Poriferan paraphyly and its implications for Precambrian palaeobiology

E. A. SPERLING[1], D. PISANI[2] & K. J. PETERSON[3]

[1]*Department of Geology and Geophysics, Yale University, P.O. Box 208109, New Haven, CT 06520, USA*

[2]*Laboratory of Evolutionary Biology, The National University of Ireland, Maynooth, County Kildare, Ireland*

[3]*Department of Biological Sciences, Dartmouth College, Hanover, NH 03755, USA (e-mail: kevin.peterson@dartmouth.edu)*

Abstract: Well-supported molecular phylogenies, combined with knowledge of modern biology, can lead to new inferences about the sequence of character acquisition in early animal evolution, the taxonomic affinity of enigmatic Precambrian and Cambrian fossils, and the Proterozoic Earth system in general. In this paper we demonstrate, in accordance with previous molecular studies, that sponges are paraphyletic, and that calcisponges are more closely related to eumetazoans than they are to demosponges. In addition, our Bayesian analysis finds the Homoscleromorpha, previously grouped with the demosponges, to be even more closely related to eumetazoans than are the calcisponges. Hence there may be at least three separate extant 'poriferan' lineages, each with their own unique skeleton. Because spiculation is convergent within 'Porifera', differences between skeletonization processes in enigmatic Cambrian taxa such as *Chancelloria* and modern sponges does not mean that these Problematica are not organized around a poriferan body plan, namely a benthic, sessile microsuspension feeding organism. The shift from the anoxic and sulphidic deep ocean that characterized the mid-Proterozoic to the well-ventilated Phanerozoic ocean occurs before the evolution of macrozooplanton and nekton, and thus cannot have been caused by the advent of faecal pellets. However, the evolution and ecological dominance of sponges during this time interval provides both a mechanism for the long-term generation of isotopically-light CO_2 that would be recorded in carbon isotopic excusions such as the 'Shuram' event, and an alternative mechanism for the drawdown and sequestration of dissolved organic carbon within the sediment.

The 'explosion' of animals and protist groups near the base of the Cambrian remains one of the most complex and important questions in historical biology. The heart of the debate is focused on timing: Is the fossil record a faithful chronicle of events, with the origin of clades closely predating their appearance, or does the event simply record the appearance of burrowing and biomineralizing organisms whose stocks diverged deep in the Precambrian (Runnegar 1982)? The ancestors of the Cambrian metazoan fauna undoubtedly existed in the Precambrian, and the search for Precambrian ancestors has focused primarily on the soft-bodied Ediacaran faunas of Newfoundland, South Australia, Russia and Namibia (Gehling 1991; Narbonne 1998, 2005). Although these faunas have traditionally been interpreted through direct morphological comparisons with the modern biota, interpretations need to be more tightly constrained by insights gained from both phylogenetic studies of the Metazoa and ecological considerations of modern taxa.

Although a clearer view of triploblast systematics is emerging, the relationships at the base of the metazoan tree are still largely unknown (Eernisse & Peterson 2004; Halanych 2004). Studies using different markers place sponges, placozoans, cnidarians and ctenophores in almost every conceivable relationship except one: monophyly—all studies (except for some early analyses with limited taxon sampling) unequivocally agree that ctenophores and cnidarians are more closely related to triploblasts than they are to sponges. Recent studies using protein-coding genes from mitochondrial genomes (e.g. Wang and Lavrov 2007, and references therein) recover 'lower' Metazoa as monophyletic, although the authors attribute this to a clear artifact related to rate changes between triploblast and the 'lower' metazoans. Sponges, which are traditionally regarded as the most basal extant metazoans, are always monophyletic in phylogenetic studies based on morphology alone (Zrzavy *et al.* 1998; Peterson & Eernisse 2001). However, analyses of ribosomal data (Cavalier-Smith *et al.* 1996; Collins 1998; Adams *et al.* 1999; Borchiellini *et al.* 2001; Medina *et al.* 2001; Cavalier-Smith & Chao 2003; Manuel *et al.* 2003; Wallberg *et al.* 2004) as well as protein

From: VICKERS-RICH, P. & KOMAROWER, P. (eds) *The Rise and Fall of the Ediacaran Biota.* Geological Society, London, Special Publications, **286**, 355–368.
DOI: 10.1144/SP286.25 0305-8719/07/$15.00

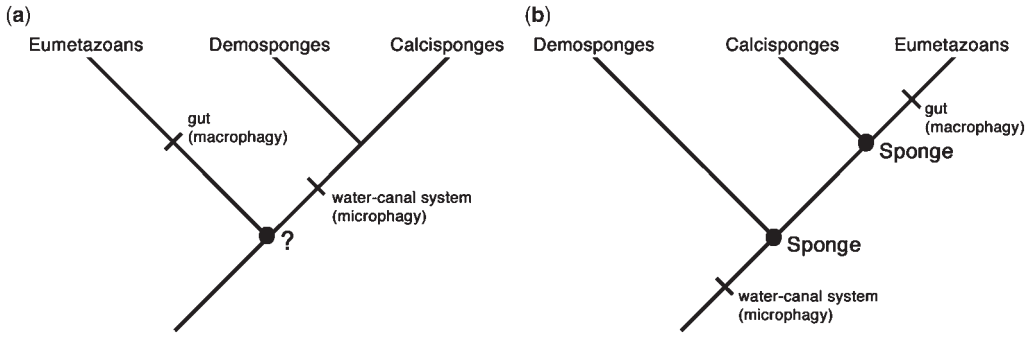


Fig. 1. The importance of paraphyly: if the eumetazoans and poriferans both represent monophyletic groups, each with a unique trophic mode, it is not possible to polarize feeding strategy, given that the outgroups are all non-metazoan (**a**). However, if calcisponges are more closely related to eumetazoans than demosponges, polarity can be established (**b**), suggesting the water-canal system is primitive and the gut is derived.

sequence data (Kruse *et al.* 1998; Peterson & Butterfield 2005), suggested that sponges are paraphyletic, with calcisponges more closely related to eumetazoans than to demosponges. The demonstration of poriferan paraphyly represents one of the most important insights that molecular systematics has given palaeontology because paraphyly means that former sponge synapomorphies (shared derived characters, e.g. water canal system [WCS] are metazoan symplesiomorphies (shared ancestral characters) (Peterson & Butterfield 2005; Peterson *et al.* 2005). Poriferan paraphyly then gives insight into the biology and ecology of the last common ancestor of all living metazoans, because we can now state with confidence that the earliest crown-group metazoans were benthic, sessile, microsuspension feeders that extracted dissolved organic matter and picoplankton out of sea water using the WCS (Fig. 1).

