The significance of moulting in Ecdysozoan evolution

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SUMMARY   Three major bilaterian clades first appear in the Early Cambrian fossil record: Deuterostomia, Lophotrochozoa, and Ecdysozoa. The taxa placed in Ecdysozoa are characterized by a moulting habit, unknown in the other major clades. The origin and consequences of moulting are of fundamental importance to the history of the ecdysozoan clade, chiefly because moulting precludes motile ectodermal cilia. Moulting may have originated as an adaptation to permit the enlargement, during growth, of secreted cuticular spines, flanges, and other structures used as ancillary locomotory devices. A combination of phylogenetic and fossil evidence suggests that the early members of these clades were small vermiform paracoelomates that likely lacked indirect-developing planktotrophic larvae. Thus, the evolution of planktotrophic larvae may have been independently achieved at least three times within Bilateria. The nonmoulting clades evolved larvae that swim and feed via ciliated tufts and bands, presumably intercalating these forms within their early developmental systems. Within Ecdysozoa, feeding larvae lacked ciliary feeding tracts and evolved by modification of early instars, employing limbs or setae to generate feeding currents. The setting aside during larval life of cells that give rise to adult features is probably an adaptation associated with metamorphosis.

INTRODUCTION

The use of molecular sequences to evaluate phylogenetic hypotheses has revealed a number of surprising relationships among the metazoan phyla. One of the more striking findings has been that there is a clade of invertebrate phyla, termed the Ecdysozoa, a principal synapomorphy of which is the possession of a moulting habit. The existence of such a clade had been postulated on morphological grounds (see Barnes et al. 1993) and was identified by 18S rRNA studies, and named, by Aguinaldo et al. (1997). This clade has since been supported in other 18S rRNA trees produced by a variety of phylogenetic methods and with different taxonomic mixtures (Giribet and Ribera 1998). Evidence for an ecdysozoan clade has also come from comparative studies of *Hox* gene assemblages (de Rosa et al. 1999). The ecdysozoans exhibit a high degree of morphological disparity (Fig. 1), ranging from worms such as the pseudocoelomate Priapulida, to Nematoda with reduced pseudocoels, and to complex limbed phyla such as the Arthropoda, which are basically hemocoelic but contain small intramesodermal spaces. Despite this disparity, some recent morphological studies have also tended to support the ecdysozoan clade (Schmidt-Rhaesa et al. 1998; Zrzavy et al. 1998b). The overwhelming majority of living animals are ecdysozoans, and it is of interest to attempt to understand the constraints and opportunities that have combined to produce the main features of this newly appreciated metazoan alliance.

Ecdysozoan phylogeny

Relationships among ecdysozoan phyla (Fig. 1) are not entirely settled (see reviews by Garey and Schmidt-Rhaesa 1998 and Giribet and Ribera 1998). An alliance of Arthropoda, Tardigrada, and Onychophora has been suggested on morphological grounds (Nielsen et al., 1996) and these clades have been linked in various combinations by 18S rRNA evidence (Garey et al. 1996; Giribet et al. 1996; Zrzavy et al. 1998a). Nematoda are generally long-branched and difficult to place with the 18S rRNA molecule, but Aguinaldo et al. (1997) discovered a relatively short-branched form, and it associated with Ecdysozoa. Nematomorpha has been placed in Ecdysozoa on 18S rRNA evidence and is linked with Nematoda on morphological grounds (Nielsen et al. 1996; Wallace et al. 1996), furnishing additional evidence that Nematoda belongs in Ecdysozoa. Priapulida and Kinorhyncha appear to form a clade that is sister to the remaining extant ecdysozoans (Aguinaldo et al. 1997; Giribet and Ribera 1998). The Loricifera may also belong to Ecdysozoa, being allied to Kinorhyncha and Priapulida on morphological grounds (Nielsen et al. 1996; Wallace et al. 1996), but we have not included this phylum in Fig. 1 because it has yet to be sampled for the 18S gene. Some long-branched phyla that are difficult to place in the phylogenetic tree have been allied with Ecdysozoa in some studies (e.g., Chaetognatha; Eernisse 1997; Zrzavy et al. 1998b), but their positions are unstable and uncertain and we do not consider them further here.

