EVOLUTION & DEVELOPMENT 5:4, 386–393 (2003)

Defining phyla: morphological and molecular clues to metazoan evolution

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SUMMARY None of the supraspecific taxonomic categories can be defined objectively. Each taxon should of course be monophyletic, but there is no morphological or molecular character that identifies, for example, the phylum level. This has led some authors to abandon the Linnaean categories, but they appear to be practical "handles" in daily communication. It has been proposed that each phylum exhibits a characteristic Bauplan, but the identification of such "types" have in practice proved difficult or impossible for several phyla. Monophyly of some of the approximately 30 morphology-based phyla has been put in question by molecular studies, but recent reports clearly show that the 18S rRNA molecule, which has been used extensively in phylogenetic analyses, cannot be used alone in identifying phyla (or other higher taxonomic groups). Some higher taxa, for example Chordata, Vertebrata, and Echinodermata, consistently show up as monophyletic in the analyses, whereas molluscan and annelidan subgroups just as consistently are mixed with each other and with a number of other protostomian phyla in varying patterns.

INTRODUCTION

Phyla, like all other monophyletic groups, also called clades, are defined as consisting of an ancestral species and all its descendants (Haeckel 1866). One of the important aims of evolutionary biology must be to infer morphological and molecular characters of these ancestors and to use this information to reconstruct early animal radiation. All characters must be taken into consideration, both morphological characters, such as ultrastructure, "paleomorphology," development, and biochemistry, and molecular characters, such as gene sequences, molecular structures, and gene expression. Collins and Valentine (2001) recently discussed a number of the latest attempts at reconstructing the patterns of origin and radiation of the metazoans, but their discussion seems to be strongly biased in favor of molecular systematics based on 18S rRNA (= small subunit rRNA) and of the opinion that the ancestral metazoan life cycle was holobenthic. A number of the points raised by Collins and Valentine (2001) are therefore discussed below to contribute to a more balanced view of early animal evolution and the definition of the living phyla.

DEFINITION OF PHYLA

Ideally, the whole phylogeny of the animal kingdom should be depicted as a tree-like pattern of repeated dichotomies, each representing a speciation event. In such a tree, the species appears to be the only rather objective systematic category that can be named. All higher taxa, from genus to phylum, reflect the various authors' personal choices of how to name smaller or larger branches. This has led some authors to abandon all higher systematic categories (Ax 1996; Westheide and Rieger 1996), except the genus, which is needed if one wants to adhere to the rules of zoological nomenclature. However, most textbooks and systematic/taxonomic articles retain the classical system with families, orders, classes, and phyla (Ruppert and Barnes 1994; Storch and Welsch 1997; Pechenik 2000), and this has obvious communicative and didactic advantages.

The category phylum is often associated with the Bauplan (or body plan) concept, which proposes that each phylum comprises species with a characteristic combination of architectural features, which is obviously different from that of other animals (e.g., see Brusca and Brusca 1990). The nematode Bauplan is easy to recognize because the phylum shows very restricted anatomical variation, whereas a molluscan Bauplan is much more difficult to envisage because of the enormous intraphyletic variation. The discussion has often been centered around the chordates, where it has been linked with the concept of a phylotypic stage (Haeckel 1874; Sander 1983; Slack et al. 1993). This idea proposes that each phylum has an ontogenetic stage, in the vertebrates called the pharyngula, which is well conserved across the groups of the phylum. This has inspired the "hourglass model," which proposes a constrained ontogenetic stage that all species of a phylum have to pass through (Elinson 1987; Duboule 1994; Richardson et al. 1997). Richardson et al. (1997) showed that Haeckel's illustration of pharyngula stages of various vertebrates was rather idealistic, but it can still be maintained that the vertebrate embryos go through a short ontogenetic period with very similar structures, although the various organs may not be present simultaneously at one characteristic developmental stage. Usually, the "phylotypic stage" is reached rather late in ontogeny, with much variation observed during early embryology and of course again in juveniles/adults. However, such a "stage" is very difficult to identify, for example, in mollusks, and a generalization of this concept to all phyla seems unwarranted. The genetically defined phylotype (Minelli and Schram 1994), which appears to be a useful tool in microbiology, is at present a hypothesis that cannot be used to identify animal phyla.

On the other hand, it should be emphasized that a "taxotypic" Bauplan is not something that characterizes only the phylum level (Raff 1996). Both higher taxa, such as Deuterostomia, and lower taxa, such as Cephalopoda, show ontogenetic stages that are highly conserved.

The highly characteristic structure of some phyla, such as nematodes and arthropods, has inspired the notion of macroevolution as the mechanism responsible for the origin of phyla. In general, phyla appear rather isolated from each other, but the rapidly increasing knowledge of the fossil record, for example of arthropods and "lobopods," apparently renders a special mechanism unnecessary (and improbable). If it was possible to identify all existing species at an early geologic point in time, each could logically be defined as the ancestor of a phylum (many of which subsequently went extinct). This would be an objective way of defining a phylum, but our paleontological knowledge is far from this level, so this is only a theoretical possibility.

