Origin of the Eumetazoa: Testing ecological predictions of molecular clocks against the Proterozoic fossil record

Kevin J. Peterson[†‡] and Nicholas J. Butterfield[‡§]

[†]Department of Biological Sciences, Dartmouth College, Hanover, NH 03755; and [§]Department of Earth Sciences, University of Cambridge, Cambridge CB2 3EQ, United Kingdom

Communicated by Eric H. Davidson, California Institute of Technology, Pasadena, CA, May 11, 2005 (received for review January 28, 2005)

Molecular clocks have the potential to shed light on the timing of early metazoan divergences, but differing algorithms and calibration points yield conspicuously discordant results. We argue here that competing molecular clock hypotheses should be testable in the fossil record, on the principle that fundamentally new grades of animal organization will have ecosystem-wide impacts. Using a set of seven nuclear-encoded protein sequences, we demonstrate the paraphyly of Porifera and calculate sponge/eumetazoan and cnidarian/bilaterian divergence times by using both distance [minimum evolution (ME)] and maximum likelihood (ML) molecular clocks; ME brackets the appearance of Eumetazoa between 634 and 604 Ma, whereas ML suggests it was between 867 and 748 Ma. Significantly, the ME, but not the ML, estimate is coincident with a major regime change in the Proterozoic acritarch record, including: (*i*) disappearance of low-diversity, evolutionarily static, pre-Ediacaran acanthomorphs; (*ii*) radiation of the high-diversity, short-lived Doushantuo-Pertatataka microbiota; and (*iii*) an order-of-magnitude increase in evolutionary turnover rate. We interpret this turnover as a consequence of the novel ecological challenges accompanying the evolution of the eumetazoan nervous system and gut. Thus, the more readily preserved microfossil record provides positive evidence for the absence of pre-Ediacaran eumetazoans and strongly supports the veracity, and therefore more general application, of the ME molecular clock.

Porifera | acritarchs | Ediacaran | coevolution

The sudden appearance of diverse metazoan fossils ≈530 million years ago (Ma) is the focus of ongoing and heated debate: Is this recording a true "Cambrian explosion" of early metazoan evolution or merely the onset of extensive burrowing and biomineralization (1)? Certainly the invisibility of microscopic, nonburrowing, and/or nonbiomineralizing metazoans in the fossil record allows for the possibility of deep Proterozoic origins, so independent lines of evidence must be sought. Molecular clocks offer a potentially powerful approach for testing such evolutionary hypotheses (2), but recent analyses have yielded conspicuously discordant results. Pisani *et al.* (3), for example, estimate that the protostome–deuterostome ancestor evolved 900–1,100 Ma, whereas Douzery *et al.* (4) place this node at 642–761 Ma and Peterson *et al.* (5) at ≈570 Ma. With estimated divergence times differing by >500 million years (myr), there is clearly a need to assess both the methods used and predictions made by individual molecular-clocks analyses.

Pisani *et al.* (3) have argued that their deep estimate for metazoan origins is robust because it agrees with a previous analysis calibrated by using different taxa, i.e., between chick and mouse (6) vs. centipedes and millipedes and spiders and horseshoe crabs (3). However, Peterson *et al.* (5) demonstrated that a significant rate reduction is associated with the vertebrate sequences, such that a vertebrate-calibrated clock produces a spurious 2-fold overestimate of invertebrate divergence times. A similar calibration artifact is also associated with the chelicerate and myriapod calibrations; the branch lengths leading to each of these four taxa are similar to those leading to the fly and mosquito [as assessed by maximum likelihood (ML) analysis], despite a Triassic divergence for the dipterans, a Silurian divergence for the myriapods, and an Ordovician divergence for the chelicerates (Fig. 1). Pisani *et al.*'s (3) analysis does indeed pass a relative rate test but only by ignoring well established palaeontological data points.

The Douzery *et al.* (4) and Peterson *et al.* (5) algorithms yield more convergent, but far from identical, predictions about early animal evolution. The differences arise primarily from how branch lengths are estimated; whereas Peterson *et al.* (5) used distance methods [minimum evolution (ME)] with a Poisson distribution, Douzery *et al.* (4) have advocated likelihood methods (ML) that take into account rate heterogeneity and yield relatively deeper divergence times (5, 7). To arbitrate between these two approaches, we propose returning to the fossil record, on the assumption that the ecological impact of early animal evolution could not have entirely evaded paleontological detection; in other words, at least some molecular clock hypotheses should be testable by reference to the Proterozoic rock record.

Of the many innovations accompanying early metazoan evolution, probably the most revolutionary was the appearance of eumetazoans from poriferan-grade ancestors. There is a strong, if not yet watertight (8, 9), case for recognizing the Porifera as paraphyletic, such that sponges with calcareous skeletons (calcisponges) are more closely related to eumetazoans (ctenophores, cnidarians, and triploblasts) than to sponges with siliceous skeletons (silicisponges: demosponges, and hexactinellids) (e.g., refs. 10–12; reviewed in refs. 13 and 14). If this is the case, then the presence of a water-canal system and choanocytes are shared-primitive characters of Metazoa and not shared-derived characters of Porifera (13, 14). Thus, because of design constraints (15, 16), the last common ancestor of both calcisponges + eumetazoans and metazoans would have been limited to a diet of bacteria-sized picoplankton and dissolved organic carbon (17). It was only with the acquisition of a eumetazoan nervous system and gut that animals began to feed on eukaryotes, a habit established (at the latest) in the last common ancestor of cnidarians and bilaterians. In this study, we demonstrate that the sponges are indeed paraphyletic and provide both ME and ML molecular clock estimates for the two nodes bracketing the evolution of Eumetazoa (i.e., the calcisponge/eumetazoan and cnidarian/bilaterian divergences). Given the profound ecological/evolutionary impact that must have accompanied the appearance of motile macrophagous metazoans, we argue that the test for these two competing molecular clock algorithms lies in

Abbreviations: DPM, Doushantuo–Pertatataka microbiota; Fm, formation; Ma, million years ago; ME, minimum evolution; ML, maximum likelihood; myr, million years.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. DQ087437–DQ087507).

