

Fossils and phylogenies: integrating multiple lines of evidence to investigate the origin of early major metazoan lineages

Pauly Cartwright^{1,*} and Allen Collins[†]

^{*}Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Avenue, Lawrence, Kansas 66045, USA; [†]National Systematics Laboratory of NOAA Fisheries Service, National Museum of Natural History, MRC-153, Smithsonian Institution, PO Box 37012, Washington, DC 20013-7012, USA

Synopsis Understanding the nature and timing of metazoan origins is one of the most important, yet elusive, questions in evolutionary biology. Fossil data provide the most tangible evidence for the origin of early animal lineages, although additional evidence from molecular phylogenetics, molecular clock studies, and development has contributed to our current understanding. We review several lines of evidence to explore the nature and timing of early metazoan evolution and discuss how these data, when considered together, provide a more cohesive picture of the origin of animal diversity. We discuss how trace fossils and biomarkers provide compelling evidence for the origins of Bilateria and siliceous sponges. Using a molecular phylogenetic framework for metazoans, we discuss how fossils can be used to date the origin of clades. We use these fossil dates to perform a relaxed molecular clock analysis for estimating dates of nodes when no fossils are available. We also discuss current data from developmental biology that suggest that early metazoans possessed a sophisticated molecular toolkit for building complex body plans. We conclude that the best evidence for the origin of major metazoan lineages lies in the careful interpretation of the fossil record and that these data, when considered with phylogenetic and developmental evidence, support the notion that the Cambrian radiation is a real phenomenon that marks a critically important time in the history of life.

Introduction

One of the grand challenges in evolutionary biology today is to understand the origins and diversification of early animal lineages. While fossils provide the only tangible evidence for the origin of animals, some key early metazoan fossils are rare and many lack informative characters, making the derivation of a rigorous phylogenetic hypothesis based solely on fossil data difficult. Molecular approaches have proven useful for elucidating phylogenetic relationships of extant metazoan taxa, although many key relationships in early diverging metazoans lineages are poorly supported or unresolved. Although there exists uncertainty regarding the timing and patterns of early animal evolution, an evaluation of different lines of evidence, including fossils, molecular phylogenies, and development, can provide insight into these questions.

Most animal phyla make their first appearance in the fossil record towards the base of the Cambrian, between 510 and 543 Ma (Valentine 2004). The relatively narrow window in which these animals appear, referred to as the Cambrian radiation, is what makes studies of the origins of early animal evolution

both intriguing and elusive (Conway Morris 1993; Fortey et al. 1996; Budd and Jensen 2000; Conway Morris 2000). The idea that the Cambrian radiation reflects a real “explosion” of animal lineages is controversial. This controversy hinges on whether or not the relatively sudden appearance of all animal phyla in the Cambrian corresponds to their actual evolutionary origin (Lieberman 1999, 2003; Conway Morris 2006). Arguments supporting the origin of metazoans well before the Cambrian purport that the appearance of a Cambrian radiation is an artifact of poor preservation in the fossil record (Darwin 1872; Davidson et al. 1995; Fortey et al. 1996; Wray et al. 1996; Xiao et al. 1998). An ancient precambrian origin for animals was supported by Darwin (1872) who found the Cambrian explosion problematic to his theory on natural selection (discussed in Lieberman 1999, 2003). Some studies using molecular clock estimations support this view (Wray et al. 1996; Blair and Hedges 2005) and hypotheses were put forth regarding early metazoans as tiny larvae-like forms with little fossilization potential (Davidson et al. 1995).

From the symposium “Key Transitions in Animal Evolution” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2007, at Phoenix, Arizona.

¹E-mail: pcart@ku.edu

Integrative and Comparative Biology, volume 47, number 5, pp. 744–751

doi:10.1093/icb/icm071

Advanced Access publication July 20, 2007

© The Author 2007. Published by Oxford University Press on behalf of the Society for Integrative and Comparative Biology. All rights reserved. For permissions please email: journals.permissions@oxfordjournals.org.

The Cambrian explosion as a real phenomenon was argued in Gould (1989) and has also been discussed in several recent papers (Donoghue and Smith 2004 and papers therein; Peterson and Butterfield 2005b; Benton and Donoghue 2006; Conway Morris 2006; Marshall 2006). Recent discussions in support of the Cambrian explosion hypothesis focus on evaluation of new fossil data from the late Neoproterozoic and Cambrian (Conway Morris and Collins 1996; Donoghue and Smith 2004; Marshall 2006) and a critical re-evaluation of molecular clock estimates (Donoghue and Smith 2004; Peterson and Butterfield 2005a; Pulquério and Nichols 2006).