In recent literature, there is a tendency to recognize about 30 phyla (as those discussed below), but numerous smaller disagreements can be found: Rotifera, Gastrotricha, Nematoda, Nematomorpha, Kinorhyncha, Priapula, and Loricifera, which are treated as separate phyla below, are united in one phylum, Aschelminthes, by Storch and Welsch (1997). Malakhov and Adrianov (1995) treat Priapula, Loricifera, Kinorhyncha, and Nematomorpha as classes of a phylum Cephalorhyncha. Ruppert and Barnes (1994) treat urochordates, cephalochordates, and vertebrates as subgroups of the phylum Chordata. Echiura and Pogonophora have been treated as separate phyla until recently but are now regarded as subgroups of the Annelida by several authors (see below).

Thus, it can be concluded that the choice of taxa to be defined as phyla is subjective. The very first criterion for the validity of a proposed taxon, such as a phylum, must of course be that it is monophyletic (i.e., holophyletic), and it seems as if a consensus about monophyly of most of the phyla discussed below is emerging. Whether groups such as Urochordata, Cephalochordata, and Vertebrata should be treated as separate phyla or subgroups of one phylum, Chordata, seems to be less important. A pragmatic approach is presented below.

MONOPHYLY OF LIVING PHYLA

The main reason for Collins and Valentine (2001) to reject the morphology-based phylogenetic hypotheses and the resulting definitions of living animal phyla is the discrepancy between these results and the results obtained through analyses of molecular data, such as the 18S rRNA sequences. However, the phylogenies of higher systematic levels obtained through the 18S studies are indeed open to discussion.

The 18S rRNA molecule has been used in numerous studies of animal phylogeny over the last decade, with a steadily increasing number of sequences becoming available and with use of more and more refined analytical methods. However, even the latest studies show considerable disagreement, and some of the generally accepted phyla, such as Annelida and Mollusca, usually come out as highly polyphyletic and mixed with each other and with other phyla. This makes the results suspect in the eyes of a morphologist. Two recent studies are discussed here to demonstrate my point.

Medina et al. (2001) studied the origin and early radiation of the Metazoa by use of 18S and 25S rRNA sequences from a number of Fungi, Mesomycetozoa, Choanoflagellata, Porifera, Cnidaria, Ctenophora, and selected Bilateria. Fungi, Mesomycetozoa, and Choanoflagellata came out as sister-groups of the (monophyletic) Metazoa in all analyses, separate as well as combined. But there were important discrepancies between the metazoan trees obtained with the 18S and the 25S sequences. Porifera were paraphyletic with Eumetazoa as sister-group of Calcarea in the 18S analyses, whereas they were paraphyletic with Bilateria as sister-group of the Silicea (Hexactinellida+Demospongia) in the 25S analyses. One of the combined analyses found a monophyletic Porifera as sister-group of the Eumetazoa, although with low bootstrap value. The 18S analyses showed the Ctenophora as sister-group of Cnidaria+Bilateria, whereas the 25S analyses showed them as sister-group of the Cnidaria. Hydrozoa were sister-group of Scyphozoa+Anthozoa in the 18S analyses, whereas a topology of Anthozoa+(Scyphozoa+Hydrozoa) was found in the 25S and in the combined analyses. Both bootstrap values and Bremer support indices of the major nodes were insignificant or very low. I believe this study clearly demonstrates that the 18S rRNA molecule alone cannot resolve the deep splits in animal evolution.

The Bilateria form a monophyletic group in almost all molecular studies and with high probabilities (Giribet and

Wheeler 1999; Peterson and Eernisse 2001; Medina et al. 2001).

Peterson and Eernisse (2001) analyzed animal radiation by studying 18S rRNA sequences and morphology of a large number of taxa. In the 18S rRNA analyses, problems were again encountered in resolving the relationships of Porifera, Cnidaria, and Ctenophora, but here I concentrate on the analyses of the Bilateria. The tree showed Acoela as sister-group of the remaining Bilateria (as shown in several other analyses; see below) and the remaining Platyhelminths (Catenulida+Rhabditophora) as sister-group of a "Lophotrochozoa without Platyhelminthes"+(Deuterostomia+Ecdysozoa). The sister-group relationship mentioned last is unexpected (although is not unique; see Giribet and Wheeler 1999), because almost all other analyses place the Deuterostomia as sister-group of Protostomia (Giribet et al. 2000; Zrzavý et al. 2001). Within the "Lophotrochozoa without Platyhelminthes," Annelida, Mollusca, and Brachiopoda were polyphyletic and mixed with each other and with the monophyletic groups Nemertini, Rotifera, and Entoprocta (each with more than one representative) and Ectoprocta, Sipuncula, and Cycliophora (each with only one representative). The Annelida showed a monophyletic Clitellata, but the "polychaete" species, pogonophorans, and an echiuran were mixed up with other "lophotrochozoan" clades. The Mollusca showed two monophyletic groups, Polyplacophora (two species) and Gastropoda (five species); the Bivalvia, which very often split up into several widely separated clades (Winnepenninckx et al. 1996), and Scaphopoda were not included in the study. In my view, these results once again demonstrate that the 18S rRNA molecule alone cannot be used to infer the structure of the basal bilaterian radiation.