Molecular studies suggest that Ecdysozoa is sister to a large clade, Lophotrochozoa (Halanych et al. 1995; Aguinaldo et al. 1997), that includes annelids, mollusks, and the lophophorate phyla. Ecdysozoa and Lophotrochozoa form Protostomia, a clade that has long been considered as sister to the Deuterostomia, a position supported by 18S rRNA studies (Halanych et al. 1995; Aguinaldo et al. 1997; Littlewood et al. 1998). Protostomia and Deuterostomia form Bilateria, which molecular studies suggest forms a clade with Cnidaria and Placozoa (Collins 1998; Littlewood et al. 1998; Kim et al. 1999). The topology of these relationships is shown in Fig. 1.

Bodyplan of the ancestral ecdysozoan

The bodyplan of the last common protostome/deuterostome (P/D) ancestor is unknown, but it is commonly reconstructed as a simple, small bilaterian (Salvini-Plawen 1978; Barnes 1980; Ax 1987), and is likely to have been benthic (Valentine et al., 1999). A group of simple, small-bodied phyla, sometimes termed aschelminths, include both acoelomates and pseudocoelomates. Inglis (1985) coined the term paracoelomate to describe a simple grade of organization that includes both of those architectures, and we use the term here without any systematic implications.

Available evidence from the fossil record and from molecular and morphological phylogenies is consistent with a paracoelomate organization of early bilaterians. The earliest fossil records that may represent bilaterians are trace fossils of Neoproterozoic age, appearing about 570 million years ago (Jensen et al. 1999). These traces are small, chiefly near 1 mm though ranging to 5 mm in width (see Crimes 1989; Droser et al. 1999), and range in cross-sectional morphology from nearly featureless grooves to complex forms with ridges and troughs. Traces of these sorts suggest trace makers of paracoelomate grade (see below).

All living paracoelomate phyla are protostomes, divided between ecdysozoans (the moulting priapulids and kinorhynchs) and the lophotrochozoans (rotifers and others). The positions of paracoelomate phyla within those bilaterian alliances are not strongly supported by molecular data. However, a paracoelomate form as the last common ecdysozoan/lophotrochozoan (E/L) ancestor is not contradicted by those data (Fig. 1) and is consistent with phylogenies based on comprehensive morphological analyses of metazoan phyla (Nielsen et al. 1996; Zrzavy et al. 1998b). The P/D ancestor, unless it was more complex than the E/L ancestor, is likely to have been a paracoelomate also.

The origin of moulting

The Late Neoproterozoic trace fossils, which provide the earliest direct evidence of animal locomotion, have been assumed, at least tacitly, to have been created by the action of pedal or body-wall muscles of bilaterians. However, trails similar to some of the earliest, simple Neoproterozoic traces can be formed by animals that employ mucociliary locomotory systems (Collins et al. 2000). Mucociliary systems are widespread among invertebrates; they are used for the transport of particles along epithelia, for example in feeding and in cleaning pulmonary surfaces (Sleigh et al. 1988; Beninger et al. 1997), and are also used in locomotion of small-bodied animals (see Clark 1964). Some members of Cnidaria, Lophotrochozoa, and Deuterostomia use mucociliary systems in creeping locomotion, usually in small-bodied forms.

Moulting animals, which shed their integuments periodically, do not possess motile ectodermal cilia, and cannot use ectodermal mucociliary systems. This constraint seems to be basic to ecdysozoan biology, and we postulate that its origin and consequences are of fundamental importance to the history of the ecdysozoan clade. Instead of mucociliary creeping, small ecdysozoan worms display several other locomotory techniques (see for example Trueman 1975; Elder 1980; Brusca and Brusca 1990). Kinorhynchs burrow by thrusting their anterior body forward and anchoring it with spines; the rest of the body is then drawn forward and the process repeated. Many priapulans burrow, chiefly using introverts and peristaltic waves; their progress is aided by cuticular flanges and other processes. Nematodes lack circular body-wall muscles and many produce serpentine body motions. Serpentine and corkscrew locomotion in some parasitic forms is probably aided by longitudinal cuticular ridges (Lee and Biggs 1990). A few free-living nematodes burrow (Stauffer 1924; Clark 1964); some have annulated cuticles that may aid in the burrowing cycle.