In this article, we investigate several lines of evidence to explore the nature and timing of metazoan divergence prior to the Cambrian radiation. Specifically, we are interested in the cladogenetic events that gave rise to the extant clades Silicea, Calcarea, Placozoa, Ctenophora, Cnidaria, and Bilateria. We review the earliest evidence for these clades and argue that this evidence is compelling enough to confidently argue that these lineages originated in the late Neoproterozoic. Next, we summarize the earliest evidence for crown-group metazoan lineages in the form of Cambrian soft-bodied fossils that are members of modern metazoan clades. We then present a phylogenetic analysis of combined small subunit of the nuclear ribosome (SSU) and large subunit of the nuclear ribosome (LSU) rDNA sequences for metazoans and map fossil dates onto the phylogeny. Lastly, we present a molecular clock analysis to extrapolate dates for the origin of major animal lineages using different calibration points. Molecular-clock approaches have a valuable, but potentially limited, role in dating the origin of metazoans as their accuracy depends on multiple calibration points and results can vary depending on several factors, including the topology of relationships, how the phylogenetic position of fossil taxa is treated, the accuracy and number of calibration points, and the validity of the rate model. We conclude that the best way to determine the origin of major metazoan lineages involves a careful, and more literal, interpretation of the fossil record. This evidence, in conjunction with phylogenetic and developmental data, supports the assertion that the Cambrian radiation is a real biological event that marks a period of relatively rapid animal diversification at the very end of the Neoproterozoic.

Evidence for the origin of early-diverging metazoan clades

While there may be opposing viewpoints on the reliability of the fossil record and how much it can

tell us about the timing of key events in the history of life, we emphasize that fossils provide the only tangible physical evidence for the existence of animal lineages in the past. In order for a fossil to be useful in indicating that a particular clade had arisen by a certain point in time, its phylogenetic affinity must be interpreted correctly. Even assuming accurate phylogenetic interpretations, the earliest fossils representing particular crown clades provide minimum ages for the clades' origins. Thus, in order to assess how close a minimum estimate based upon a first appearance in the fossil record is to the actual origin of a particular clade, the subsequent fossil record of the clade must be carefully examined. We suggest that accuracy in estimating the actual origin of a clade based upon a first fossil occurrence is dependent on the extent of continuity of the clade in the fossil record following its first appearance.

The main sponge clades, Silicea (=Demospongia plus Hexactinellida), and Calcarea, have excellent preservation potential because their members typically possess mineralized skeletons, mainly in the form of spicules. Not surprisingly, the fossil record of sponges is significant throughout the Phanerozoic (Krautter 2002; Pickett 2002; Reitner and Wörheide 2002; Pisera 2006). In terms of body fossils, the record of Calcarea (both stem and putative crown members) goes back to the early Cambrian (Pickett 2002). Diverse siliceans are similarly well represented in the early Cambrian, but there are also a number of reports of likely crown-group members from the late Neoproterozoic, perhaps going back to 570 Ma (Krautter 2002; Reitner and Wörheide 2002). Taken at face value, the sponge-body fossil record accords well with the hypothesis that sponges are paraphyletic with Calcarea being more closely related to eumetazoans than is Silicea (reviewed by Nicholls and Wörheide 2005). An alternative type of fossil, in the form of biomarkers, indicative of Silicea goes back to roughly 710 Ma and persists into the Phanerozoic (Love et al. 2006). Because this record is continuous after its inception, it should provide a reasonably close estimate of its origin.

Ctenophores are early diverging soft-bodied animals. A few likely crown-group ctenophores are known from the Devonian (Stanley and Stürmer 1983, 1989), whereas a small number of putative stem-group members of Ctenophora have been reported from the Cambrian (Conway Morris and Collins 1996; Chen 2004; Chen et al. 2007). A number of different cnidarian groups have skeletonized or "hard" body parts, particularly within Anthozoa, and these tend to have a rich fossil record. Nevertheless, the vast majority

of major cnidarian clades do not have hard parts. The best evidence for the earliest cnidarians comes from fossil tubes of the Doushantuo, dated to about 570 Ma (Xiao et al. 2000), but the subsequent record of Cnidaria through the Cambrian and the rest of the Phanerozoic is fragmented, considering the breadth of known extant diversity. Not surprisingly, given their small size and soft-bodied architecture, placozoans have no fossil record.