Other molecules, such as 25S rDNA and elongation factor-1α, and the arrangement of genes on the chromosome have been used in phylogenetic analyses, but these trees also show considerable disagreements (Nielsen 2003). It should also be mentioned that the statistical analyses of sequence data are undergoing important refinement, demonstrating that some of the older analyses may be methodologically flawed (Murphy et al. 2001; Whelan et al. 2001).

With the above information in mind, the status of the metazoan phyla, as defined in Nielsen (2001), is listed with remarks on information from molecular studies. "Monophyletic" is used to indicate phyla where monophyly has not been questioned (at least not recently). In this connection it appears counterproductive to describe, for example, the phylum Annelida as paraphyletic with regards to the phyla Pogonophora and Echiura, as done by Collins and Valentine (2001). The logical consequence of the additional information must be to state that the (monophyletic) phylum Annelida includes the subordinate (subphyletic) groups Pogonophora and Echiura. Phyla comprising only one (described) species can of course only be characterized as monophyletic.

Acanthocephala: see Rotifera.

Annelida: probably monophyletic. The annelids are a morphologically well-defined group; some of the conflicting molecular analyses are discussed above. The inclusion of Pogonophora is now becoming generally accepted, and Rouse and Fauchald (1997) even placed them as a family, Siboglinidae, within the "polychaete" group Sabellida. This is in good agreement with analyses of elongation factor-1α (McHugh 1997, 1999; Kojima 1998). The inclusion of Echiura is more controversial, but the recent finding of segmentally arranged units in the ventral nerve cord of juvenile *Bonellia* and *Urechis* (Purschke et al. 2000; Hessling 2002; Hessling and Westheide 2002) definitely supports it, and this is also the case with analyses of elongation factor-1α (McHugh 1997, 1999). A more specific position within the phylum has not been identified. The Myzostomida are usually included too, but this has been questioned both by morphological and molecular studies (Eeckhaut et al. 2000; Zrzavý et al. 2001), some of which indicate a position close to the Rotifera and *Symbion*. New studies of the development of myzostomes could probably throw important light on this problem.

Arthropoda: monophyletic (although a few authors still adhere to the idea of separate annelid origins of chelicerates, "uniramians," and crustaceans; see Margulis and Schwartz 1998). Analyses of 18S and partial 28S rRNA sequences showed some of the outgroups (tardigardes, onychophorans, and nematomorphs) nested within the Arthropoda (Giribet and Ribera 2000). Also, the large study of Peterson and Eernisse (2001) using 18S sequences gave trees with onychophorans as an arthropod ingroup. However, a large analysis based on sequences from eight genes of a tardigrade, two onychophorans, and many arthropods gave a tree that is in good accordance with more traditional morphological ideas (Giribet et al. 2001)

Brachiopoda: probably monophyletic. This is supported by some studies of 18S sequences (Campbell 2000), whereas other studies either weakly indicate that Phoronida is an ingroup (Cohen et al. 1998; Cohen 2000) or show a complete mix of brachiopod groups, phoronids, and other "lophotrochozoan groups" (Peterson and Eernisse 2001).

Cephalohordata: monophyletic.

Chaetognatha: monophyletic.

Cnidaria: monophyletic.

Ctenophora: monophyletic.

Cycliophora: monophyletic.

Echinodermata: monophyletic. The calcichordate theory (Jefferies et al. 1996) does not question the monophyly of the living echinoderms.

Echiura: See Annelida.

Ectoprocta (Bryozoa s. str.): probably monophyletic. Some 18S studies indicate that Gymnolaemata and Phylactolaemata+Cyclostomata are not closely related (Giribet et al. 2000); however, morphology and fossils indicate that the cyclostomes may be an ingroup of the Gymnolaemata (Todd 2000).

Enteropneusta: Generally regarded as monophyletic, but Pterobranchia is shown as an ingroup in the study of Cameron et al. (2000).

Entoprocta: monophyletic. (The indication of an ectoproct within the Entoprocta in Littlewood et al. [1998] is an error; *Pedicellina* is an entoproct.)

Gastrotricha: monophyletic.

Gnathostomulida: monophyletic.

Kinorhyncha: monophyletic.

Loricifera: monophyletic.

Mollusca: monophyletic. The mollusks are very well defined morphologically, but the studies of 18S sequences show them as highly polyphyletic and mixed up with many other "lophotrochozoan" clades; see for example Giribet and Wheeler (1999), Giribet et al. (2000), Peterson and Eernisse (2001), and several older studies.

Myzostomida: see Annelida.

Nematoda: monophyletic.

Nematomorpha: monophyletic.

Nemertini: monophyletic.

Onychophora: monophyletic.

Phoronida: monophyletic. See also Brachiopoda

Placozoa: monophyletic (probably only one species).

Platyhelminthes: possibly diphyletic. The group Rhabditophora is well characterized both morphologically and in 18S rRNA analyses (Littlewood et al. 1999) and through special codons for asparagine and isoleucine (Telford et al. 2000). Catenulida is usually regarded as its sister-group, and this is also seen in the 18S tree of Peterson and Eernisse (2001). The Acoela (plus Nemertodermatida) have always been more problematic but are regarded as platyhelminths in most "classic" studies. This finds support from some studies of 18S and elongation factor-1α sequences (Berney et al. 2000; Littlewood et al. 2001). However, other studies of 18S rRNA and other molecules place them as the sister-group of the remaining bilaterians (Zrzavý et al. 1998; Ruiz-Trillo et al. 1999; Littlewood et al. 1999; Peterson and Eernisse 2001).