[‡]To whom correspondence may be addressed. E-mail: kevin.j.peterson@dartmouth.edu or njb1005@esc.cam.ac.uk.

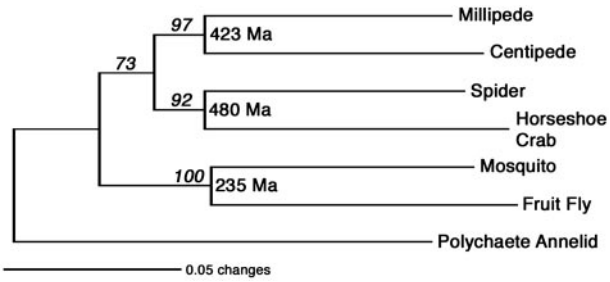

Fig. 1. ML analysis of a concatenated sequence of enolase and glyceraldehyde-3-phosphate dehydrogenase (668 amino acids) from six different arthropods by using the polychaete annelid *Nereis* as the outgroup. The branches leading to each of the six different arthropod taxa are similar, despite a Triassic divergence for the two dipertans and Ordovician and Silurian divergences for the chelicerates and myriapods, respectively. Hence, calibrating a molecular clock analysis to the myriapod and/or chelicerate divergence will result in spurious overestimates of divergence times between deuterostomes and protostomes, given the similar rate of molecular evolution between dipterans, echinoderms, and molluscs (5).

recognizing the coevolutionary impact of animals on contemporaneous, but more readily preservable, organisms.

Materials and Methods

The molecular methods are those used in Peterson *et al.* (5). *Crepidula fornicata*, *Amphiporus angulatus*, *Lineus viridis*, *Cerebratulus lacteus*, *Scypha lingua*, *Leucosolenia sp.*, and *Microciona prolifera* were purchased from the Marine Biological Laboratory (Woods Hole, MA). *Nereis vexillosa* was collected by K. Halanych (Auburn University, Auburn, AL) in False Bay, Friday Harbor, WA. *Haliotis rufenscens* was purchased from the Cultured Abalone (Santa Barbara, CA). *Trochospongilla pennsylvanica* was collected by J. Addis (Carroll College, Helena, MT) from Mud Pond, NH (43°48′N/72°05′W).

ML used QUARTET PUZZLE V. 5.0 (18) with the VT matrix of amino acid substitution; distance and parsimony analyses used PAUP* V. 4.0b10 (19). Distance analysis used mean character difference for the standard distances and ME for the objective function; we note that the branch lengths are similar to those found with MEGA V. 2.1 (20) by using pairwise deletion and a Poisson correction. The maximum parsimony (MP) analysis used the option of heuristic search with tree–bisection–reconnection. The heuristic search used random addition sequence (100 replications) to estimate the best tree for both MP and ME. Bootstrap values were derived from 1,000 replications, and 1,000 puzzling steps were performed. The ML analysis in Fig. 2 used the topology as found with ME as a constraint tree (i.e., likelihood mapping analysis).

Date estimates for uncalibrated nodes were derived by using the software package R8S V. 1.5 (M. J. Sanderson, http://ginger.ucdavis.edu/r8s). Echinoderm, insect, and bivalve calibrations are listed in Peterson *et al.* (5); the 530-Ma divergence between bivalves and gastropods is from Pojeta (21), and the 500-Ma divergence between the vetigastropod *Haliotis* and the

