35: circulatory system), and the scoring of one taxon should have been explicitly justified in view of a viable alternative interpretation. Outside the Trochozoa the hemal system is scored as present in deuterostomes and panarthropods and as reduced in nemerteans. However, ZEA incorrectly scored hemal system in vertebrates as “unknown.” The vertebrate hemal system is directly comparable to that of other coelomates, apart from the secondarily attained (both ontogenetically and phylogenetically) endothelium (Ruppert and Carle, 1983). Furthermore, chaetognaths also possess a hemal system (Shinn, 1997). The correct scoring of this information is essential for the proper phylogenetic placement of chaetognaths. For example, when chaetognaths are rescored for presence of a hemal system in the original matrix of Nielsen et al. (1996), they shift from within the protostomes to a sister group position to the deuterostomes. This illustrates that changing only a tiny fraction of a data matrix (0.05% of the total information content in this
case) may have nontrivial consequences for the results. Because GEA identified chaetognaths as one of the most phylogenetically problematic bilaterians that group among protostomes in their morphological analysis, this crucial information could bear on their conclusions. Further notable mis scorings are sipunculids and entoprocts, which both lack a hemal system (Ruppert and Carle, 1983; Nielsen, 1995), and nemertanes, which do not possess a reduced hemal system. Structural and developmental data (Turbeville, 1986) indicate that the nemertean circulatory system is coelomic and not homologous to the hemal system of other bilaterians (in contrast to the claim by GEA, the morphological matrix of ZEA simply scores absence of hemal system for nemertanes). Finally, the scoring of Bryozoa (Ectoprocta) as lacking a hemal system clearly should be defended, because available morphological data allows the alternative scoring based on the proposed morphology of the bryozoan funiculus and hemal system (Carle and Ruppert, 1983; Ruppert and Carle, 1983).

Third, the scoring of primary larvae (Z133) is even more problematic because no clear morphological definition is at present available. Primary larvae commonly appear to be interpreted as ciliated (free swimming) larvae, a rather imprecise definition. Adopting such a definition leaves unexplained why, for example, catenulid platyhelmints are not scored as possessing primary larvae. Catenulids do possess a ciliated larva known as a Luther’s larva (Ruppert, 1978), which has been considered as a primary larva (Gallenii and Gremigni, 1989). Lack of a precise morphological definition might also explain the apparent conflict between the scoring of some taxa for the presence of a primary larva and for the presence of a monophasic or biphasic life cycle (Z131). A biphasic life cycle is commonly defined as including a primary larva, while a monophasic life cycle is said to lack a primary larva. However, urochordates are scored as possessing a biphasic life cycle but lacking a primary larva. Currently, the concept of primary larvae must be regarded as being largely phylogenetic rather than structural (Jenner, 2000). Obviously, that makes meaningful discussions on character scoring very problematic at this time.

A final comment concerns the reliability of GEA’s (and ZEA’s) results as a balanced representation of all pertinent information. Including all applicable data would be especially relevant for the highly unstable taxa in the analyses, for which variable analysis parameters yield inconsistent phylogenetic placements. For example, GEA cannot confidently place the Nemertodermatida. In their summary cladogram, GEA place nemertodermatids (together with chaetognaths) at the base of the Protostomia, far removed from the other platyhelminths. The difficulty of placing the nemertodermatids in the total evidence analysis may be largely caused by GEA’s recognition of the sequence of Nemertinoides elongatus as a sequence artifact. However, equally important, ZEA’s morphological matrix did not include all pertinent characters. The complex pattern of interconnecting epidermal ciliary rootlets (distinct from the terminal axoneme structure) in acelomorph (Acoela and Nemertodermatida) platyhelmints is not included in the matrix, although this distinctive character has been widely regarded as one of the few synapomorphies of Acelomorpha (e.g., Smith et al., 1986; Ax, 1995; Haszprunar, 1996b; Rieger, 1996; Littlewood et al., 1999). Similarly, although ZEA’s and GEA’s analyses found no support for a close relationship between annelids and arthropods, they did not include one potentially important synapomorphy that yielded a close relationship between these taxa in previous studies: longitudinal body musculature organized into bands (Rouse and Fauchald, 1995, 1997). Thus, despite the comprehensiveness of the morphological data matrix, the objectivity of the analyses could be enhanced by including an explicit character selection protocol, which is conspicuously lacking in many phylogenetic analyses (Poe and Wiens, 2000). Notably, explicating unacknowledged differences in character selection plays a pivotal role in understanding current conflicting phylogenetic hypotheses of the animal kingdom (Jenner and Schram, 1999, and in prep).

**Morphology and Metazoan Phylogenetics**

GEA’s study is impressive in its scope, its incorporation of newly sequenced taxa, and its use of sensitivity analyses and character congruence tests to yield the most robustly supported and maximally congruent phylogenetic hypothesis. Unfortunately,
GEA also inherited the greatest weakness of ZEA: an uncritically assembled morphological data matrix. Introducing a poorly constructed morphological matrix into a total evidence approach of the Bilateria can scarcely be expected to add weight to the analysis or to infuse confidence in the espoused clades.

Naturally, the performance of a new analysis with a properly adjusted morphological matrix will be imperative to ascertain the full effects of the many problems involved (a voluminous task that lies outside the scope of this paper). Moreover, the analysis will have to take place in the context of a detailed discussion of the results of other phylogenetic studies. In their concluding paragraph, GEA point out that their results may be subject to revision on the basis of increased taxon sampling and the inclusion of diploblast taxa. Although these recommendations are certainly valuable, the above discussion shows there is an even more urgent need for improving the quality of the morphological data set used in ZEA and GEA.

The phylogenetic data sets assembled in previous studies provide a valuable foundation available for further refinement and extension. The continual evaluation and reevaluation of pertinent phylogenetic data will have to play a central role in future studies. Although molecular data are playing an increasingly important role in metazoan phylogenetics, our understanding of animal evolution is, and always will be, critically dependent on morphological data as well, including total evidence analyses. Therefore, molecular and morphological data deserve to be treated with equal care.

ACKNOWLEDGMENTS

I thank Arne Mooers, Ronald Sluys, Sven Lange, Frederick Schram, Claus Nielsen, Gonzalo Giribet, and two anonymous reviewers for their incisive comments, and Arne Mooers for his encouragement to write this paper. My work is supported by grant 805-33.431-P from the Earth and Life Sciences Foundation (ALW) of the Netherlands Organization for Scientific Research (NWO) and is publication No. 8 of the Dutch national program in systematics and developmental biology.

REFERENCES

ADRIANOVA, A. V., AND V. V. MALAKHOV. 1996. Priapulids (Priapulida): Structure, development, phylogeny, and classification. KMK Scientific Press, Moscow, Russia. [In Russian with English summary.]


DONAGAN, T. T., AND D. M. MILLER. 1991. Acanthocephala. Pages 299-332 in Microscopic anatomy of


Received 30 October 2000; accepted 2 January 2001
Associate Editor: R. Olmstead