Tracks and trails of the late Neoproterozoic provide excellent evidence for the origin of Bilateria. Beginning at about 560 Ma, the first creeping traces appear in the fossil record (Jensen 2003). These are simple trails made at or near the surface of the sediment and were most certainly created by small vermiform bilaterians (Valentine 1994; Collins et al. 2000). Between the time when fossil trails appear in the late Neoproterozoic and the base of the Cambrian, they become more robust and complex in terms of architecture and the underlying behavior they represent (Jensen 2003). During this time period, however, the trail makers did not disturb the sediment enough to generate ichnofabrics indicative of bioturbation (Droser et al. 2002). It is not until the base of the Cambrian that burrowing and bioturbation by putative bilaterian worms begins, and throughout the Cambrian burrowing depth and bioturbation intensity increases (Droser et al. 2002). Taken together, this sequence and development of the metazoan-trace fossil record, which began at about 560 Ma, and shows burrowing began at 543 Ma, and continued throughout the Phanerozoic, provides the strongest evidence for the origin and persistence of one of the early diverging, major metazoan lineages, namely Bilateria.

Exceptional glimpses of early animal diversity

The Cambrian fossil record also provides excellent evidence for the origin of several crown-group phyla within Bilateria. This record includes skeletonized organisms and also Burgess Shale-type soft-bodied fossils. Burgess Shale-type soft-bodied fossil deposits are known for their remarkable preservational detail across a diversity of body forms, and these fossil formations give us extraordinary insight into early animal evolution. The most famous of these is the Burgess Shale fossil fauna of British Columbia, Canada that contains remarkably preserved fossils including representatives of the sponge, ctenophore, arthropod, annelid, priapulid, and chordate clades (Gould 1989). These fossils provide compelling evidence that much of the modern-day animal diversity was present by the Middle

Cambrian (500 Ma) (Briggs and Fortey 1989; Conway Morris 1989; Briggs 1991). More recently, fossil finds in the early Cambrian Chengjiang fossil biota of Yunnan Province, China, have revealed an equally well-preserved and diverse fauna, that predates the Burgess Shale by over 20 million years. The Chengjiang fauna includes the earliest known vertebrate, *Haikouella* (Chen et al. 1999) as well as sponges, priapulids, arthropods, annelid-like forms, and hemichordates (Hou et al. 2004).

Other less well-known soft-bodied fossil formations have contributed to our increased understanding of early animal evolution. Most notably, we (in collaboration with other biologists and paleontologists) have recently reported on Middle Cambrian cnidarian jellyfish from the Marjum formation of Utah (Cartwright 2006; Hendricks et al. 2006). We have described fossils representing three separate classes of medusozoan cnidarians. Remarkably, many of these fossil jellyfish are diagnosable to deeply derived modern clades, suggesting that at least some medusozoans have undergone little change over the past half a billion years. In addition, the demonstration that the breadth of medusozoan diversity was present 500 Ma also provides further evidence that much of the diversification of major animal lineages had already occurred by the Middle Cambrian. To better illustrate the narrow window of time in which many of these crown-group taxa appear in the fossil record, we have assembled a summary of some of the earliest fossil representatives from major animal lineages: records presented here include biomarkers, trace fossils, and soft-bodied fossils (Table 1).

A phylogenetic hypothesis of metazoan relationships

We assembled a data matrix of published and unpublished sequences from nuclear SSU and LSU rDNA comprising 159 metazoan taxa and nine nonmetazoan outgroup taxa. These data were aligned using Muscle v3.6 (Edgar 2004) and “conserved” positions were chosen with Gblocks v0.91 (Castresana 2000). We derived a topology for which the data were most likely (Fig. 1), running 10 replicate searches with taxa added randomly, using GARLI v0.951 (Zwickl 2006). For these analyses, we assumed the GTR+I+G model of nucleotide evolution, as indicated by the AIC criterion implemented in Modeltest v3.7 (Posada and Crandall 2000). We used GARLI to run 100 bootstrap replicate searches to calculate bootstrap indices. Many of the major animal taxa