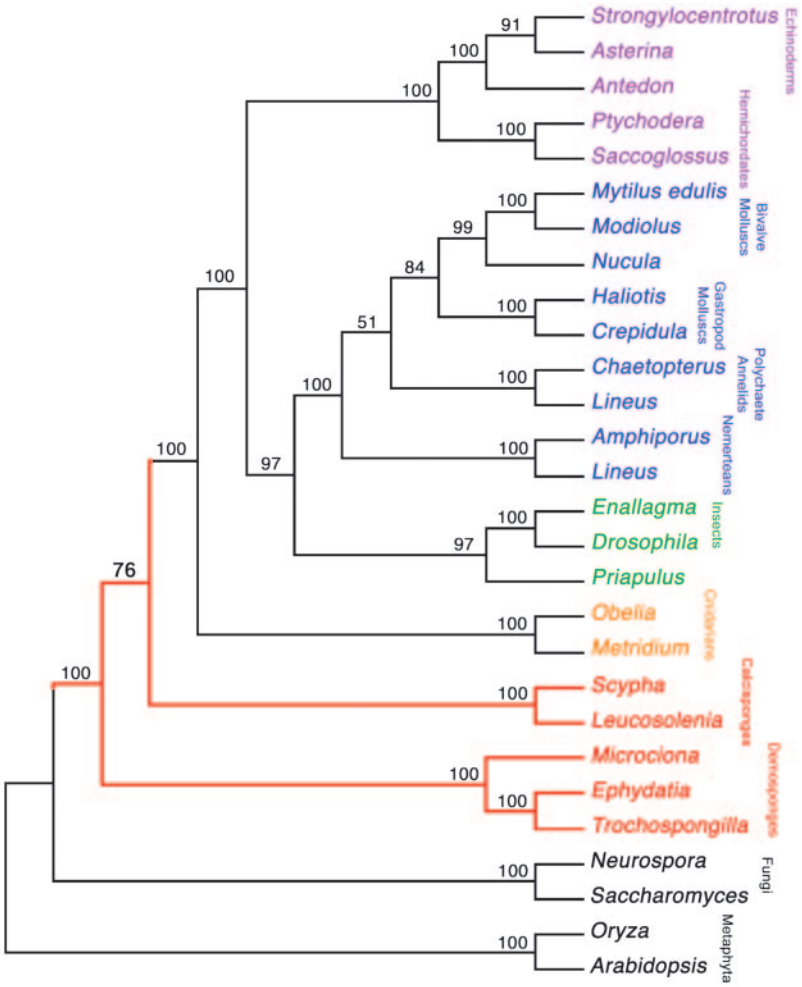

Fig. 2. Maximum parsimony analysis of a total-evidence data set consisting of 2,039 amino acids derived from the seven different housekeeping genes used in Fig. 2, 228 amino acids from the cytochrome oxidase I gene, 1,747 nucleotides from the 18S rDNA gene, and 150 morphological characters coded for the genus where possible, for 24 metazoan taxa by using two fungal and two plant taxa as outgroups (see *Appendices* 1–3, which are published as supporting information on the PNAS web site). Purple taxa are the ambulacrarians (echinoderms + hemichordates), blue taxa are the spiralian protostomes (molluscs, annelids, and nemerteans), green taxa are the ecdysozoans (insects and priapulid), orange taxa are the cnidarians, and red taxa are the sponges. This is one of two trees at 10,765 steps (number of parsimony informative characters, 1,889); consistency index = 0.54; retention index = 0.52; rescaled consistency index = 0.28. Bootstrap values are derived from 1,000 replicates. Note that Porifera is paraphyletic, with calcisponges more closely related to eumetazoans than to demosponges. Because of the clear homology between the water-canal systems of calcisponges and silicisponges (15, 16), sessile microsuspension feeding must have evolved sometime before the last common ancestor of metazoans (13. 14) and lost sometime before the last common ancestor of eumetazoans (indicated by the red line).

sorbeoconch *Crepidula* is from Lindberg and Guralnick (22). Because of the difficulty in assigning maximum values to divergence times based on an incomplete fossil record, all calibrations were fixed as minima (contrary to ref. 23; see ref. 5 for further details). We note, however, that setting the maximum of each of our calibrated nodes at 1.5 billion years increases the protostome–deuterostome ancestor from 579 to just 646 Ma, still 100 myr younger than estimated by Blair and Hedges (23). This discrepancy cannot be explored further due to the absence of methods provided (23).

Results

The Paraphyly of Porifera. Contrary to the results derived from most ribosomal sequence analyses (10–13), sponge monophyly is always realized when coding only morphological characters (24, 25). Thus, we asked whether paraphyly would still be realized from a maximum parsimony analysis of a total-evidence matrix consisting of 2,039 amino acids derived from seven different nuclear-encoded housekeeping genes, 228 amino acids from the cytochrome oxidase I gene, 1,747 nucleotides from the *18S rDNA* gene, and 150 morphological characters coded for the genus where possible (see *Supporting Text* for the character matrix, character descriptions, and accession numbers) for 23 metazoan taxa including three demosponges and two calcisponges (Fig. 2). All expected relationships (13, 14) are recovered with high precision, including the monophyly of the three major groups of triploblasts [ambulacrarian deuterostomes (echinoderms and hemichordates, purple), spiralians (annelids, molluscs, and nemerteans, blue), and ecdysozoans (insects and the priapulid, green)], Protostomia, Triploblastica, Eumetazoa, and Metazoa, as well as Calcispongia, Demospongia, and the two freshwater demosponges (*Ephydatia cooperensis* and *T. pennsylvanica*) (Fig. 2). Importantly, this total-evidence analysis suggests with moderate precision (75%) that Porifera (red) is paraphyletic; the two calcisponges are more closely related to the eumetazoans than to the three demosponges, despite the inclusion of several putative morphological synapomorphies for Porifera (*Appendices 2* and *3*). This result implies that the last common ancestor of both metazoans and calcareous sponges + eumetazoans had a water-canal system, and that both would have fed intracellularly on free organic matter and demersal bacteria, as both groups of sponges still do today (15, 16).

We also asked whether the paraphyly of sponges would be realized from a phylogenetic (ME) analysis of only the nuclear-encoded amino acid sequences. Again, where known (13, 14), the topology is accurate and, for most nodes, precise (Fig. 3). Significantly, sponges (shown in red) are not monophyletic; the two calcisponges group with the eumetazoans with moderate precision (78%), whereas the three demosponges are monophyletic and basal to the Calcispongia + Eumetazoa clade. Although not strongly supported, the congruence between this analysis and the numerous (and independent) ribosomal sequence analyses (e.g., refs. 10–13), as well as the total-evidence analysis (Fig. 2), is compelling evidence for the paraphyly of Porifera.