Pogonophora: see Annelida.

Porifera: probably monophyletic. The general body architecture with many incurrent openings, collar chambers, and one to several excurrent openings is unique within the Metazoa, and it is difficult to envisage the evolution of a eumetazoan body plan from this type. The peculiar demosponge *Asbestopluma*, which lacks collar chambers and feeds on small crustaceans (Vacelet and Boury-Esnault 1995), demonstrates an unexpected plasticity within the phylum but gives no hint of a route toward eumetazoan organization. However, as mentioned above, some 18S analyses show the Calcarea as sister-group of the Eumetazoa, and other analyses even indicate that the Hexactinellida are the sister-group of Calcarea+Eumetazoa (Borchiellini et al. 2001).

Priapula: monophyletic.

Pterobranchia: monophyletic (see also Enteropneusta).

Rotifera: monophyletic. The inclusion of Acanthocephala is supported by several molecular analyses (Garey et al. 1998; Giribet and Wheeler 1999; García-Varela et al. 2000). Ahlrichs (1995) introduced the name Syndermata for Rotifera plus Acanthocephala, but because the acanthocephalans are an ingroup of the old Rotifera, the new name seems superfluous (Segers 2002).

Sipuncula: monophyletic.

Tardigrada: monophyletic.

Urochordata: monophyletic.

Vertebrata: monophyletic.

PELAGO-BENTHIC LIFE CYCLE AND ANIMAL PHYLOGENY

Studies of morphology and embryology have until recently been the foundation of phylogeny and of definition of phyla. Early studies were based on morphology of adult animals, but as knowledge of larval stages and whole life cycles increased, ontogenetic characters became central in phylogenetic discussions (Hatschek 1891; Jägersten 1972; Rouse 1999; Nielsen 1998).

The pelago-benthic life cycle with ciliated larvae is the dominating type in marine invertebrates, except in ecdysozoans, which lack locomotory cilia completely. This lack of cilia undoubtedly represents a specialization because locomotory cilia are found in sponge larvae and in ctenophores, cnidarians, lophotrochozoans, and the nonchordate deuterostomes (Nielsen 1998, 2001).

According to Collins and Valentine (2001), the fossil record may indicate that the early metazoans were holobenthic with direct development. New findings of fossil embryos do not support this opinion, because both embryos, which resemble gastrulae of amphioxus and living echinoderms with planktotrophic larvae and large apparently yolk-rich embryos indicative of direct development, are now known from the Precambrian and Earliest Cambrian (Bengtson and Zhao 1997; Chen et al. 2000). Collins and Valentine (2001, p. 439) further argue that some colonial choanoflagellates are benthic and that the "entirely benthic sponges" are derived from a benthic ancestor. This is misleading. Choanoflagellate life cycles are not well known (sexual reproduction has not been observed), but a solitary species has been shown to change between a benthic and a pelagic phase (Leadbeater 1977), and another species was found to have a colonial motile phase (*Proterospongia*), a sedentary solitary phase ("*Choanoeca*"), and two types of