Table 1 Earliest fossil representative of major metazoan clades

Earliest fossil representative	Clade	Date (Ma) formation	Stem/crown	Reference
Unnamed	Porifera, Silicea	710 (Biomarkers)	Stem	(Love et al. 2006)
<i>Palaeophragmodictya</i>	Porifera	560	Crown	(Gehling and Rigby 1996)
Unnamed	Cnidaria	570	Stem	(Xiao et al. 2000)
Unnamed	Scyphozoa, Semaestome	500 Marjum	Crown	(Cartwright 2006; Hendricks et al. 2006)
Unnamed	Scyphozoa, Coronate	500 Marjum	Crown	(Cartwright 2006; Hendricks et al. 2006)
Unnamed	Hydrozoa, Narcomedusae	500 Marjum	Crown	(Cartwright 2006; Hendricks et al. 2006)
Unnamed	Hydrozoa, Filifera	500 Marjum	Crown	(Cartwright 2006; Hendricks et al. 2006)
<i>Tripedalia</i>	Cubozoa, Carybdea	500 Marjum	Crown	(Cartwright 2006; Hendricks et al. 2006)
<i>Fasciculus</i>	Ctenophora	500 Burgess	Stem	(Conway Morris and Collins 1996)
<i>Maotianoascus</i>	Ctenophora	540 Meishucun	Stem	(Chen et al. 2007)
Trace fossil	Bilateria	560	Stem	(Narbonne and Aitken 1990)
<i>Anomalocaris</i>	Arthropoda	530	Stem	(Collins 1996)
<i>Rusophycus</i> trace fossil	Arthropoda	531 or 534	Stem	(Crimes 1992; Budd and Jensen 2000)
Many	Brachiopoda	525 cosmopolitan	Crown	
<i>Shankouclava</i>	Urochordata	525 Chengjiang	Stem	(Chen et al. 2003)
<i>Yunnanozoon</i> , <i>Haikouichthys</i>	Chordata	525 Chengjiang	Stem	(Chen et al. 1995; Shu et al. 1999)
Many	Chordata, Agnathan	495	Crown	

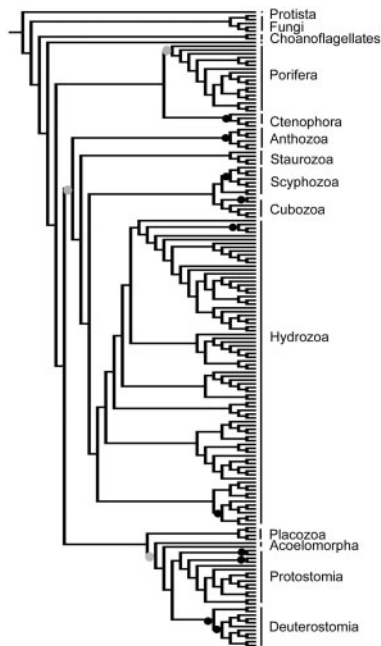


Fig. 1 Maximum-likelihood analysis of combined published and unpublished SSU and LSU rDNA. Black circles represent minimum-age-fossil calibrations using crown-group fossils. Gray circles represent maximum-age calibrations based on biomarkers and trace fossils.

have strong support for monophyly. These include the cnidarians and within cnidarians, the medusozoan/anthozoan sister-group relationship. This is consistent with previously published analyses (Bridge

et al. 1992; Collins 2002; Collins et al. 2006). In addition, there is strong support for the monophyly of Bilateria with the choanoflagellates as a sister group to the metazoans. These nodes have bootstrap values of greater than 95 (data not shown) and these results, and the consensus in the literature, implies that biologists may be converging on a robust phylogenetic framework for at least some of the early metazoan relationships. It is also worth noting that in our analysis, placozoans and bilaterians form a clade that arose after the other early metazoan divergences, a finding contradictory to recent analyses of mitochondrial genome data which suggested that these two lineages arose earliest in metazoan history (Dellaporta et al. 2006). These results suggest that additional markers are needed for a more robust phylogenetic placement of these lineages. Current efforts identify nuclear-protein-coding loci from expressed tag sequence (EST)-directed or PCR-directed approaches that should prove useful in this regard (Rokas et al. 2003, 2005; Philippe et al. 2004).