Dating the Origin of Eumetazoa. Given the paraphyly of "Porifera," the maximal age for eumetazoans, and thus eumetazoan characters such as the gut and nervous system, is the divergence between calcisponges and eumetazoans. Because the housekeeping genes used in Figs. 2 and 3 evolved in a clock-like fashion in echinoderms, molluscs, and insects (5), this divergence can potentially be addressed by using these protein sequences. Fig. 3 shows the 12 calibration points (black boxes) and the divergence estimates (white boxes) as determined by ME (Fig. 3 top) and ML (Fig. 3 bottom). The regression coefficient between distance and time for both analyses is >0.96. In addition, both analyses give reasonable estimates for nodes where a meaningful comparison to the fossil record can be made, e.g., the paleontological estimate for the origin of freshwater sponges (*Trochospongilla* and *Ephydatia*) is Early Jurassic (≈150 myr; ref. 16), and ME estimates this divergence at 183 Ma, whereas ML estimates it at 141 Ma. The paleontological estimate for the origin the calcaronean sponges (*Scypha* and *Leucosolenia*) is Early Carboniferous (≈340 myr; ref. 16), and ME estimates this divergence at 366 Ma, whereas ML estimates it at 348 Ma.

In general, ML gives shallower estimates than ME for more recent divergences but much deeper estimates for ancient divergences (Fig. 3). Using ME, we date the origin of Metazoa at 664 Ma and find demosponge divergences to be relatively deep; e.g., *Microciona* diverges from the two freshwater species at 632 Ma, in good agreement with the post-"Sturtian" but pre-Ediacaran record of demosponge biomarkers (26).[¶] By contrast, the corresponding ML estimates for metazoan and demosponge divergence are 867 and 723 Ma, respectively. The ME and ML divergences between calcisponges and eumetazoans are estimated at 634 and 826 Ma, whereas the divergence between cnidarians and triploblasts is estimated at 604 and 748 Ma, respectively. Thus, the ME clock proposes that the eumetazoan apomorphies, including the presence of tissues, a nervous system, and a gut, evolved between 634 and 604 Ma, whereas the ML clock brackets them evolving between 826 and 748 Ma.

Incorporating the Proterozoic Fossil Record. The ME estimate of 634–604 Ma for the origin of eumetazoan characters places it in the early part of the newly established Ediacaran period (27), an unusually eventful interval of Earth history. The beginning of the Ediacaran, defined by the top of the Marinoan cap carbonate, is associated with the second of three globally expressed cycles of Neoproterozoic glaciation, the Sturtian [710–670 Ma (28)], Marinoan [635 Ma (29, 30)], and Gaskiers [580 Ma (31)]. Interestingly, it is also when animals make their first appearance in the fossil record, first as taxonomically unresolved embryos in rocks from the mid-upper Doushantuo Formation (Fm) of South China (32), inferred to be no younger than ≈580 Ma (30), followed by unambiguously eumetazoan trace fossils in the >558-Ma Verkhovka Fm of Northwest Russia (33, 34). Neither of these data points, however, can be used as an even approximate measure of first appearance, due to their dependence on exceptional preservation and/or macroscopic size (35).

The key to stratigraphic confidence at any size lies instead with fossils of relatively unexceptional preservation (35). In the Proterozoic, the "unexceptional" fossil record of eukaryotes is limited largely to acritarchs, organic-walled vesicular microfossils of unknown taxonomic affiliation, which are sufficiently common to serve as a proxy for major evolutionary trends. Most Proterozoic acritarchs are small nondescript spheroids of essentially unlimited stratigraphic range; however, beginning in the Mesoproterozoic, shallow-water assemblages are joined by a modest assortment of larger, more distinctive forms, including macroscopic *Tawuia* and *Chuaria* (36), concentrically ornamented *Valeria* (37, 38), and process-bearing (acanthomorphic) *Tappania* (refs. 38 and 39; Fig. 4*A*) and *Trachyhystrichosphaera* (ref. 40; Fig. 4*B*). Remarkably, these taxa exhibit stratigraphic ranges of hundreds of millions of years, neither going extinct nor giving rise to daughter species (41, 42). At least some of these acanthomorphs, e.g., *Trachyhystrichosphaera* and *Cymatiosphaeroides*, demonstrably survived the Sturtian glaciation (43), but none are known from the Ediacaran.

The early Ediacaran witnessed a major radiation of entirely novel acanthomorphic acritarchs, best known from the Doushantuo (44) and Pertatataka (45) microbiotas (DPM) (Fig. 4*C*). Like their

[¶]Love, G. D., Grosjean, E., Fike, D. A., Grotzinger, J. P., Bowring, S. A., Condon, D., Lewis, A. N., Stalvies, C., Snape, C. E. & Summons, R. E. (2005) 22nd International Meeting on Organic Geochemistry, September 12–16, 2005, Seville, Spain.