The origin of ecdysozoan moulting may lie in the use of such cuticular features as spines, flanges, scales, annulations, and rugae as ancillary locomotory devices in vermiform body plans. Secreted as part of the cuticular integument, these structures cannot be enlarged as body size increases through growth, but the integument can be moulted and replaced by one with larger structures that function appropriately at larger body sizes. As the evolution of moulting forecloses the use of mucociliary locomotion, it is likely that moulting evolved in a lineage that had cuticular anchoring mechanisms and that moved by using peristaltic waves and/or an introvert; it could have been either a creeper or a burrower. Moulting presumably evolved between the last E/L common ancestor and the last common ecdysozoan ancestor (Fig. 1). It should be possible to identify traces left by nonmucociliary locomotory techniques. While nonmucociliary traces could not be unequivocally assigned to ecdysozoans, their first appearance would form an estimate of the earliest possible time of origin of that clade.

Moulting and ecdysozoan body plans

A notable feature of Ecdysozoa is that the hydrostatic skeletons are based chiefly on fluids within the pseudocoel compartment, and perhaps on tissues in some cases, but not on in-
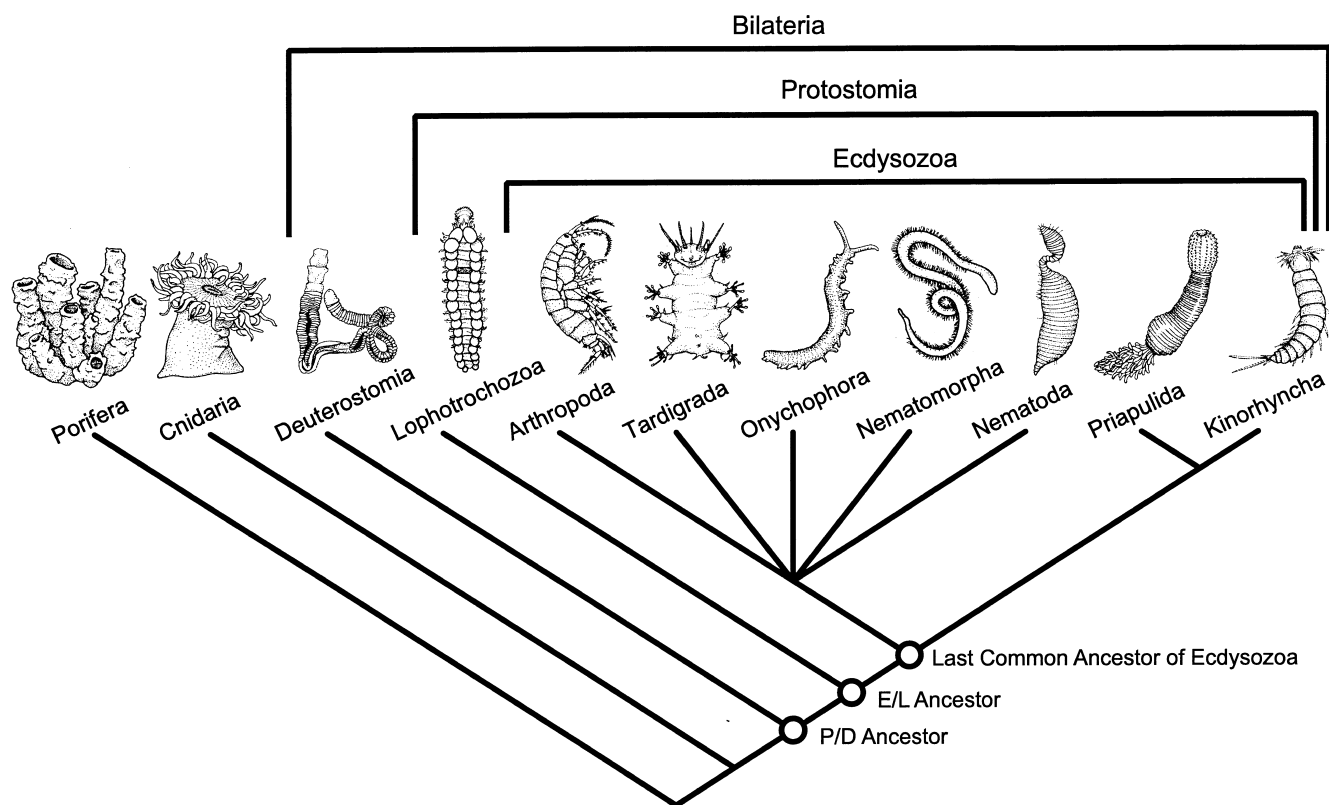
Fig. 1. A phylogenetic hypothesis of the relationships among ecdysozoan phyla, and between Ecdysozoa and other major clades. P/D ancestor, last common protostome-deuterostome ancestor; E/L ancestor, last common ecdysozoan-lophotrochozoan ancestor. See text for references. Drawings by Crissy Huffard, from various sources.