Rocks and clocks: the limited role of molecular rate analyses for dating the origin of metazoan lineages

Using the molecular phylogeny as a framework, we mapped many of the fossils listed in Table 1 onto nodes of the clade that includes the crown-group. By definition, crown-group fossils possess the

Table 2 Results of molecular-clock analysis for estimated dates of origin of several metazoan lineages, using several fossil calibrations and different maximum-age constraints

Taxon	Estimated date of origin using different maximum age calibrations		
	A, C	A and C	A, B, and C
Metazoa	12,539, 10,364	1633	1147
Choanoflagellates	9198, 7630	1195	837
Ctenophores	390, 1178	390	390
Placozoa	944, 132	85	61
Silicea	710, 6254	710	710
Cnidarians	8368, 4806	1162	570
Scyphozoans	5425, 3115	837	540
Cubozoans	3326, 1900	567	508
Hydrozoans	5477, 3183	932	538
Hexacorals	5024, 2866	658	377
Bilaterians	7301, 560	560	560
Deuterostomes	4850, 540	540	540
Hemichordates	2795, 367	363	361
Chordates	2274, 495	495	495
Protostomes	4729, 543	543	543
Ecdysozoa	3906, 530	530	530
Lophotrochozoa	4382, 538	538	537

A = Biomarker date of 710 Ma for the origin of sponges, B = Fossil date of 570 Ma for origin of cnidarians, C = Trace-fossil data of 560 Ma for origin of bilaterians.

morphological synapomorphies for that clade, so the clade must have evolved by the time such a fossil appeared. Therefore, the presence of a crown-group at a given time can only serve as a minimum age calibration for the clade that includes the fossil taxon. This can be somewhat problematic because analyses that utilize fossils to calibrate molecular clocks that are subsequently used to date the origin of clades require that either the ages of nodes be fixed in time, or that a maximum age be set. We used the biomarker and trace-fossil evidence listed in Table 1 to assign a maximum-age date to the siliceous sponge and bilateria clades, respectively. Assigning maximum-age dates at these nodes assumes that no crown-group taxon appeared before the appearance of the fossil. The assumptions in these two instances rely on negative evidence, but we contend that these particular cases represent the least egregious assumptions in this regard because each of these clades has an exceptional, continuous record in subsequent fossil deposits (as discussed earlier); this suggests that their first appearances most likely closely correspond to the actual origins of these clades.

Mapping fossil dates onto the phylogeny is useful for dating the origins of the clades to which the fossils belong, but this alone cannot provide insight

at nodes where no fossil data are available. It was therefore necessary to apply a model of molecular evolution to extrapolate dates of origin for several nodes that lacked fossil data. We chose the penalized likelihood model (Sanderson 2002) that uses a semi-parametric approach to relax the stringency of a clock by allowing for rate smoothing, but provides a likelihood penalty for rate variation across the phylogeny. The model was implemented in the program r8s (Sanderson 2003) that allows one to specify minimum and maximum age constraints on any given node. Constraining maximum ages assumes that the clade did not exist before that date. Our justification for these maximum dates is discussed earlier, with the caveat that the extrapolated dates are contingent on the accuracy of the maximum-date calibrations. To test the effect of maximum-date calibrations on the model, we performed several different rate analyses using different combinations of maximum-age calibrations.

The results of these analyses are shown in Table 2 and demonstrate that the accuracy of the rate model is highly dependent on the maximum-age calibrations. Without applying at least two maximum-age calibration points, the r8s analyses dated the origin of metazoan lineages well before one billion years. Given the known Proterozoic record of fossils

and paleoclimatic information (Knoll et al. 2006), we view such an early date as biologically unrealistic. For example, using just one maximum calibration point, the model predicted that metazoans originated over 10 bya, a date preceding all accepted dates for the origin of the earth! By adding one additional calibration point, the model predicted metazoan origins at 1.6 bya and two additional calibration points estimated an origin at 1.1 bya (Table 2).

The early metazoan molecular toolkit

Recent evidence from comparative genomics and developmental biology has provided much insight into the possible genetic composition of early animals. Recently, the first complete assembled genome from an early-diverging metazoan, the sea anemone *Nematostella vectensis*, was released. Assembled genome sequences from the demosponge *Amphimedon queenslandica* (often mistakenly referred to as *Reniera*) and the placozoan *Trichoplax* (sequenced at the United States Department of Energy's Joint Genome Institute) are expected to be released in the near future. Also underway at the National Human Genome Research Center at NIH is the genome-sequencing effort for *Hydra magnipapillata*. In addition to whole genome projects, dozens of large-scale EST projects are underway in a variety of bilaterian and nonbilaterian taxa. Comparisons of genome and EST sequences have revealed unexpected insights into the genetic makeup of early-diverging animals.