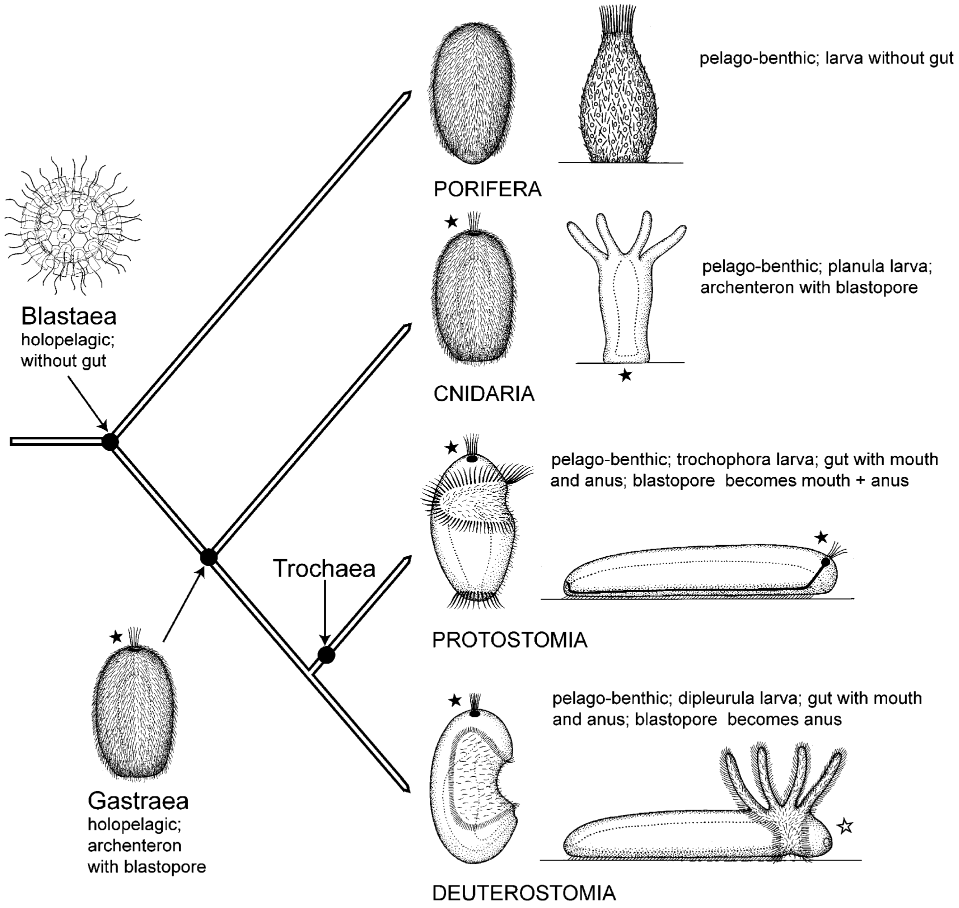

Fig. 1. The modified trochaea theory, showing the evolution of the four main pelago-benthic clades from holopelagic ancestors. The position of the apical pole is indicated by an asterisk. The morphology of the bilaterian ancestor has not been envisaged. (Modified from Nielsen 2001.)

solitary swarmers (Leadbeater 1983). A shift between sedentary and free-living stages is thus demonstrated in the few species where life-cycle studies have been carried out. It should be emphasized further that all sponges have a pelago-benthic life cycle with a ciliated nonfeeding larva. These larvae apparently all have a short pelagic phase, but there is nothing to indicate that a ciliated larva was not part of the life cycle of the ancestral sponge. Collins and Valentine (2001) further state that " . . . sponges stand in the direct ancestry of diploblastic organisms . . . ," but it is indeed questionable if the eumetazoans are an ingroup of the sponges (see above).

If the planktonic larvae represent later additions to the life cycles of a number of groups, one would expect that the genes of the Hox cluster were active from an early developmental stage and in the organization of the whole body. However, in many groups the *Hox* genes are not active in the head region but are involved exclusively in the organization of body regionation along the anteroposterior body axis of the body (Bruce and Shankland 1998). This could indicate that the head region of the planktonic larvae represents the ancestral, holopelagic, radial ancestor and that the bilateral body organized through the *Hox* genes represents the bilateral benthic stage "added on" to the pelagic ancestor (Peterson and Davidson 2000).

A competing view, viz. that a pelago-benthic life cycle was ancestral in metazoans and that a planktotrophic larva was ancestral in the eumetazoans, has been central in a number of classic studies (such as Hatschek 1891), and in several of my own publications (Nielsen 1979, 1985, 1995; Nielsen and Nørrevang 1985). It should be pointed out that my current view of metazoan phylogeny and of the status of the ancestor called trochaea (Nielsen 1998, 2001) (Fig. 1) is somewhat different from the earlier version discussed by Collins and Valentine (2001). The occurrence of planktotrophic larvae, lecithotrophic larvae, and direct development has been discussed in detail in Nielsen (1998). The hypothesized evolution of the pelago-benthic life cycle of the protostomes from the holoplanktonic trochaea nicely visualizes the adaptive changes leading from the ancestral holopelagic trochaea with a circumblastoporal ring of compound cilia working as a downstream-collecting filtering system and a circumblastoporal nerve ring to the protostomian trochophora larva with a downstream-collecting prototroch–metatroch complex and adults with a paired mid-ventral nerve cord.

Evolution of lecithotrophy/direct development from planktotrophy is well documented in groups of echinoderms and mollusks (Wray 1995a,b; Nielsen 1998), whereas I do not know of any good example of evolution in the opposite direction (Davidson et al. 1995). Parsimony arguments seem less relevant here, because the life cycles of only some species have been studied, and known numbers of each of the two types are therefore rather haphazard. Furthermore, it should be emphasized that studies of ascidians clearly demonstrate that losses of larval characters are phylogenetically much "cheaper" than gains (Hadfield et al. 1995; Swalla and Jeffery 1996); this is not considered in most parsimony analyses.

The original meaning of the recapitulation theory has been abandoned long ago, but this should not lead to a total rejection of larval/developmental characters in animal phylogeny. By a priori choosing the direct development as the ancestral, a whole suite of informative larval characters is lost, and several of these characters figure prominently in modern morphological cladistic analyses of animal phylogeny (Zrzavý et al. 1998; Peterson and Eernisse 2001).

CONCLUSION

In their conclusion, Collins and Valentine (2001, p. 440) state that " . . . there is a long Neoproterozoic and Early Cambrian fossil record that is replete with positive evidence of benthic inhabitation, which is amenable to interpretation as suggesting that nearly all metazoan body plans arose as adaptations to benthic environments." This is in complete agreement with the hypothesis that the benthic stages of ancestral sponges, cnidarians, protostomes, and deuterostomes are additions (and therefore adaptations) to the ancestral holopelagic life cycles. It is also in agreement with major parts of the set-aside cell hypothesis (Davidson et al. 1995; Peterson et al. 1997), especially of the idea that emphasizes the ancestral character of planktotrophic larvae (and maximal indirect development).