EVOLUTION

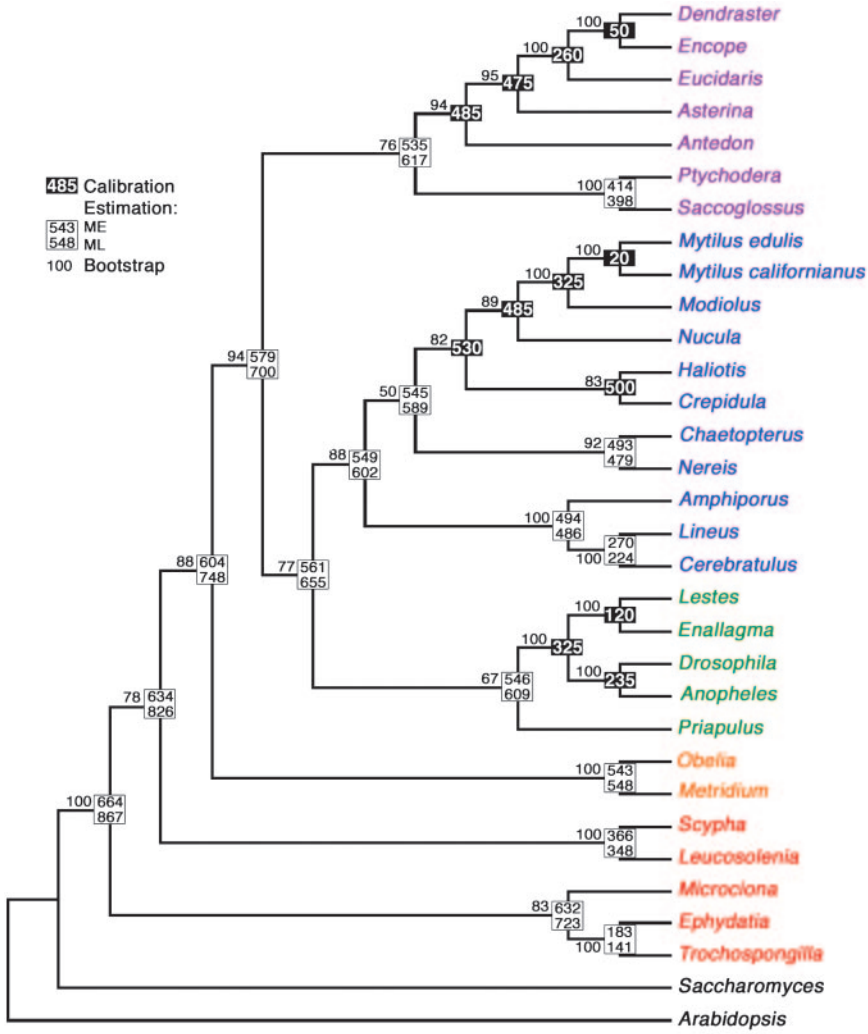

Fig. 3. Cladogram derived from ME analysis of the seven concatenated protein sequences from 29 metazoan taxa by using a fungus and a plant as outgroups. Color coding is the same as in Fig. 2. Numbers in black boxes are calibration points, and numbers in white boxes are molecular clock estimates derived from R8S V. 1.5; ME estimate is on the top and the ML estimate is on the bottom (see the key). Bootstrap values (1,000 replications) are given to the left of the boxes. Note that eumetazoan apomorphies (e.g., the gut) arose between 634 and 604 Ma according to ME but 826–748 Ma according to ML.

pre-Ediacaran counterparts, these DPM fossils are characteristically large and limited to shallow-water environments, suggesting a benthic habit (46). They differ fundamentally, however, in their diversity and evolutionary dynamics (41, 47). Knoll (41), for example, has calculated the total and per-taxon rates of first and last appearances in the early Ediacaran to exceed the highest pre-Ediacaran rates by ≈1 order of magnitude. This is almost certainly an underestimate, given that increased sampling has corroborated the limited age range of DPM acritarchs (635–580 Ma; e.g., refs. 44–48) but has exponentially expanded that of pre-Ediacaran acanthomorphs, to the extent that there is little, if any, biostratigraphic resolution in the billion-year interval leading up to the Ediacaran (37, 42). At the same time, acritarch form taxonomy has disproportionately inflated the true diversity of pre-Ediacaran biotas (see refs. 39 and 42), artificially blurring the distinction between pre-Ediacaran and DPM biotas.

The mutually exclusive distributions of pre- and post-Marinoan acanthomorphic acritarchs mark a fundamental shift in the Proterozoic biosphere, from an archaic world of limited morphological diversity and extreme evolutionary stasis to one of high diversity and rapid Phanerozoic-like turnover, notable in particular for the elimination of hitherto extinction-proof taxa. Indeed, this interval marks both the first resolvable extinction and radiation in the whole of the fossil record.

The coincidence of this biotic turnover with our ME molecular clock estimate for the evolution of eumetazoans is striking but so, too, is its correlation with Marinoan glaciation (48, 49) and the post-Marinoan Acraman impact (47). Identifying the causal connection lies in the correct interpretation of acritarch paleobiology. As an artificial group of nonmineralizing problematica, acritarchs clearly represent a disparate range of clades and habits but, by the same token, they offer an unusually comprehensive view of early eukaryotic life; apart from a handful of identified algae and amoebozoa (see ref. 39), acritarchs encompass the entire body-fossil record of pre-580-Ma eukaryotes. As far as the extinction of pre-Ediacaran forms is concerned, extreme physical/environmental perturbation provides a possible mechanism; however, the subsequent polyphyletic radiation of complex morphologies can be realistically understood only in terms of biological interaction and accompanying ecological/evolutionary feedback (42). We emphasize here that pre-Ediacaran and DPM biotas are entirely distinct both taxonomically and stratigraphically, ruling out the possibility of a Cretaceous–Tertiary-type scenario of ecological incumbency and replacement.