tramesodermal (coelomic) compartments. Intramesodermal spaces are found in a number of ecdysozoan phyla, commonly providing reservoirs within or around organs, or functioning as ducts. However, the hydrostatic functions of the eucoeloms in the trunks of many protostomes (as in annelids) and deuterostomes (as in cephalochordates) are performed in Ecdysozoa by fluids on the site of the blastocoel compartment, as pseudocoels or hemocoels. Furthermore, the hydraulic functions of coelomic luminae in feeding tentacles, as found in protostomes (e.g., phoronids) and deuterostomes (e.g., pterobranchs), have no counterparts in ecdysozoans. The evolution of ciliated feeding tentacles in Ecdysozoa is clearly precluded by the absence of ectodermal mucociliary tracts. It is possible that the lack of coelomic trunk compartments in Ecdysozoa may also be associated with the moulting habit. Just as moulting permits the periodic enlargement of ancillary locomotory devices and other cuticular structures, it also affords the opportunity to strengthen and even armor the general cuticular surface. Fluid spaces on the site of the blastocoel, which may also ramify through tissues, provide turgor against which body wall muscles, reinforced by the strong surrounding cuticle, may operate. The presence of this system may have mitigated against the evolution of additional fluid compartmentalization to support ecdysozoan activities.

Moulting and early ecdysozoan development

The last common P/D and E/L ancestors may have been direct developers, as postulated by a number of workers (Salvini-Plawen 1978; Barnes 1980; Grell et al. 1980; Ax 1987). Fossil embryos of the Neoproterozoic are of a size that suggests direct development (Xiao et al. 1998). Furthermore, Neoproterozoic traces indicate small body sizes, and there is a strong correlation between small body size and direct development. It has been argued that since fecundities are necessarily low in small-bodied metazoans, planktotrophy becomes too risky a developmental option, and nonplanktotrophic modes of development, especially direct development, are favored (Jablonski and Lutz 1983 and references therein; Chaffee and Lindberg 1986; see also Haszprunar 1992). Certainly, most of the metazoan phyla that are restricted to comparable body sizes today are direct developers, including all aschelminth paracoelomates. The exceptions are Bryozoa and Entoprocta. It may be that for bryozoans, the energetics of reproduction involves the mass of the colony rather than the mass of the individuals. Entoprocta, some of which are solitary, produce ciliated feeding larvae (see Nielsen 1995), though clutches are small and larval life is short for most taxa. Olive (1985) suggests that entoproct larvae are specialized to permit colo-

nization of highly dispersed and localized habitats for these sessile organisms. Recently, members of Acoela, which have a direct mode of development, have been hypothesized to be the sister group to all other living bilaterians (Ruiz-Trillo et al. 1999). If this relationship is confirmed, it would provide additional support for a direct developmental mode in early members of the Bilateria.