Nonetheless, the importance of poriferan paraphyly extends beyond these palaeoecological insights, as it sheds light on interpreting the phylogenetic affinities of Ediacaran organisms, as well as Cambrian 'Problematica' including *Chancelloria*. In addition, sponges provide an alternative mechanism for the oxidation of the Proterozoic ocean. Here, sponge paraphyly is explored by analysing with Bayesian phylogenetics a concatenated data set consisting of seven protein sequences from 30 eumetazoan and 12 sponge taxa including one homoscleromorph. Paraphyly is again found, with the interesting result that the homoscleromorph *Oscarella* is more closely related to the eumetazoans (cnidarians and triploblasts) than it is to any other sponge lineage. We then explore the palaeobiological implications of this result and argue that poriferan paraphyly gives insight into several disparate areas of Precambrian and Cambrian palaeobiology.

Material and methods

Although the Peterson & Butterfield (2005) tree contained several exemplars of both demosponges and calcisponges, the taxonomic coverage within these groups was not widespread—the two included calcisponges group closely within the Calcaronea (Manuel *et al.* 2003), and the three demosponges group in the G4 clade of Borchiellini *et al.* (2004: this study found four main demosponge clades, which they labelled G1–G4). Although we were unable to obtain any calcinean calcisponges, we were able to analyse two G1 sponges, *Darwinella mulleri* and *Dysidea camera*, the G2 *Chondria* sp., as well as the putative G3 sponge *Xestospongia* sp. (putative because, although not analysed by Borchiellini *et al.* 2004, it groups with the G3 *Haliclona* in Nichols 2005) and the G4 sponge *Halichondria* sp. All were purchased from Gulf Specimens Marine Laboratory (Panacea, Florida); except for *Halichondria*, which was purchased from the Marine Biological Laboratory (Woods Hole, MA). Total RNA from these taxa was prepared from live animals by using a one-step TRIzol method (GIBCO-BRL). Total RNA from the homoscleromorph *Oscarella carmela* was kindly provided by Dr Scott Nichols (University of California, Berkeley). cDNA synthesis was performed with RETROSCRIPT (Ambion, Austin, TX) using 1–2 μg of total RNA.

Partial sequences of seven nuclear-encoded genes were PCR amplified, cloned, and sequenced using standard techniques: aldolase (ALD), ATP synthase beta chain (ATPB), catalase (CAT), elongation factor 1-alpha (EF1a), methionine adenosyltransferase (MAT), phosphofructokinase (PFK), and triosephosphate isomerase (TPI). Primer sequences are as follows (5′-3′): ALDf: GGGAARGGNATH YTNGCNGC; ALDr: GGGGTNACCATRTTNG

GYTT; ATPBf: GTNGAYGT NCARTTYGAYGA; ATPBr: NCCNACCATRTARAANGC; CATf: GAYGARATGDSNCAYTTYGAYMG; CATr: CCNARNCKRTGNMDRTGNGTRTC; EF1Af: AAYATYGTNGTNATYGGNCAYGT; EF1Ar: ACNGCNACNGTYTGNCKCATRTC; MATf: GGNGARGGNCAYCCNGAYAA; MATr: CCNGGNCKIARRTCRAARTT; PFKf: GAYWSNCARGGNATGAAYGC; PFKr: CCRCARTGNCKNCCCATNACYTC; TPIf: GGNGGNAAYTGGAARATGAAYGG; TPIr: GCNCCNCCNACIARRAANCC. Gene-specific primers (50 pmol) and 2 μL of cDNA, plus the *Amplitaq* system (using the 10× buffer with 15 mM MgCl2, Applied Biosystems) were mixed and used in a touchdown style PCR. The first touchdown (TD 1) procedure started the annealing temperature at 52°C and then after two cycles dropped one degree for another two cycles, all the way to 40°C, followed by a final ten cycles at 52°C. A second touchdown procedure using 35 cycles at 52°C using 1 μL of the TD1 as template followed if the genes of interest were not amplified during TD1. PCR fragments of the predicted sizes were excised, purified (Qiagen, Valencia, CA), ligated at 16°C overnight into the pGEM-T-Easy vector according to manufacturer's instructions (Promega, Madison, WI), and electroporated into DH10B cells. Clones containing the correct insert size were sequenced with an ABI373 model sequencer according to manufacturer's instructions (Applied Biosystems, Foster City, CA). We were unable to obtain MAT from *Dysidea* and *Halichondria*, and PFK from *Xestospongia* and *Chondrilla*.