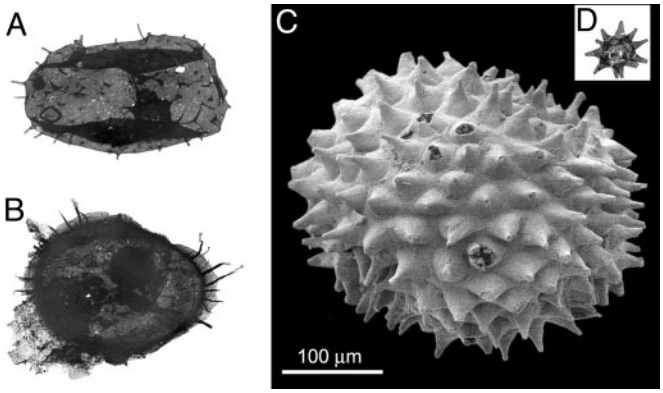

Fig. 4. Acanthomorphic acritarchs from before (*A* and *B*) and after (*C* and *D*) the Marinoan turnover. (*A*) *Tappania* sp. from the 850-Ma Wynniatt Fm, Northwestern Canada (39), but also known from ≈1,450-Ma (38) strata, giving it a ≈600-myr age range. (*B*) *Trachyhystrichosphaera aimica* from the 850-Ma Wynniatt Fm (39), but also known from 1,000-Ma (40) and post-Sturtian (43) strata, giving it a ≈350-myr age range. (*C*) *Meghystrichosphaeridum chadianesis* from the Doushantuo Fm, Southern China; age range, <55 my. [Image courtesy of Shuhai Xiao (Virginia Polytechnic Institute and State University, Blacksburg, VA).] (*D*) Unidentified Lower Cambrian acanthomorph, which, as a class, have a mean age range of 7.7 my (41); note the fundamentally smaller dimensions relative to the Precambrian acanthomorphs, indicative of a planktic habit. (Scale bar in *C* applies to all images.)

Biologically induced polyphyletic radiation is a conspicuous feature of the Phanerozoic record, including the Early Cambrian "explosions" of biomineralization (50) and phytoplankton. In the latter case, the sudden appearance of diverse rapidly evolving ornamentation after some billion years of morphological stasis has been convincingly ascribed to the evolution of herbivorous mesozooplankton (35, 46, 51). Spinose ornamentation is also a common adaptation among epibenthic organisms exposed to metazoan predation (e.g., refs. 52 and 53) and presents a compelling explanation for the early Ediacaran replacement of acritarch biotas, an adaptive response of newly vulnerable eukaryotes to the appearance of benthic eumetazoans. Such defensive responses are, of course, liable to be countered by coevolutionary adaptations in predators, leading to further morphological diversification and escalating evolutionary turnover (42, 52). We interpret the extreme stasis exhibited by pre-Ediacaran acritarchs as positive evidence for the absence of pre-Ediacaran eumetazoans, and the novel morphologies and Phanerozoic-like dynamics of DPM acritarchs, combined with the conspicuous extinction of pre-Ediacaran acanthomorphs, as positive evidence for their appearance at or around 635 Ma (30).

Discussion

The construction of accurate molecular clocks is contingent upon robustly supported calibration points from the fossil record and realistic assumptions and algorithms for addressing molecular data. Recent opinion has embraced parametric methods such as ML (e.g., ref. 4); however, there is a strong possibility that ML overestimates the lengths of internal branches (54), resulting in artificially deep divergence times. For accurate recovery of deep metazoan divergence times, we advocate methods that use multiple primary calibration points and analyze amino acid sequences without a γ correction. The suitability of this approach has been borne out in the present case; our molecular clock estimate of 634–604 Ma for the first appearance of motile macrophagous predators coincides conspicuously with the first documented turnover in the acritarch record, indeed the first documented turnover in the entire fossil record. Insofar as eumetazoans have a unique capacity to drive coevolutionary escalation (42, 52) and coincident extinction, such congruence presents a powerful case for the accuracy of our clock.

In addition to exploiting the coevolutionary responses to eumetazoan evolution, this palaeoecological test of our molecular clock requires robust stratigraphic constraints. Both pre-Ediacaran- and DPM-type acanthomorphic acritarchs exhibit near-global but mutually exclusive distributions in shallow marine facies, separated by the 635-Ma (29, 30) Marinoan glaciation. The oldest DPM-type acritarchs first appear in the lower Doushantuo Fm (55), in association with an ash bed recently dated at 635–627 Ma (30, 56, 57), and disappear from the record before 580 Ma (48). We note that the appearance of the DPM precedes the first (direct) record of eumetazoan fossils by as much as 55 myr (30), a not-unexpected gap, given the taphonomic challenges of preserving small nonbiomineralizing metazoans. By contrast, the plant protists and other eukaryotes with which they interacted have fundamentally greater preservation potential and provide a much more precise estimate of their first appearance (see ref. 35).

Recognizing the Ediacaran acritarch turnover as the consequence of newly introduced eumetazoans adds substantially to the resolution of early metazoan evolution and its relationship to Proterozoic earth history. For example, the development of significant spinose ornamentation in predominately benthic (46, 51) DPM-type acritarchs, whereas contemporaneous phytoplankton remain unornamented until the Early Cambrian, suggests that novel predation pressures first appeared in the shallow-water benthos. This in turn points to the benthos as the site of early eumetazoan evolution (ref. 17 and refs. therein).