If it is indeed the case that P/D and E/L ancestors were direct developers, then indirect development through the sort of ciliated planktonic larvae that have been termed primary larvae (Jägersten 1972) has evolved independently at least twice in Bilateria, once in Deuterostomia and once in Lophotrochozoa. None of the Ecdysozoa have this style of larvae. Kinorhyncha and Nematoda are direct developers. Priapulida (Storch 1991) and Loricifera (Kristensen 1991) have early benthic stages with introverts, somewhat resembling the adults; these stages may be considered as larvae but they are likely to be elaborations of the juveniles of a direct-developing ancestor and are in any case not comparable to the primary planktonic larvae with their ciliated locomotory and feeding bands and tufts. Cilia in ecdysozoan larval forms are restricted to sensilla. Modern Onychophora and Tardigrada are terrestrial and their development appears to be highly derived. Marine arthropods have a variety of planktonic larval types but they all seem to be juvenile instars modified for larval life.

Jägersten (1972) regarded the arthropod and priapulid larvae as secondary (the Loricifera were unknown at that time), by which he meant that primary larvae had been present ancestrally but had been lost, and new larvae had evolved within juvenile stages, perhaps through forms of heterochrony. However, if the ecdysozoans are descended from direct-developing paracoelomates, then their larvae are not secondary in Jägersten's sense. Rather, ecdysozoans have produced moulting stages that are fully as primary as dipleurulae and trochophores are within their clades, but that are either benthic or are adapted to pelagic life through morphological modifications that do not involve ciliary bands but that permit planktotrophy.

If indirect-developing larvae have evolved independently in the three major bilaterian clades, then developmental evolutionary hypotheses that rely upon planktonic larval forms to be plesiomorphic for those clades, either present in the clade ancestries as larvae (e.g., Nielsen 1995) or representing the ancestors themselves (e.g., Davidson et al. 1995; Peterson et al. 1997), should be reconsidered. The hypothesis by Davidson et al. is partly based on the observation that many adult organs in bilaterians are not derived from the cells of larval organs but rather from pleuripotent cells that are sequestered during larval life and employed as primordia for adult structures, such as imaginal discs in insects. Those workers suggest that the larval type is generally an ancestral form, perhaps resembling Rotifera, and that the modern adults are derived forms whose complexity was achieved through the evolutionary strategy of development in set-

aside cells. Arenas-Mena et al. (1998) report that only two *Hox* genes are employed in development of the primary larvae of a sea urchin, and not in axial patterning in the larvae, and suggest that this favors the set-aside cell hypothesis, for a large cluster of *Hox* genes is used only in patterning the complex adult. However, the P/D ancestor had at least seven *Hox* genes and probably more (de Rosa et al. 1999). We suggest that the planktonic larval forms have been evolutionarily intercalated within the ontogenies of their lineages, and that a couple of *Hox* genes were among the regulatory genes enlisted to this end, at least in deuterostomes. Set-aside cells are most likely to have evolved as a strategy to permit rapid metamorphosis, connecting newly evolved larval types to their adult body plans (see Wolpert 1999).

Although we argue that the larval forms of the major bilaterian clades are likely to have been independently evolved, the evidence is not conclusive, though further tests are possible. For example, in order to determine whether the specification of sea urchin embryogenesis represents a general case, it would be useful to learn which *Hox* genes are active during embryogenesis in many other metazoan clades, particularly in protostomes. It is also possible that evidence bearing on the set-aside hypothesis can be recovered from the fossil record. Fossil larvae have been described from Neoproterozoic (Xiao et al. 1998) and Cambrian (Zhang and Pratt 1994; Bengtson and Yue 1997; Kouchinsky et al. 1999) deposits. The discovery of additional larval assemblages, especially in the Neoproterozoic, could greatly advance understanding of early metazoan developmental diversification.

Acknowledgments

REFERENCES

Aguinaldo, A. M. A., Turbeville, J. M., Linford, L. S., Rivera, M. C., Garey, J. R., Raff, R. A., and Lake, J. A. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 387:489–493.