Prior to the availability of genomic data in nonbilaterian animals, it was thought that many developmental regulatory genes/gene pathways were specific to vertebrates because the invertebrate model organisms *Drosophila* and *Caenorhabditis elegans* lacked many of these genes. Immediately following the release of the *Nematostella* genome, however, a series of papers was published that suggested that *Nematostella* possesses a complex genome and contains many developmental regulatory genes/gene families previously thought to be specific to vertebrates (Ball et al. 2004; Kusserow et al. 2005; Technau et al. 2005; Chourrout et al. 2006; Ryan et al. 2006). Multiple members of many developmental regulatory pathways have been identified, including the Wnt, TGF, Hedgehog, and Notch pathways (Technau et al. 2005). These data support the idea that the metazoan ancestor was equipped with the molecular tools necessary for the specification of complex body plans. Thus, currently available genomic data supports the origin of a complex genome predating the Cambrian radiation, with the

ancestral genome possessing the molecular toolkit necessary for an "explosion" of body plans and complex traits. While the availability of genomes from other early-diverging lineages will allow for a more precise reconstruction of the genetic makeup in the lineages leading to the first modern metazoans, the trigger for the Cambrian radiation likely lies in the changing ecological circumstances resulting from the origin of the body plans associated with the early metazoan lineages.

Conclusions

The biological significance of the Cambrian radiation has been debated ever since the publication of "The Origin of Species" (Darwin 1872) and was in fact the subject of some concern to Darwin (Lieberman 1999), causing him to claim that there must be a long-hidden history of animals; others have argued that there was a real explosion of animals during the late Neoproterozoic/early Cambrian transition (Valentine 2004). We conclude that a careful interpretation of all available evidence, particularly paleontological data, presents strong evidence that most major animal lineages originated in a relatively short period of time and therefore that the Cambrian radiation represents a real and significant event in the history of life, and not some artifact of taphonomy or of a poor fossil record.

Acknowledgments

We would like to thank Bernd Schierwater and Rob DeSalle for inviting us to participate in this symposium. We thank Scott Nichols, Kord Kober, and Meg Daly for sharing unpublished sequence data. We are grateful to B. S. Lieberman for comments on earlier versions of this article and to two anonymous reviewers for helpful comments. We acknowledge funding from NSF ATOL EF0531779.

References

- Ball EE, Hayward DC, Saint R, Miller DJ. 2004. A simple plan - cnidarians and the origins of developmental mechanisms. *Nat Rev Gen* 5:567-77.
- Benton MD, Donoghue PCJ. 2006. Paleontological evidence to date the tree of life. *Mol Biol Evol* 24:26-53.
- Blair JE, Hedges SB. 2005. Molecular clocks do not support the Cambrian explosion. *Mol Biol Evol* 22:387-90.
- Bridge D, Cunningham CW, Schierwater B, DeSalle R, Buss LW. 1992. Class-level relationships in the phylum Cnidaria: evidence from mitochondrial genome structure. *Proc Natl Acad Sci USA* 89:8750-3.