Morphology-based studies of animal evolution have not reached a generally accepted phylogeny, but the phyla listed above are generally accepted as natural groups, the only serious exception being the Acoela (plus Nemertodermatida), which are difficult to fit in because of a general lack of synapomorphies. The molecular studies have not yet led to the stable classification that had been hoped for. Some phyla, such as Nematoda, Echinodermata, and Vertebrata, are monophyletic in almost all analyses, whereas Annelida, Mollusca, and Ectoprocta are usually mixed up in a most confusing fashion. At this point it appears that the traditional morphology-based definition of the about 30 bilaterian phyla is the only operational possibility.

In general, I believe that the words *falsify* and *parsimonious* should be used with much more care than has been usual. From the above discussion of molecular trees, it should be clear that only few hypotheses can be falsified by such studies (but they can be rendered more or less probable) and to characterize one hypothesis as the most parsimonious will in most cases just indicate which theory is preferred by the author.

REFERENCES

Ahlrichs, W. H. 1995. Ultrastruktur und Phylogenie von *Seison nebaliae* (Grube 1859) und *Seison annulatus* (Claus 1876). Dissertation, Georg-August-University, Göttingen, Cuvillier Verlag, Göttingen.

Ax, P. 1996. *Multicellular Animals. A New Approach to the Phylogenetic Order in Nature*. Vol. 1. Springer, Berlin.

Bengtson, S., and Zhao, Y. 1997. Fossilized metazoan embryos from the Earliest Cambrian. *Science* 277: 1645–1648.

Berney, C., Pawlowski, J., and Zaninetti, L. 2000. Elongation factor 1-alpha sequences do not support an early divergence of the Acoela. *Mol. Biol. Evol.* 17: 1032–1039.

Borchiellini, C., Manuel, M., Alivon, E., Boury-Esnault, N., Vacelet, J., and Le Parco, Y. 2001. Sponge paraphyly and the origin of the Metazoa. *J. Evol. Biol.* 14: 171–179.

Bruce, A. E. E., and Shankland, M. 1998. Expression of the head gene *Lox22-Otx* in the leach *Helobdella* and the origin of the bilaterian body plan. *Devl. Biol.* 201: 101–112.

Brusca, R. C., and Brusca, G. J. 1990. *Invertebrates*. Sinauer Associates, Sunderland, MA.

Cameron, C. B., Garey, J. R., and Swalla, B. J. 2000. Evolution of the chordate body plan: new insights from phylogenetic analyses of deuterostome phyla. *Proc. Natl. Acad. Sci. USA* 97: 4469–4474.

Campbell, D. C. 2000. Molecular evidence on the evolution of the Bivalvia. In E. M. Harper, J. D. Taylor, and J. A. Crame (eds.). *The Evolutionary Biology of the Bivalvia*. Special publication no. 177. Geological Society of London, London, pp. 31–46.

Chen, J. -Y., Oliveri, P., Li, C. -W., Zhou, G. -Q., Gao, F., and Hagadorn, J. W., et al. 2000. Precambrian animal diversity: putative phosphatized embryos from the Doushanto Formation of China. *Proc. Natl. Acad. Sci. USA* 97: 4457–4462.

Cohen, B. L. 2000. Monophyly of brachiopods and phoronids: reconciliation of molecular evidence with Linnaean classification (the subphylum Phoroniformea nov.). *Proc. R. Soc. Lond. B* 267: 225–231.

Cohen, B. L., Gawthrop, A., and Cavalier-Smith, T. 1998. Molecular phylogeny of brachiopods and phoronids based on nuclear-encoded small subunit ribosomal RNA gene sequences. *Philos. Trans. R. Soc. B* 353: 2040–2060.

Collins, A. G., and Valentine, J. W. 2001. Defining phyla: evolutionary pathways to metazoan body plans. *Evol. Dev.* 3: 432–442.

Davidson, E. H., Peterson, K. L., and Cameron, R. A. 1995. Origin of bilaterian body plans: evolution of developmental regulatory mechanisms. *Science* 270: 1319–1325.

Duboule, D. 1994. Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphogenesis through heterochrony. *Development* (Suppl): 135–142.

Eeckhaut, I., McHugh, D., Mardylun, P., Tiedemann, R., Monteyne, D., and Jangoux, M., et al. 2000. Myzostomida: a link between trochozoans and flatworms? *Proc. R. Soc. Lond. B* 267: 1383–1392.

Elinson, R. P. 1987. Change in developmental patterns: embryos of amphibians with large eggs. In R. A. Raff, and E. C. Raff (eds.).

Development as an Evolutionary Process. Alan R. Liss, New York, pp. 1–21.

García-Varela, M., Pérez-Ponce de Leon,, G., de la Torre, P., Cummings, M. P., Sarma, S. S. S., and Laclette, J. P. 2000. Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *J. Mol. Evol.* 50: 523–540.

Garey, J. R., Schmidt-Rhaesa, A., Near, T. J., and Nadler, S. A. 1998. The evolutionary relationships of rotifers and acanthocephalans. *Hydrobiologia* 387/388: 83–91.

Giribet, G., and Ribera, C. 2000. A review of arthropod phylogeny: new data based on ribosomal DNA sequences and direct character optimization. *Cladistics* 16: 204–231.