Sequences for the demosponge *Amphimedon queenslandica* (formerly *Reniera*, see Hooper & Van Soest 2006) another putative G3 taxon (again not analysed by Borchiellini *et al.* 2004, but traditionally classified as a chalinid haplosclerid closely related to *Haliclona*), as well as the anthozoan cnidarian *Nematostella vectensis*, the chordates *Branchiostoma floridae* and *Ciona intestinalis*, and the polychaete annelid *Capitella* sp. were downloaded from genomic traces from the NCBI Genbank database. Sequences were edited, translated, and aligned by using MACVECTOR, v. 7.2.3 (Genetics Computer Group). Twelve different sponge lineages plus 30 eumetazoan lineages taken from Peterson *et al.* (2004) and Peterson & Butterfield (2005) and two outgroups were analysed using Bayesian phylogenetics. Bayesian analyses were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) under a mixed model, with the parameters of seven unlinked evolutionary models (one for each gene) independently estimated during tree search (including the best fitting amino acid substitution matrix). Two independent runs of four linked MCMC chains were set up, sampling the chains every 1000 cycles. Convergence was monitored by controlling the standard deviation of the split sequences (Ronquist *et al.* 2005). The results of the two Bayesian runs were summarized by calculating a majority rule consensus of all trees sampled after stationarity was reached.

Results and discussion

Poriferan systematics

A Bayesian analysis was completed for the 12 sponge taxa plus the 30 eumetazoan taxa and the two outgroups (Fig. 2). All expected higher-metazoan relationships (e.g. Metazoa, Eumetazoa, Triploblastica, Protostomia, Ecdysozoa, Spiralia, Deuterostomia, Ambulacraria) (indicated with labelled nodes and the light grey boxes) are recovered, as are the expected internal relationships within phyla (e.g. Cnidaria, Echinodermata, Nemertea, Insecta). The recovery of known external nodes is important in sponge phylogenetics, as hypotheses regarding the phylum are undergoing such flux that there are few internal nodes that can confidently be used as an accuracy check for trees. The posterior probabilities for all nodes are indicated on Figure 2.

The addition of the new sponge taxa does not change the paraphyly of Porifera: calcisponges are more closely related to eumetazoans than they are to demosponges (Fig. 2, dark grey box). And similar to previous molecular studies (Borchiellini *et al*. 2004; Nichols 2005), the G1 clade of keratose sponges (which here includes *Darwinella* and *Dysidea*) is the sister taxon of the G3 + G4 sponges (Fig. 2). Unlike these previous analyses, though, we find strong support for a G1 + G2 (which here includes the taxon *Chondrilla*) clade (Fig. 2). In addition, our analysis does not support the monophyly of the G3 group as *Amphimedon* does not group with *Xestospongia* but instead groups with the two G4 freshwater sponges *Ephydatia* and *Trochospongilla*. Thus, the topology found with ribosomal DNA analyses (Borchiellini *et al.* 2004; Nichols 2005) is partially supported with an independent data set.

Previous molecular studies based on ribosomal data (Borchiellini *et al.* 2004; Nichols 2005) placed the Homoscleromorpha in an unresolved polytomy that included Demospongiae, Calcarea, Ctenophora and Cnidaria. Wang & Lavrov (2007), using sequences from the complete mitochondrial genome of *Oscarella carmela*, recovered this taxon as the sister group of the other sequenced demosponges. As mentioned above, current mitochondrial trees recover a monophyletic 'Lower'