- Briggs DEG. 1991. Extraordinary fossils. *American Scientist* 79:130–41.
- Briggs DEG, Fortey RA. 1989. The early radiation and relationships of the major arthropod groups. *Science* 256:1670–3.
- Budd GE, Jensen DA. 2000. A critical reappraisal of the fossil record of the Bilaterian phyla. *Biol Revs* 75:253–95.
- Cartwright P. 2006. Patterns and processes in the phylum Cnidaria: evidence from phylogeny, development and fossils. In: Yang Q, Wang Y, Weldon EA, editors. 2nd International paleontological congress. Beijing, China: University of Science and Technology of China Press. p 48.
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–52.
- Chen J-Y. 2004. The dawn of animal world. Nanjing: Jiangsu Publishing House of Science and Technology.
- Chen J-Y, Dzik J, Edgecomb GD, Ramskold L, Zhou GQ. 1995. A possible early Cambrian chordate. *Nature* 377:720–22.
- Chen J-Y, Huang D-Y, Li C-W. 1999. A early Cambrian craniate-like chordate. *Nature* 518–22.
- Chen J-Y, Huang D-Y, Peng Q-Q, Chi H-M, Wang X-Q, Feng M. 2003. The first tunicate from the Early Cambrian of South China. *Proc Natl Acad Sci USA* 100:8314–8.
- Chen J-Y, Schopf WJ, Bottjer DJ, Zhang C-Y, Kudryavtsev II AB, Tripathi AB, Wang X-Q, Yang Y-H, Gao X, Yang Y. 2007. Raman spectra of a lower Cambrian ctenophore embryo from southwestern Shaanxi, China. *Proc Natl Acad Sci USA* 104:6289–92.
- Chourrout D, et al. 2006. Minimal ProtoHox cluster inferred from bilaterian and cnidarian Hox complements. *Nature* 442:684–7.
- Collins D. 1996. The “evolution” of anomalocaris and its classification in the arthropod class dinocarida (nov.) and order radiodonta (nov.). *J Paleont* 70:280–93.
- Collins AG. 2002. Phylogeny of medusozoa and the evolution of cnidarian life cycles. *J Evolution Biol* 15:418–32.
- Collins AG, Lipps JH, Valentine JW. 2000. Modern mucociliary gliding trails and the body plans of Neoproterozoic trace-makers. *Paleobiology* 26:47–55.
- Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Syst Biol* 55:97–115.
- Conway Morris S. 1989. Burgess shale faunas and the Cambrian explosion. *Science* 246.
- Conway Morris S. 1993. The fossil record and the early evolution of the Metazoa. *Nature* 361:219–25.
- Conway Morris S. 2000. The Cambrian “explosion”: slow-fuse or megatonnage? *Proc Natl Acad Sci USA* 97:4426–9.
- Conway Morris S. 2006. Darwin’s dilemma: the realities of the Cambrian “explosion”. *Phil Trans R Soc B* 361:1069–83.
- Conway Morris S, Collins DH. 1996. Middle Cambrian ctenophores from the Stephen formation, British Columbia, Canada. *Phil Trans Roy Soc London B* 351:279–308.
- Crimes TP. 1992. Changes in the trace fossil biota across the proterozoic-phanerozoic boundary. *J Geol Soc London* 149:637–46.
- Darwin C. 1872. On the origin of species by means of natural selection; or the preservation of favored races in the struggle for life. New York: Mentor.
- Davidson EH, Peterson KJ, Cameron RA. 1995. Origin of bilaterian body plans: evolution of developmental regulatory mechanisms. *Science* 270:1319–25.
- Dellaporta SL, Xu A, Sagasser S, Jakob W, Moreno MA, Buss LW, Schierwater B. 2006. Mitochondrial genome of *Trichoplax adhaerens* supports placozoa as the basal lower metazoan phylum. *Proc Natl Acad Sci USA* 103:8751–6.
- Donoghue PCJ, Smith MP. 2004. Telling the evolutionary time: molecular clocks and the fossil record. London Boca Raton: Taylor & Francis, CRC Press.
- Droser ML, Jensen S, Gehling JG. 2002. Trace fossils and substrates of the terminal Proterozoic-Cambrian transition: implications for the record of early bilaterians and sediment mixing. *Proc Natl Acad Sci USA* 99:12572–6.
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–7.
- Fortey RA, Briggs DEG, Wills MA. 1996. The Cambrian evolutionary “explosion”: decoupling cladogenesis from morphological disparity. *Biol J Linn Soc* 57:13–33.
- Gehling JG, Rigby JK. 1996. Long expected sponges from the neoproterozoic ediacara fauna of South Australia. *J Paleont* 2:185–95.
- Gould SJ. 1989. Wonderful life. New York: WW Norton.
- Hendricks JR, Halgedahl SL, Lieberman BS, Jarrard RD. 2006. Crown-group cnidarians from the Cambrian of Utah. GSA Abstracts with Programs. Philadelphia, PA: Geological Society of America. p 383.
- Hou X-G, Aldridge RJ, Bengstrom J, Wiveter DJ, Feng X-H. 2004. The Cambrian fossils of Chengjiang. China: Blackwell Science Ltd.
- Jensen S. 2003. The proterozoic and earliest Cambrian trace fossil record; patterns problems and perspectives. *Integ Comp Biol* 43:219–28.
- Knoll AH, Javaux EJ, Hewitt D, Cohen P. 2006. Eukaryotic organisms in proterozoic oceans. *Phil Trans Biol Sci* 361:1023–38.
- Krautter M. 2002. Fossil hexactinellida: an overview. In: Hooper JNA, van Soest RWM, editors. *Systema porifera. A guide to the classification of sponges*. New York: Kluwer Academics/Plenum Press. p 1211–23.
- Kusserow A, et al. 2005. Unexpected complexity of the Wnt gene family in a sea anemone. *Nature* 433:156–60.
- Lieberman BS. 1999. Testing the darwinian legacy of the Cambrian radiation using trilobite phylogeny and biogeography. *J Paleont* 72:176–81.