Giribet, G., and Wheeler, W. C. 1999. The position of arthropods in the animal kingdom: Ecdysozoa, islands, trees, and the "parsimony ratchet". *Mol. Phyl. Evol.* 13: 619–623.

Giribet, G., Distel, D. L., Polz, M., Sterrer, W., and Wheeler, W. C. 2000. Triploblastic relationships with emphasis on the acoelomates and the position of Gnathostomulida, Cycliophora, Plathelminthes, and Chaetognatha: a combined approach of 18S rDNA sequences and morphology. *Syst. Biol.* 49: 539–562.

Giribet, G., Edgecombe, G. D., and Wheeler, W. C. 2001. Arthropod phylogeny based on eight molecular loci and morphology. *Nature* 413: 157–161.

Hadfield, K. A., Swalla, B. J., and Jeffery, W. R. 1995. Multiple origins of anural development in ascidians inferred from rDNA sequences. *J. Mol. Evol.* 40: 413–427.

Haeckel, E. 1866. *Generelle Morphologie der Organismen*. Two volumes Georg Reimer, Berlin.

Haeckel, E. 1874. *Anthropogenie oder Entwicklungsgeschichte des Menschen*. Engelmann, Leipzig.

Hatschek, B. 1891. *Lehrbuch der Zoologie*, 3. Lieferung. Gustav Fischer, Jena, pp. 305–432.

Hessling, R. 2002. Metameric organisation of the nervous system in developmental stages of *Urechis caupo* (Echiura) and its phylogenetic implications. *Zoomorphology* 121: 221–234.

Hessling, R., and Westheide, W. 2002. Are Echiura derived from a segmented ancestor? Immunohistochemical analysis of the nervous system in developmental stages of *Bonellia viridis*. *J. Morphol.* 252: 100–113.

Jägersten, G. 1972. *Evolution of the Metazoan Life Cycle*. Academic Press, London.

Jefferies, R. P. S., Brown, N. A., and Daley, P. E. 1996. The early phylogeny of chordates and echinoderms and the origin of chordate left-right asymmetry and bilateral symmetry. *Acta Zool. (Stockh.)* 77: 101–122.

Kojima, S. 1998. Paraphyletic status of Polychaeta suggested by phylogenetic analysis based on amino acid sequences of elongation factor-1α. *Mol. Phyl. Evol.* 9: 255–261.

Leadbeater, B. S. C. 1977. Observations on the life-history and ultrastructure of the marine choanoflagellate *Choanoeca perplexa* Ellis. *J. Mar. Biol. Assoc. UK* 57: 285–301.

Leadbeater, B. S. C. 1983. Life-history and ultrastructure of a new marine species of *Proterospongia* (Choanoflagellida). *J. Mar. Biol. Assoc. UK* 63: 135–160.

Littlewood, D. T. J., Telford, M. J., Clough, K. A., and Rohde, K. 1998. Gnathostomulida—an enigmatic metazoan phylum from both morphological and molecular perspectives. *Mol. Phyl. Evol.* 9: 72–79.

Littlewood, D. T. J., Rohde, K., and Clough, K. A. 1999. The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biol. J. Linn. Soc.* 66: 75–114.

Littlewood, D. T. J., Olson, P. D., Telford, M. J., Herniou, E. A., and Riutort, M. 2001. Elongation factor 1-alpha sequences alone do not assist in resolving the position of the Acoela within the Metazoa. *Mol. Biol. Evol.* 18: 437–442.

Malakhov, V. V., and Adrianov, A. V. 1995. *Cephalorhyncha—A New Phylum of the Animal Kingdom.* (In Russian, with English summary.) KMK Scientific Press Ltd., Moscow.

Margulis, L., and Schwartz, K. V. 1998. *Five Kingdoms*. 3rd Ed. W. H. Freemann, New York.

McHugh, D. 1997. Molecular evidence that echiurans and pogonophorans are derived annelids. *Proc. Natl. Acad. Sci. USA* 94: 8006–8009.

McHugh, D. 1999. Phylogeny of the Annelida: Siddall *et al.* (1998) rebutted. *Cladistics* 15: 85–89.

Medina, M., Collins, A. G., Silberman, J. D., and Sogin, M. L. 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *Proc. Natl. Acad. Sci. USA* 98: 9707–9712.

Minelli, A., and Schram, F. R. 1994. Owen revisited: a reapprisal of morphology in evolutionary biology. *Bijdr. Dierk*. 64: 65–74.

Murphy, W. J., Eizirik, E., O'Brien, S. J., Madsen, O., Scally, M., and Douady, C. J., et al. 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 294: 2348–2351.

Nielsen, C. 1979. Larval ciliary bands and metazoan phylogeny. *Fortschr. Zool. Syst. Evolutionsforsch.* 1: 178–184.

Nielsen, C. 1985. Animal phylogeny in the light of the trochaea theory. *Biol. J. Linn. Soc.* 25: 243–299.

Nielsen, C. 1995. *Animal Evolution: Interrelationships of the Living Phyla*. Oxford University Press, Oxford.

Nielsen, C. 1998. Origin and evolution of animal life cycles. *Biol. Rev.* 73: 125–155.

Nielsen, C. 2001. *Animal Evolution: Interrelationships of the Living Phyla.* 2nd Ed. Oxford University Press, Oxford.