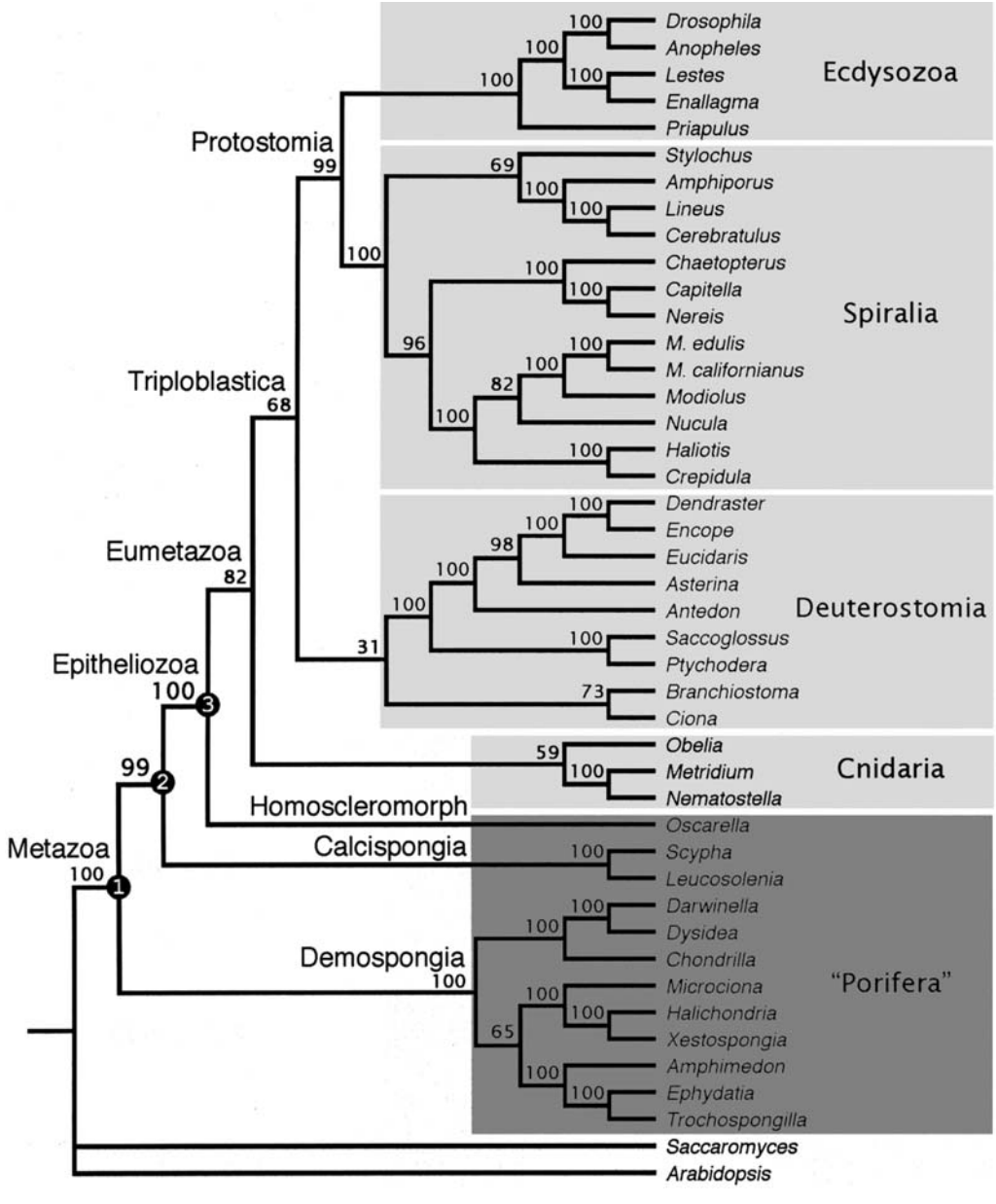


Fig. 2. Bayesian analysis of 30 eumetazoan taxa and 12 'sponges' rooted on the plant *Arabidopsis* and the yeast *Saccaromyces*. Note the paraphyly of 'Porifera' (dark grey box) with the homoscleromorph *Oscarella* more closely related to eumetazoans than to other sponges, forming the clade Epitheliozoa (node 3), and the calcisponges more closely related to the epitheliozoans than to demosponges (node 2). In addition, note the division of demosponges into two clades: G1 (*Dysidea*, *Darwinella*) + G2 (*Chondrilla*); and G3 (*Xestospongilla*, *Amphimedon*) + G4 (*Microciona*, *Halichondria*, *Ephydatia*, and *Trochospongilla*). Major eumetazoan groups are indicated with light grey boxes. *M. edulis* and *californianus* are members of the genus *Mytilus*.

Metazoa, a result that morphological, ribosomal, nuclear protein coding and microRNA (Sempere *et al.* 2006) data sets strongly refute, and it is difficult to evaluate the placement of taxa within an erroneous topology. The Bayesian tree recovered here places the homoscleromorph *Oscarella* as the sister taxon to Eumetazoa (Cnidaria + Triploblastica). Phyla have both a constructional

and a historical context, and although the homoscleromorph body plan is not exceptionally different from other sponges, this result establishes, in effect, a new phylum-level lineage if further analyses with more homoscleromorph taxa continue to find Homoscleromorpha + Eumetazoa to the exclusion of the other two sponge lineages. This result is not completely unexpected, given that homoscleromorphs are unique among sponges in possessing two eumetazoans characters—true epithelia (Boury-Esnault *et al.* 1984; 2003; Boute *et al.* 1996), and a distinct acrosome (Harrison & De Vos 1991; Boury-Esnault & Jamieson 1999). Although this result must be tested with more taxa, congruence of independent morphological and molecular data sets provides strong support for phylogenetic inferences.

Poriferan paraphyly and character acquisition

Donoghue (2005), in his discussion of plant phylogeny, demonstrated the usefulness of paraphyly in unravelling the sequential evolution of what had once appeared to be a number of phylogenetically coincident character changes. Key changes in plant evolution occurred not at a single node, but were spread along many steps. The demonstration that Porifera is paraphyletic and therefore represents an evolutionary grade has important implications for the polarization of character states and understanding the sequence of character acquisition at the base of Metazoa.

Metazoa (Fig. 2, node 1) is characterized, in part, by the acquisition of multicellularity and the presence of the extracellular matrix, a complex of collagen, proteoglycan, adhesive glycoprotein and integrin, which mediates cell motility and transitions between epithelial and motile cell types (Morris 1993). Because of sponge paraphyly, the WCS with choanocytes (itself a likely plesiomorphic cell type), had also evolved by this point as well. The unnamed clade Calcarea + Epitheliozoa (epitheliozoans are the homoscleromorph + eumetazoans, see below) (Fig. 2, node 2) is potentially characterized by the presence of cross-striated rootlets. Most metazoan ciliated cells have a system of cross-striated rootlets that originates in the ciliary basal body and extends into the cytoplasm. Calcisponge larvae, as well as those of homoscleromorphs, have long, cross-striated cell rootlets (Woollacott & Pinto 1995; Amano & Hori 2001; Boury-Esnault *et al.* 2003) that were perhaps incorporated into adult eumetazoans through neotenous evolution (Maldonaldo 2004). However, the choanoflagellate *Monosiga*, the placozoan *Trichoplax*, as well as several other protistan taxa, also have striated rootlets (Nielsen 2001; Boury-Esnault *et al.* 2003) implying that either the rootlets of Calcarea + Epitheliozoa are not homologous with those of choanoflagellates or this trait is plesiomorphic for Metazoa and has been secondarily lost in Hexactinellida and Demospongiae.

The clade Homoscleromorpha + Eumetazoa is herein recognized as Epitheliozoa (Fig. 2, node 3). Ax (1996) defined the clade Epitheliozoa for the clade of epithelial animals, and it is usually considered to include Ctenophora, Cnidaria and triploblasts (e.g. Wallberg *et al.* 2004). The position of the homoscleromorphs as the sister taxa to Eumetazoa, as well as the presence of basal laminae (Boute *et al.* 1996; Boury-Esnault *et al.* 2003), suggests that the Epitheliozoa should include the Homoscleromorpha. A second potential apomorphy of the Epitheliozoa is the presence of an acrosome (Harrison & De Vos 1991; Boury-Esnault & Jamieson 1999). Thus, of the four primary eumetazoan characters—tissues, an acrosome, a nervous system and a gut—the acquisition of tissues and the acrosome antedated the last common ancestor of homoscleromorphs and eumetazoans. The expression of features such as epithelia in the adult, along with the acquisition of these new characters (nervous system and gut), and the loss of the WCS, could be due to a coordinated character change (Jenner 2004) accompanying the neotenous evolution of a non-feeding sponge larva to a predatory eumetazoan. Based on molecular clocks, this coordinated character loss and acquisition accompanying the dramatic change in trophic strategies from sponge to eumetazoan is likely to have occurred sometime during, or soon after, the melting of the Marinoan glaciers *c*. 635 Ma (Peterson & Butterfield 2005), and may be tied to the oligotrophic ocean conditions associated with the Marinoan glaciation.