Arenas-Mena, C., Martinez, P., Cameron, R. A., and Davidson, E. H. 1998. Expression of the *Hox* gene complex in the indirect development of a sea urchin. *Proc. Natl. Acad. Sci. USA* 95:13062–13067.

Ax, P. 1987. *The Phylogenetic System*. Wiley, London.

Barnes, R. D. 1980. *Invertebrate Zoology*, 4th Ed. W. B. Saunders, Philadelphia.

Barnes, R. S. K., Calow, P., and Olive, P. J. W. 1993. *The Invertebrates, a New Synthesis*, 2nd Ed. Blackwell Scientific, Oxford.

Bengtson, S., and Yue, Z. 1997. Fossilized metazoan embryos with blastomeres. *Science* 266:1645–1648.

Beninger, P. G., Lynn, J. W., Dietz, T. H., and Silverman, H. 1997. Mucociliary transport in living tissue: the two-layer model confirmed in the mussel *Mytilus edulis* L. *Biol. Bull.* 193:4–7.

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

Chaffee, C., and Lindberg, D. R. 1986. Larval biology of Early Cambrian molluscs: the implications of small body size. *Bull. Mar. Sci.* 39:536–549.

Clark, R. B. 1964. *Dynamics in Metazoan Evolution*. Clarendon Press, Oxford.

Collins, A. G. 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: an analysis of 18S molecular evidence. *Proc. Natl. Acad. Sci. USA* 95:15458–15463.

Collins, A. G., Lipps, J. H., and Valentine, J. W. 2000. Modern mucociliary creeping trails and the body plans of Neoproterozoic trace makers. *Paleobiology* (in press).

Crimes, T. P. 1989. Trace fossils. In J. W. Cowie and M. D. Brasier (eds.). *The Precambrian-Cambrian Boundary*. Clarendon Press, Oxford, pp. 166–185.

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

de Rosa, R., Grenier, J. K., Andreeva, T., Cook, C. E., Adoutte, A., Akam, M., Carroll, S. B., and Belavoine, G. 1999. *Hox* genes in brachiopods and priapulids: implications for protostome evolution. *Nature* 399:772–776.

Droser, M. L., Gehling, J. G., and Jensen, S. 1999. When the worm turned: concordance of Early Cambrian ichnofabric and trace-fossil record in siliciclastic rocks of South Australia. *Geology* 27:625–629.

Eernisse, D. J. 1997. Arthropod and annelid relationships re-examined. In R. A. Fortey and R. H. Thomas (eds.). *Arthropod Relationships*. Chapman and Hall, London, pp. 43–56.

Elder, H. Y. 1980. Peristaltic mechanisms. In H. Y. Elder and E. R. Trueman (eds.). *Aspects of Animal Movement*. Cambridge University Press, Cambridge, pp. 71–92.

Garey, J. R., Krotec, M., Nelson, D. R., and Brooks, J. 1996. Molecular analysis supports a tardigrade-arthropod association. *Invert. Biol.* 115:79–88.

Garey, J. R., and Schmidt-Rhaesa, A. 1998. The essential role of "minor" phyla in molecular studies of animal evolution. *Am. Zool.* 38:907–917.

Giribet, G., and Ribera, C. 1998. The position of arthropods in the animal kingdom: a search for a reliable outgroup for internal arthropod phylogeny. *Mol. Phylogenet. Evol.* 9:481–488.

Giribet, G., Carranza, S., Baguna, J., Riutort, M., and Ribera, C. 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Mol. Biol. Evol.* 13:76–84.

Grell, K. G., Gruner, H.-E., and Kilian, E. F. 1980. Einführung. In H.-E. Gruner (ed.). Lehrbuch der Speziellen Zoologie, Vol. 1. Fischer, Jena, pp. 15–156.

Halanych, K. M., Bacheller, J. D., Aguinaldo, A. M. A., Liva, S. M., Hillis, D. M., and Lake, J. A. 1995. Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science* 267:1641–1643.

Haszprunar, G. 1992. The first mollusks—small animals. *Boll. Zool.* 59:1–16.