Nielsen, C. 2003. Animal evolution: the solution to the Articulata-Ecdysozoa controversy? *Zool. Scr.* (in press).

Nielsen, C., and Nørrevang, A. 1985. The trochaea theory: an example of life cycle phylogeny. In S.Conway Morris, J. D. George, R. Gibson, and H. M. Platt (eds.). *The Origin and Relationships of Lower Invertebrate Groups*. Oxford University Press, Oxford, pp. 28–41.

Pechenik, J. A. 2000. *Biology of the Invertebrates*. 4th Ed. McGraw-Hill, Boston.

Peterson, K. J., and Davidson, E. H. 2000. Regulatory evolution and the origin of the bilaterians. *Proc. Natl. Acad. Sci. USA* 97: 4430–4433.

Peterson,, K. J., and Eernisse, D. J. 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA sequences. *Evol. Dev.* 3: 170–205.

Peterson, K. J., Cameron, R. A., and Davidson, E. H. 1997. Set-aside cells in maximal indirect development: evolutionary and developmental significance. *BioEssays* 19: 623–631.

Purschke, G., Hessling, R., and Westheide, W. 2000. The phylogenetic position of the Clitellata and the Echiura—on the problematic assessment of absent characters. *J. Zool. Syst. Evol. Res.* 38: 165–173.

Raff, R. A. 1996. *The Shape of Life*. University of Chicago Press, Chicago.

Richardson, M. K., Hanken, J., Gooneratne, M. L., Pieau, C., Raynaud, A., and Selwood, L., et al. 1997. There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development. *Anat. Embryol.* 196: 91–106.

Rouse, G. W. 1999. Trochophore concepts: ciliary bands and the evolution of spiralian Metazoa. *Biol. J. Linn. Soc.* 66: 411–464.

Rouse, G. W., and Fauchald, K. 1997. Cladistics and polychaetes. *Zool. Scr.* 26: 139–204.

Ruiz-Trillo, I., Riutort, M., Littlewood, D. T. J., Herniou, E. A., and Baguñà, J. 1999. Acoel flatworms: earliest extant bilaterian metazoans, not members of Platyhelminthes. *Science* 283: 1919–1923.

Ruppert, E. E., and Barnes, R. D. 1994. *Invertebrate Zoology*. 6th Ed. Saunders College Publishing, Fort Worth, TX.

Sander, K. 1983. The evolution of patterning mechanisms: gleanings from insect embryogenesis and spermatogenesis. In B. C. Goodwin, H. Holder, and C. C. Wylie (eds). *Development and Evolution*. Cambridge University Press, Cambridge, pp. 137–159.

Segers, H. 2002. The nomenclature of the Rotifera: annotated checklist of valid family- and genus-group names. *J. Nat. Hist.* 36: 631–640.

Slack, J. M. W., Holland, P. W. H., and Graham, C. F. 1993. The zootype and the phylotypic stage. *Nature* 361: 490–492.

Storch, V., and Welsch, U. 1997. *Systematische Zoologie*. 5th Ed. Gustav Fischer, Stuttgart.

Swalla, B. J., and Jeffery, W. R. 1996. Requirement of the *Manx* gene for expression of chordate features in a tailless ascidian larva. *Science* 274: 1205–1208.

Telford, M. J., Herniou, E. A., Russell, R. B., and Littlewood, D. T. J. 2000. Changes in mitochondrial genetic codes as phylogenetic characters: two examples from the flatworms. *Proc. Natl. Acad. Sci. USA* 97: 11359–11364.

Todd, J. A. 2000. The central role of ctenostomes in bryozoan phylogeny. In A.Herrera Cubilla, and J. B. C. Jackson (eds.). *Proceedings of the 11th International Bryozoology Association Conference*. Smithsonian Tropical Research Institute, Balboa, Panama, pp. 104–135.

Vacelet, J., and Boury-Esnault, N. 1995. Carnivorous sponges. *Nature* 373: 333–334.

Westheide, W., and Rieger, R. 1996. *Spezielle Zoologie, Teil 1: Einzeller und wirbellose Tiere*. Gustav Fischer, Stuttgart.

Whelan, S., Lió, P., and Goldman, N. 2001. Molecular phylogenetics: state-of-the-art methods for looking into the past. *Trends Genet*. 17: 262–272.

Winnepenninckx, B., Backeljau, T., and De Wachter,, R. 1996. Investigation of molluscan phylogeny on the basis of 18S rRNA sequences. *Mol. Biol. Evol*. 13: 1306–1317.

Wray, G. A. 1995a. Evolution of larvae and developmental modes. In L. McEdward (ed.). *Ecology of Marine Invertebrate Larvae*. CRC Press, Boca Raton, FL, pp. 413–447.

Wray, G. A. 1995b. Punctuated evolution of embryos. *Science* 267: 1115–1116.

Zrzavý, J., Hypsa, V., and Tietz, D. E. 2001. Myzostomida are not annelids: molecular and morphological support for a clade of animals with anterior sperm flagella. *Cladistics* 17: 170–198.

Zrzavý, J., Mihulka, S., Kepka, P., Bezdek, A., and Tietz, D. 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics* 14: 249–285.