Poriferan paraphyly and Precambrian palaeobiology

Precambrian fossils are often confusing and contentious and among some of the more enigmatic forms are the rangeomorph fauna, preserved primarily in Ediacaran age strata in the Avalon Zone of Newfoundland. These fossils provide an example of the usefulness of modern systematic studies in constraining the possible phylogenetic hypotheses for Precambrian fossils.

Ediacaran fossils were first discovered in the middle of the 19th century in Newfoundland, but these structures were not recognized as biogenic until very recently (Gehling *et al.* 2000). These fossils were deposited between 575 Ma and 560 Ma (Benus 1988; Bowring *et al.* 2003; Narbonne &

Gehling 2003) in a deep-water setting well beneath the photic zone (Wood *et al.* 2003). The rangeomorphs, or 'fractal vendobionts' (Seilacher *et al.* 2003), which make up more than 85% of specimens in the classic Mistaken Point Formation, are composed of fractally-repeating architectural elements (Narbonne 2004). The organisms grew by pure inflation, with the first-order branches eventually turning into second-order. Fractal or infaltionary systems are a method for increasing the surface area-to-volume ratio, as might be expected for an organism feeding directly from the water-column without orifices. In the presence of a 'soup' of dissolved organic carbon (DOC) that likely existed during the Proterozoic (Rothman *et al.* 2003), rangeomorphs likely fed primarily on this DOC pool using direct absorption. Noting their environmental setting beneath the photic zone, indeterminate growth and lack of movement or taphonomic shrinkage, Peterson *et al.* (2003) suggested that many members of the Newfoundland fauna, such as *Aspidella*, *Charnia* and *Charniodiscus*, resembled Fungi rather than Metazoa. Nonetheless, recent studies have considered the degree of tiering similarity between the Newfoundland Ediacaran ecosystems and both Palaeozoic and Modern ecosystems sufficient to place the rangeomorphs with the Metazoa (Clapham & Narbonne 2002; Clapham *et al.* 2003). The lack of any eumetazoan features such as a gut or mouth, depsite the exceptional preservation, prompted Narbonne (2005) to consider the rangeomorphs as an extinct architecture that may lie between poriferans and cnidarians on the metazoan tree, a position first proposed by Buss and Seilacher (1994).

However, in light of the results of sponge paraphyly as discussed above, this phylogenetic placement of the rangeomorphs, while not impossible, does not represent the most likely evolutionary scenario. Given that the last common ancestor of metazoans was something akin to a modern sponge, this implies that a WCS is primitive for Metazoa, and thus must have been lost early in the phylogenetic history of rangeomorphs if the group is apical to sponges. Although eumetazoans must have also lost the WCS, the key difference here is that acquisition of the gut allowed eumetazoans to change trophic modes and feed macrophagously, whereas rangeomorphs would still feed on the abundant DOC in the Proterozoic ocean in a manner analogous to sponges. While evolution is not always a predictable pathway, the loss of the WCS by a sponge-like organism in favour of a fractal construction to feed on the same food source does not represent a parisimonious interpretation of the data. The ecological studies of Clapham and colleagues showing similarity to modern and ancient animal ecosystems is merely sufficient to demonstrate that they are heterotrophs that partitioned the water column for suspension feeding, but does not necessarily imply that they were, in fact, metazoans. Community tiering to extract nutrients more efficiently from the water column is a simple ecological strategy, likely to be adopted regardless of phylogenetic affinity, as shown by the convergent strategy used by Early Cambrian sponges (Yuan *et al.* 2002). Considering: (1) the deep-water setting which precludes a plant or algal status; (2) the lack of any eumetazoan apomorphies despite exceptional preservation; (3) their fractal organization; (4) the heterotrophic tiering of the community; and (5) the low probability that the WCS would have been lost in favour of a fractal design to feed on the same material, the Newfoundland rangeomorph fauna probably represent members of the opisthokonts (the group defined by the last common ancestor of fungi and animals; Cavalier-Smith 1998), but were not crown-group metazoans. It is worth emphasizing that they still could be stem-group metazoans, but our point is that the most likely explanation of the trophic data is that the last common ancestor of metazoans and rangeomorphs was unicellular, and the fractal and water canal architectures are two different solutions to achieve the same goal, namely feeding upon the abundant DOC available during the Ediacaran.

Poriferan paraphyly and Cambrian 'problematica'

Modern poriferan systematics and biology can also be used to infer the phylogenetic placement of several groups of enigmatic Cambrian fossils, and suggest guidelines for the taxonomy of sponge fossils in general. For example, chancelloriids are a group of early Cambrian spongiform organisms that were originally considered to be sponges but have since been the subject of a long history of taxonomic speculation. They range from the earliest Cambrian to the early Late Cambrian, flourishing in shallow marine environments, often as components of archaeocyathan mounds (Janussen *et al.* 2002). Although they are usually found as disassociated sclerites, complete scleritomes can be found in low-energy depositional environments. The scleritomes show sessile, attached, sac-like fossils that are covered by spiny sclerites. The non-anchored end contains a thick tuft of sclerites that likely surrounded an apical orifice (Bengtson 2005). The sclerites have thin, originally aragonitic walls surrounding a cavity with a restricted basal opening (Bengtson & Missarzhesky 1981). The outer surface was covered by a soft epithelial integument, perhaps indicating the presence of

desmosomal cell junctions (Bengtson & Hou 2001; Janussen *et al.* 2002). Chancelloriids were originally described as poriferans due to their sponge-like body form (Walcott 1920), and some workers still adhere to this position (Butterfield & Nicholas 1996). Nonetheless, in recent years others have suggested possible affinities with ascidians (Mehl 1996), cnidarians (Randall *et al.* 2005), and with the extinct and most likely polyphyletic (Conway Morris & Chapman 1997) Coeloscleritophora (Bengtson & Missarzhevsky 1981). Most arguments against a sponge affinity have focused on the sclerites (Bengtson 2005; Randall *et al.* 2005), and because there are clear differences between the spicules of chancelloriids and sponges, most authors agree that the two are not homologous. We agree. Most workers argue further that if the spicules are not homologous, then chancelloriids are not sponges. We disagree.