- Lieberman BS. 2003. Taking the pulse of the Cambrian radiation. *Integ Comp Biol* 43:229–37.
- Love GD, Fike DA, Grosjean E, Stalvies C, Grotzinger J, Bradley AS, Bowring S, Condon D, Summons RE. 2006. Constraining the timing of basal metazoan radiation using molecular biomarkers and U-Pb isotope dating. Goldschmidt Conference, Melbourne, Australia.
- Marshall CR. 2006. Explaining the Cambrian “explosion” of animals. *Ann Rev Earth Pl Sc* 34:355–84.
- Narbonne GM, Aitken JD. 1990. Ediacaran fossils from the Sekwi Brook area, Mackenzie mountains, northwestern Canada. *Palaeontology* 945–80.
- Nicholls S, Wörheide G. 2005. Sponges: new views of old animals. *Integ Comp Biol* 45:333–4.
- Peterson KJ, Butterfield NJ. 2005a. Origin of the eumetazoa: testing ecological predictions of molecular clocks against the proterozoic fossil record. *Proc Natl Acad Sci USA* 102:9547–52.
- Peterson KJ, Butterfield NJ. 2005b. Origin of the eumetazoa: testing ecological predictions of molecular clocks against the proterozoic fossil record. *Proc Natl Acad Sci USA* 102:9547–52.
- Philippe H, Snell EA, Baptiste E, Lopez P, Holland PWH, Casane D. 2004. Phylogenomics of eukaryotes: impact of missing data on large alignments. *Mol Biol Evol* 21:1740–52.
- Pickett J. 2002. Fossil calcarea. An overview. In: Hooper JNA, van Soest RWM, editors. *Systema porifera. A guide to the classification of sponges*. New York: Kluwer Academics/Plenum Press. p 1117–9.
- Pisera A. 2006. Palaeontology of sponges—a review. *Can J Zool* 84:242–61.
- Posada D, Crandall KA. 2000. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–8.
- Pulquério MJF, Nichols RA. 2006. Dates from the molecular clock: how wrong can we be? *Trends Ecol Evol* 22:180–4.
- Reitner J, Wörheide G. 2002. Non-lithistid fossil Demospongiae—origin of their paleobiodiversity and highlights in history of preservation. In: Hooper JNA, van Soest RWM, editors. *Systema porifera. A guide to the classification of sponges*. New York: Kluwer Academics/Plenum Press. p 1377–85.
- Rokas A, King N, Finnerty J, Carroll SB. 2003. Conflicting phylogenetic signals at the base of the metazoan tree. *Evol Dev* 5:346–59.
- Rokas A, Kruger D, Carroll SB. 2005. Animal evolution and the molecular signature of radiations compressed in time. *Science* 310:1933–8.
- Ryan JF, Burton PM, Mazza ME, Kwong GK, Mullikin JC, Finnerty JR. 2006. The cnidarian-bilaterian ancestor possessed at least 56 homeoboxes: evidence from the starlet sea anemone, *Nematostella vectensis*. *Genome Biol* 7:R642–19.
- Sanderson MJ. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol Biol Evol* 19:101–9.
- Sanderson MJ. 2003. r8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19:301–2.
- Shu D-G, Luo H-L, Conway Morris S, Zhang X-L, Hu S-X, Han J, Zhu M, Li Y, Chen L-Z. 1999. Lower Cambrian vertebrates from south China. *Nature* 402:42–6.
- Stanley GD, Stürmer W. 1983. The first fossil ctenophore from the lower devonian of West Germany. *Nature* 303:518–20.
- Stanley GD, Stürmer W. 1989. A new fossil ctenophore discovered by X-rays. *Nature* 327:61–3.
- Technau U, et al. 2005. Maintenance of ancestral complexity and non-metazoan genes in two basal cnidarians. *Trends Genet* 21:633–9.
- Valentine JW. 1994. Late Precambrian bilaterians: grades and clades. *Proc Natl Acad Sci USA* 91:6751–7.
- Valentine JW. 2004. *On the origin of phyla*. Chicago: University of Chicago Press.
- Wray GA, Levinton JS, Shapiro LH. 1996. Molecular evidence for deep recambrian divergences among metazoan phyla. *Science* 274:568–73.
- Xiao S, Yuan X, Knoll AH. 2000. Eumetazoan fossils in terminal proterozoic phosphorites? *Proc Natl Acad Sci USA* 97:13684–9.
- Xiao S, Zhang Y, Knoll AH. 1998. Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature* 351:553–8.
- Zwickl DJ. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Austin: The University of Texas.