Shallow temporal origins for the Eumetazoa also undermine the case for oxygen as the principal control on the Cambrian explosion (e.g., ref. 58). In this view, eumetazoans are seen as having diverged early, but constrained to small size because of the elevated oxygen demand of large energetic organisms. Both the microfossil record and our molecular clock, however, point to a genuine absence of pre-Ediacaran eumetazoans. Even small eumetazoans are expected to drive morphological coevolution in associated (eukaryotic) organisms, but such a pattern is demonstrably absent before the DPM.

Insofar as our molecular clock provides an accurate estimate for the origin of Eumetazoa by using the radiation of acanthomorphic acritarchs as a proxy, it is worth interrogating it at other key points, particularly where these offer further coevolutionary tests. After the appearance of eumetazoans, probably the most significant development in metazoan ecology/evolution was the appearance of triploblastic metazoans, which, according to our ME clock, occurred by ≈580 Ma. Intriguingly, 580 Ma also marks the first [and taphonomically unexceptional (35)] appearance of Ediacaran-type macrofossils (59). Although they were certainly not bilaterians themselves, it is worth considering these early Ediacaran organisms as the consequence of novel ecological challenges, their macroscopic dimensions potentially representing a size-refuge response to the introduction of small but ecologically disruptive triploblasts. The value of our corroborated molecular clock in this context is that it proposes such testable hypotheses and offers reliable estimates for divergences that may not have been accompanied by ecosystem-wide changes (e.g., the origin of metazoans themselves).

Conclusion

Molecular clocks make predictions about events in the fossil record and, for major ecological innovations, should be testable against the record. Eumetazoans have a profound ecological and evolutionary impact on the organisms around them, not least coevolutionary escalation leading to large size, high diversity, and rapid evolutionary turnover. Thus, the most reliable measure for the first appearance of (difficult to preserve) eumeta-

zoans will occur in the (easy-to-preserve) record of plant protists, etc., with which they interacted. The conspicuous stasis exhibited by pre-Ediacaran acritarch assemblages does not accord with the presence of eumetazoans, even small eumetazoans, and thus stands as *bona fide* evidence of absence, whereas the early Ediacaran shift to diverse rapidly evolving DPM biotas provides compelling evidence of presence. In this light, the Pisani *et al.* (3) hypothesis fails the test by ≈500 myr, whereas Douzery *et al.* (4) miss the mark by at least 50 myr. The consilience of our molecular clock results and independent fossil data rejects the hypothesis of pre-Ediacaran crown-group eumetazoans and suggests that the modern biosphere was initiated by the evolution of the eumetazoan gut and nervous system in the early Ediacaran.

We thank S. Xiao for Fig. 4*C* and helpful discussion. We also extend our gratitude to J. Lyons for technical assistance, M. McPeek for analytical assistance and discussion, K. Halanych for the kind gift of *Nereis* material, J. Addis for collecting *Trochospongilla*, and Yin Chongyu for discussion of Doushantuo acritarch distribution. We also thank S. Conway Morris, D. Erwin, A. Knoll, one anonymous referee, and especially E. Davidson for useful comments on an early draft. K.J.P. is supported by the National Science Foundation, National Aeronautical and Space Administration–Ames, and Dartmouth College. This is Cambridge Earth Sciences contribution 8128.