First, despite the superficial morphological similarity of spicules, clear structural and developmental differences exist between silicisponges and calcisponges (Harrison & De Vos 1991; Reitner & Mehl 1996; Brusca & Brusca 2002). Silicisponges deposit siliceous spicules intracellularly, first secreting an organic carbon axial filament within an elongated vacuole in a sclerocyte. As the axial filament elongates, hydrated silica is secreted into the vacuole and around the filament. Calcareous sponges, on the other hand, deposit their spicules extracellularly and without an organic axis; each spicule is essentially a single crystal of calcium carbonate. Second, the presence of silicified spicules does not characterize all demosponges. The G1 sponges form their skeleton entirely of spongin fibres and do not secrete a siliceous skeleton. The same is true for most, but not all of the G2s—*Chondrilla* lacks megascleres but possesses aster microscleres that are homoplasic with respect to other demosponges (Borchiellini *et al.* 2004). In fact, if hexactinellids are nested near or within the G3/G4 sponges, as suggested by some rDNA studies (Cavalier-Smith & Chao 2003; Nichols 2005), then this would strongly suggest that siliceous megascleres arose only once; differences between the spicules of hexactinellids and demosponges could be due to the radically different cellular organizations of the two (Leys 2003). And third, the presence of silicified spicules does not characterize all of Homoscleromorpha. Two genera (*Oscarella* and *Pseudocorticium*) are aspiculate (Muricy & Diaz 2002), but whether this is primitive or not is unknown, as the internal phylogeny of Homoscleromorpha remains unexplored by modern molecular means. Therefore, it is clear from the emerging sponge phylogeny that spicules arose at least three times within 'Porifera': at least once within Silicispongia, once within Calcispongia and once at either at the base or within Homoscleromorpha. Given the clear homoplasy of massive calcareous skeletons within demo- and calcisponges (Chombard *et al.* 1997), convergence of spicule structure as well should not be too surprising.

The inescapable conclusion is that an organism cannot be removed from the poriferan grade based simply on spicule characteristics. An organism is a 'sponge' (taxon in quotes to represent a paraphyletic grade) if it feeds using a WCS, pumping water through a chamber via the power of choanocytes and extracting dissolved organic matter and picoplankton from the current. Fluid physics causes water flow velocities to slow as cross-sectional area increases. Sponges generally have a combined diameter of choanocyte chambers greater than the incurrent pores, and the combined diameters of the excurrent oscula are less than both the choanocyte chambers and incurrent pores (Brusca & Brusca 2002). This causes water to enter at velocity x, slow dramatically over the choanoderm for maximum absorption of nutrients, and then exit the sponge at a velocity far greater than x, sending the water clear of the sponge and avoiding recycling problems. Fossil sponges can be recognized, therefore, as organisms with combined incurrent pores greater in diameter than the combined excurrent openings.

Chancelloriids were sessile, benthic, radially symmetrical organisms constructed with presumed excurrent openings having a lesser diameter than the presumed combined incurrent pores. No specimen shows evidence of a mouth, gut or any other eumetazoan apomorphy, despite their co-occurrence with soft-bodied forms in localities such as Chengjiang (China), the Burgess Shale (Canada) and the Wheeler Shale (USA) that commonly preserve such anatomical features. Finally, the recognition of tissues as a eumetazoan plesiomorphy removes an additional difficulty with a poriferan affinity (Janussen *et al.* 2002) given that, similar to homoscleromorphs, chancelloriids were likely to possess an integument (Bengtson & Hou 2001; Janussen *et al.* 2002).

Archaeocyaths, another Early Cambrian fossil group, provide a historical example of how a focus on skeletal characteristics may incorrectly preclude a group from the poriferan grade. As sessile, conical marine organisms, archaeocyaths were first formally described as possible sponges, but later classified as coelenterates, algae, foraminifera or an extinct phylum or kingdom, with a non-poriferan taxonomic affinity often preferred because their unique double-walled massive calcareous skeleton was unknown in modern sponges (see review in Rowland 2001). However, the discovery of massive, calcareous sponges (sclerosponges and *Vaceletia*) demonstrated that the archaeocyathan

skeleton was not incompatible with a 'sponge' affinity. Studies of archaeocyath ultrastructure (Kruse & Debrenne 1989) and functional morphology (Savarese 1992; Wood *et al.* 1992) further cemented their status as sponges, a point now agreed upon by nearly all archaeocyath and sponge workers (Rowland 2001). The convergent evolution of both spicules and massive skeletons in different 'sponge' lineages suggests the skeletonization process in archaeocyaths and chancelloriids is largely irrelevant to their broad-scale taxonomic position as compared to recognizing the biological constraints afforded by a sessile, radially constructed organism with a probable excurrent osculum and no visible gut or mouth.