Inglis, W. G. 1985. Evolutionary waves: patterns in the origins of animal phyla. *Aust. J. Zool.* 33:153–178.

Jablonski, D., and Lutz, R. A. 1983. Larval ecology of marine benthic invertebrates: paleobiological implications. *Biol. Rev.* 58:21–89.

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

Jensen, S., Gehling, J. G., and Droser, M. L. 1999. Trace fossils and bioturbation in the Proterozoic. *J. Conf. Abst.* 4:264–265.

Kim, J., Kim, W., and Cunningham, C. W. 1999. A new perspective on lower metazoan relationships from 18S rDNA sequences. *Mol. Biol. Evol.* 16:423–427.

Kouchinsky, A., Bengtson, S., and Gershwin, L. 1999. Cnidarian-like embryos associated with the first shelly fossils in Siberia. *Geology* 27:609–613.

Kristensen, R. M. 1991. Loricifera. In F. W. Harrison and E. E. Ruppert (eds.). *Microscopic Anatomy of Invertebrates*, Vol. 4, Aschelminthes. Wiley-Liss, New York, pp. 351–375.

Lee, D. L., and Biggs, W. D. 1990. Two- and three-dimensional locomotion of the nematode *Nippostrongylus brasiliensis*. *Parasitology* 101:301–308.

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

Nielsen, C. 1995. Animal evolution: interrelationships of the living phyla. Oxford University Press, Oxford.

Nielsen, C., Scharff, N., and Eibye-Jacobsen, D. 1996. Cladistic analyses of the animal kingdom. *Biol. J. Linn. Soc.* 57:385–410.

Olive, P. J. W. 1985. Covariability of reproductive traits in marine invertebrates: implications for the phylogeny of the lower invertebrates. In S. Conway Morris, J. D. George, R. Gibson, and H. M. Platt (eds.). *The Origins and Relationships of Lower Invertebrates*. Oxford University Press, Oxford, pp. 42–59.

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.

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

Salvini-Plawen, L. von. 1978. On the origin and evolution of the lower Metazoa. *Z. Zool. Syst. Evolutionsforsch.* 16:40–88.

Schmidt-Rhaesa, A., Bartolomaeus, T., Lemburg, C., Ehlers, U., and Garey, J. R. 1998. The position of the Arthropoda in the phylogenetic system. *J. Morph.* 238: 263–285.

Sleigh, M. A., Blake, J. R., and Liron, N. 1988. The propulsion of mucus by cilia. *Am. Rev. Resp. Dis.* 137:726–741.

Stauffer, H. 1924. Die Lokomotion der Nematoden. *Zool. Jb.* 49:119–130.

Storch, V. 1991. Priapulida. In F. W. Harrison and E. E. Ruppert (eds.). *Microscopic Anatomy of Invertebrates*, Vol. 4, Aschelminthes. Wiley-Liss, New York, pp. 335–350.

Trueman, E. R. 1975. *The Locomotion of Soft-Bodied Animals*. Edward Arnold, London.

Valentine, J. W., Jablonski, D., and Erwin, D. H. 1999. Fossils, molecules and embryos: new perspectives on the Cambrian explosion. *Development* 126:851–859.

Wallace, R. L., Ricci, C., and Melone, G. 1996. A cladistic analysis of pseudocoelomate (aschelminth) morphology. *Invert. Biol.* 115:104–112.

Wolpert, L. 1999. From egg to adult to larva. *Evol. Dev.* 1:3–4.

Xiao, S., Zhang, Y., and Knoll, A. H. 1998. Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature* 391:553–558.

Zhang, X., and Pratt, B. R. 1994. Middle Cambrian arthropod embryos with blastomeres. *Science* 266:627–639.

Zrzavy, J., Hypso, V., and Vlaskova, M. 1998a. Arthropod phylogeny: taxonomic congruence, total evidence and conditional combination approaches to morphological and molecular data sets. In R. A. Fortey and R. H. Thomas (eds.). *Arthropod Relationships*. Chapman and Hall, London, pp. 97–107.

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