1. Runnegar, B. (1982) *J. Geol. Soc. Aust.* **29,** 395–411.
2. Runnegar, B. (1982) *Lethaia* **15,** 199–205.
3. Pisani, D., Poling, L. L., Lyons-Weiler, M. & Hedges, S. B. (2004) *BMC Evol. Biol.* **2,** 1–10.
4. Douzery, E. J. P., Snell, E. A., Bapteste, E., Delsuc, F. & Philippe, H. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 15386–15391.
5. Peterson, K. J., Lyons, J. B., Nowak, K. S., Takacs, C. M., Wargo, M. J. & McPeek, M. A. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 6536–6541.
6. Wang, D. Y.-C., Kumar, S. & Hedges, S. B. (1999) *Proc. R. Soc. Lond. Ser. B* **266,** 163–171.
7. Bromham, L., Rambaut, A., Fortey, R., Cooper, A. & Penny, D. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 12386–12389.
8. Adams, C. L., McInerney, J. O. & Kelly, M. (1999) *Mem. Queensl. Mus.* **44,** 33–43.
9. Manuel, M., Borchiellini, C., Alivon, E., Le Parco, Y., Vacelet, J. & Boury-Esnault, N. (2003) *Syst. Biol.* **52,** 311–333.
10. Cavalier-Smith, T., Allsopp, M. T. E. P., Chao, E. E., Boury-Esnault, N. & Vacelet, J. (1996) *Can. J. Zool.* **74,** 2031–2045.
11. Medina, M., Collins, A. G., Silberman, J. D. & Sogin, M. L. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 9707–9712.
12. Cavalier-Smith, T. & Chao, E. E.-Y. (2003) *J. Mol. Evol.* **56,** 540–563.
13. Eernisse, D. J. & Peterson, K. J. (2004) in *Assembling the Tree of Life*, eds. Cracraft, J. & Donoghue, M. J. (Oxford Univ. Press, Oxford), pp. 197–208.
14. Halanych, K. M. (2004) *Annu. Rev. Ecol. Syst.* **35,** 229–256.
15. Brusca, R. C. & Brusca, G. J. (2002) *Invertebrates* (Sinauer, Sunderland, MA), 2nd Ed.
16. Finks, R. M., Reid, R. E. H. & Rigby, J. K. (2004) *Treatise on Invertebrate Paleontology, Part E, Porifera* (Geological Society of America, Boulder, CO), Rev. Ed., Vol. 3.
17. Peterson, K. J., McPeek, M. A. & Evans, D. A. D. (2005) *Paleobiology*, in press.
18. Strimmer, K. & von Haeseler, A. (1996) *Mol. Biol. Evol.* **13,** 964–969.
19. Swofford, D. L. (2002) PAUP* *Phylogenetic Analysis Using Parsimony (* and Other Methods) V. 4.0b10 for Macintosh* (Sinauer, Sunderland, MA).
20. Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001) MEGA2: *Molecular Evolutionary Genetics Analysis V. 2.1* (Arizona State University, Tempe).
21. Pojeta, J. J. (2000) *Am. Malacol. Bull.* **15,** 157–166.
22. Lindberg, D. R. & Guralnick, R. P. (2003) *Evol. Dev.* **5,** 494–507.
23. Blair, J. E. & Hedges, S. B. (2005) *Mol. Biol. Evol.* **22,** 387–390.
24. Zrzavy, J., Mihulka, S., Kepka, P., Bezdek, A. & Tietz, D. (1998) *Cladistics* **14,** 249–285.
25. Peterson, K. J. & Eernisse, D. J. (2001) *Evol. Dev.* **3,** 170–205.
26. McCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A. & Watt, D. S. (1994) *Geochim. Cosmochim. Acta* **58,** 529–532.
27. Knoll, A. H., Walter, M. R., Narbonne, G. M. & Christie-Blick, N. (2004) *Science* **305,** 621–622.
28. Fanning, C. M. & Link, P. K. (2004) *Geology* **32,** 881–884.
29. Hoffmann, K. H., Condon, D. J., Bowring, S. A. & Crowley, J. L. (2004) *Geology* **32,** 817–820.
30. Condon, D., Zhu, M., Bowring, S., Wang, W., Yang, A. & Jin, Y. (2005) *Science* **308,** 95–98.
31. Bowring, S., Myrow, P., Landing, E., Ramezani, J. & Grotzinger, J. (2003) *Geophys. Res. Abstr.* **5,** 13219.
32. Xiao, S., Zhang, Y. & Knoll, A. H. (1998) *Nature* **391,** 553–558.
33. Martin, M. W., Grazhdankin, D. V., Bowring, S. A., Evans, D. A. D., Fedonkin, M. A. & Kirschvink, J. L. (2000) *Science* **288,** 841–845.
34. Grazhdankin, D. (2004) *Paleobiology* **30,** 203–221.
35. Butterfield, N. J. (2003) *Int. Comp. Biol.* **43,** 166–177.
36. Maithy, P. K. & Babu, R. (1988) *J. Geol. Soc. India* **31,** 584–590.
37. Hofmann, H. J. (1999) *Acta Micropalaeont. Sinica* **16,** 215–224.
38. Javaux, E. J., Knoll, A. H. & Walter, M. R. (2001) *Nature* **412,** 66–69.
39. Butterfield, N. J. (2005) *Paleobiology* **31,** 165–182.
40. Samulsson, J. & Butterfield, N. J. (2001) *Precamb. Res.* **107,** 235–251.
41. Knoll, A. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6743–6750.
42. Butterfield, N. J. (2004) *Paleobiology* **30,** 231–252.
43. Kaufman, A. J., Knoll, A. H. & Awramik, S. M. (1992) *Geology* **20,** 181–185.
44. Zhang, Y., Yin, L., Xiao, S. & Knoll, A. H. (1998) *Paleont. Soc. Mem. 50* **72,** 1–52.
45. Zang, W. & Walter, M. R. (1992) *Association of Australasian Palaeontologists Memoir* **12,** 1–132.
46. Butterfield, N. J. (1997) *Paleobiology* **23,** 247–262.
47. Grey, K., Walter, M. R. & Calver, C. R. (2003) *Geology* **31,** 459–462.
48. Xiao, S. (2004) in *The Extreme Proterozoic: Geology, Geochemistry, and Climate*, eds. Jenkins, G., McMenamin, M., Sohl, L. & McKay, C. (Geophysical Monograph Series), Vol. 146, pp. 199–214.
49. Hoffman, P. F. & Schrag, D. P. (2002) *Terra Nova* **14,** 129–155.
50. Vermeij, G. J. (1989) *Palaios* **4,** 585–589.
51. Butterfield, N. J. (2001) in *The Ecology of the Cambrian Radiation*, eds. Zhuravlev, A. Y. & Riding, R. (Columbia Univ. Press, New York), pp. 200–216.
52. Vermeij, G. J. (1993) *Evolution and Escalation: A Ecological History of Life* (Princeton Univ. Press, Princeton).
53. Harper, E. M. & Skelton, P. W. (1993) *Scripta Geologica, Special Issue* **2,** 127–153.
54. Kolaczkowski, B. & Thornton, J. W. (2004) *Nature* **431,** 980–984.
55. Yin, C. (1999) *Continental Dynamics* **4,** 1–18.
56. Zhang, S., Jiang, G., Zhang, J., Song, B., Kennedy, M. J. & Christie-Blick, N. (2005) *Geology* **33,** 473–476.
57. Yin, C., Tang F., Liu, Y., Gao, L., Liu, P., Xing, Y., Yang, Z., Wan, Y. & Wang, Z. (2005) *Episodes* **28,** 48–49.
58. Knoll, A. H. (2003) *Life on a Young Planet* (Princeton Univ. Press, Princeton).
59. Narbonne, G. M. & Gehling, J. G. (2003) *Geology* **31,** 27–30.