Poriferan paraphyly and Precambrian oxygenation

Geochemical evidence suggests that the partial pressure of atmospheric oxygen rose substantially circa 2.3 billion years ago (Holland & Beukes 1990; Farquhar *et al.* 2000). While the increase in atmospheric oxygen helped to oxygenate the surficial layers of the ocean, the mid-Proterozoic deep ocean was likely anoxic and sulphidic (Canfield 1998; Anbar & Knoll 2002). Between the mid-Proterozoic and the Phanerozoic there was a transitional period from ancient to modern ocean, the latter characterized by a well-oxygenated mixed or surface layer, a mildly dysoxic to anoxic oxygen-minimum zone from *c*. 200–1000 metres where the mineralization of organic matter descending from surface productivity draws down dissolved oxygen levels, and an oxygenated deep ocean. Evidence from carbon isotope studies indicates that the Proterozoic ocean was a 'soup' of dissolved organic matter (Rothman *et al.* 2003). Logan *et al.* (1995), in their seminal study of sedimentary organic carbon, suggested that hydrocarbons in Proterozoic sediments were derived primarily from bacteria and heterotrophic organisms rather than from photosynthetic organisms as in the modern ocean. Due to the lack of faecal pellets, which increase transport speed to the ocean bottom, the degradation of algal products was unusually complete. Logan *et al.* (1995), therefore, suggested that the slowly sinking algal products were re-mineralized before they could be sequestered in the sediment, and it was the advent of planktonic organisms with faecal pellets that allowed for the drawdown of the organic carbon pool and transition to the Phanerozoic ocean. The timing of this shift as described in Logan *et al.* (1995) is not clear due to the lack of available radiometric dates at that time, occurring at some point between the Late Neoproterozoic and the Early Cambrian (Peterson *et al.* 2005). New data from carbon and sulphur isotopes in the Huqf Supergroup in Oman suggests that this reorganization and the oxidation of the Proterozoic ocean took place during the 'Shuram' excursion. Chemostratigraphic correlations with other sections worldwide suggest that the Shuram event likely occurred prior to 551 Ma (Condon *et al.* 2005), while detrital zircons from Oman date it younger than 610 Ma (Le Guerroue *et al.* 2006*a*). The faecal pellets of mesozooplankton do not sink, and only the faecal pellets of macrozooplankton or nekton are capable of rapidly sinking (Peterson *et al.* 2005). Given that the fossil record of planktic predation (Butterfield 1997), and the actual fossil record of the possible predators themselves (Butterfield 1994), indicates that the base of the zooplankton food chain did not exist until after the Tommotian, the evolution of macrozooplankton could not have occurred until significantly after the major oxidation of the dissolved organic carbon reservoir and transition to the Phanerozoic ocean. The origin and diversification of sponges and associated organisms might, however, represent an alternative mechanism for the drawdown of the dissolved organic carbon.

The Shuram negative carbon isotope excursion, preserved in mid-Ediacaran strata in Oman, shows a $\delta^{13}C_{carb}$ shift on the order of $>15^{0/00}$ to values of $-12^{0/00}$ (Le Guerroue *et al.* 2006*b*; Fike *et al.* 2006). It is unique in Earth history in recording long-lasting marine carbonate carbon values with presumed primary signal well below the canonical mantle value of $-6^{0/00}$. Negative excursions of extremely large magnitude, such as those during Snowball Earth episodes (Halverson *et al.* 2005), in the Early Cambrian (Maloof *et al.* 2005) or Early Triassic (Payne *et al.* 2004), which do not show carbonate carbon values below $-6^{0/00}$, can be explained through normal agents such as changes in the fraction of carbon buried as organic carbon and/or changes in isotopic fractionation during photosynthesis. The Shuram excursion, however, demands a fundamentally different explanation for the long-term production of isotopically-light carbonates. Fike *et al.* (2006) suggested that the Shuram excursion was caused by the oxidation of a large dissolved organic carbon (DOC) pool hypothesized to have existed throughout much of the Proterozoic (Rothman *et al.* 2003), but they did not identify any mechanism to account for this event. Here, we posit that the advent of novel modes of heterotrophy, especially the orgin and radiation of sponges and rangeomorphs, could account for the Shuram excursion.

According to our hypothesis (Fig. 3), in the early Neoproterozoic, the isotopic composition of marine carbonates is controlled by the normal Phanerozoic paradigm: $\delta^{13}C_{carb} = \delta w - (\Delta\delta x\, f\, org)$ where δw is equal to the isotopic composition of mantle-derived

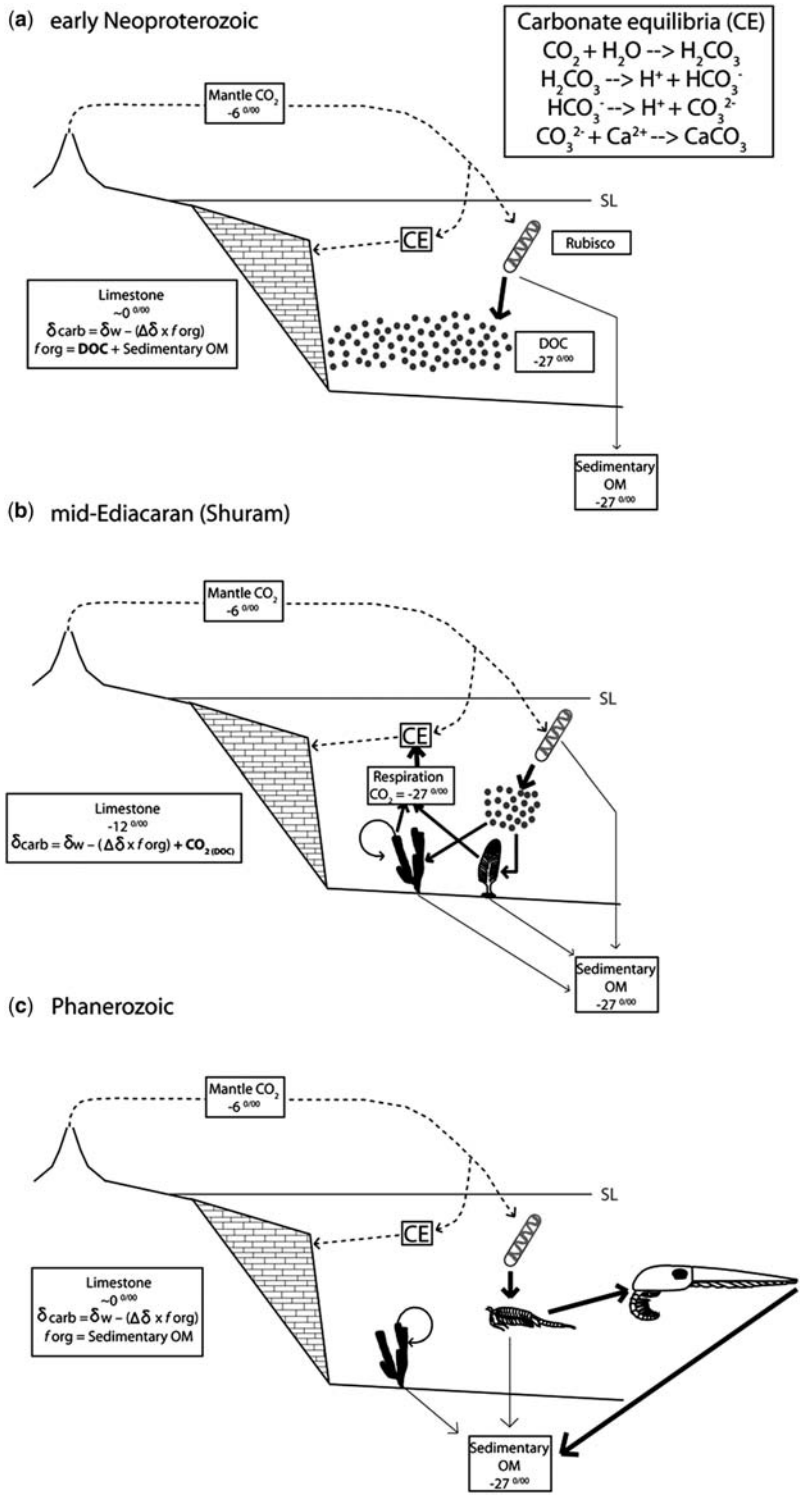
CO_2 (generally interpreted to be *c.* $-6^{0/00}$ throughout Earth history), $\Delta\delta$ is the fractionation introduced during photosynthesis, and *f* org is the fraction converted to organic carbon during photosynthesis. Unlike the Phanerozoic, where *f* org $\cong$ sedimentary organic matter, a significant percentage of primary production is not buried in the sediment (Logan *et al*. 1995) but is partly degraded in the water column and accumulates as dissolved organic carbon (DOC). By the early- to mid- Ediacaran ('Shuram' time), feeding by sponges and rangeomorphs on DOC led to volumetrically-significant heterotrophic respiration on this DOC pool for the first time in Earth history. Unlike in photosynthesis there is no isotopic fractionation during respiration (oxidation of the DOC), thereby producing large quantities of isotopically-light (*c.* $-27^{0/00}$) CO_2 that equilibrated with the water and caused a long-lived isotopic excursion below the canonical mantle value. Eventually the DOC reservoir was depleted, leading to a long isotopic recovery and explaining why the Shuram is a singular event in Earth history. The processing of DOC by sponges after the Shuram excursion would still have been important, although the amount of DOC available for feeding would be directly related to production. The evolution of the modern zooplankton food chain with faecal pellets near the base of the Cambrian finally allowed for the direct consumption of algae and the export of carbon to the sediment (Logan *et al.* 1995) without an intermediate DOC step.

This hypothesis is supported by the temporal correlation of the Shuram isotope excursion with the origination and ecological dominance of two unrelated grades of organism, sponges and rangeomorphs. Molecular clocks (Peterson & Butterfield 2005) and the fossil record (McCaffrey *et al.* 1994; Love *et al*. 2006) show that the origin of the sponge-grade is not much older than the Ediacaran, and the first rangeomorphs are dated to *c.* 575 Ma (Narbonne & Gehling 2003). Both were extremely abundant and physiologically well-suited to feed directly on DOC, unlike virtually all other clades of eukaryotes including eumetazoans. The WCS of sponges is extremely efficient at moving water through the body via the power of the choanocytes. For example, a large sponge can filter its own volume of water every 10 to 20 seconds (Brusca & Brusca 2002). *In situ* feeding studies on modern sponges demonstrate that some derive the majority of their food from DOC, and can remove an average of 10% of the DOC in the water in a single pass through the water canal system (Yahel *et al.* 2003). Rangeomorphs, which must also have fed on dissolved organic carbon using a convergent solution based on passive absorption (see above), would also have contributed to the drawdown. The absolute dominance of rangeomorphs in the older (*c.* 575–565 Ma) Newfoundland Ediacaran fauna, and their almost complete absence in the younger (and shallower) Ediacara/White Sea (555 Ma) and Namibian (*c.* 549–543 Ma) faunas (Narbonne 2005) may be related to their reliance on passive absorption on the disappearing pool of dissolved organic carbon in the deep ocean, whereas sponges, which can essentially bring the ocean through their body with the water-canal system, would have been less affected by a declining reservoir.

Canfield *et al.* (2006) suggested the deep ocean may have become oxygenated around the Gaskiers glaciation, *c.* 580 Ma. Oxidation of the Neoproterozoic DOC pool as discussed above must necessarily have resulted in a *de*-oxygenation of the ocean-atmosphere system, unless a different oxidant is invoked, and so oxygenation and oxidation are not necessarily linked. Nonetheless, the advent of the sponge and rangeomorph body plans allowed DOC to be incorporated into benthic biomass that could be buried and removed as an oxygen sink. Given that algal products could not be buried as faecal pellets until the Cambrian, the reorganization of biogeochemical cycles, as evidenced by the Shuram excursion, could reflect the origin and subsequent radiation of sponges and rangeomorphs.

Conclusions

Our molecular results provide further evidence for the paraphyly of Porifera and suggest that there are three 'sponge' phyla: Silicispongia, Calcispongia and Homoscleromorpha. This strengthens the conclusions of Peterson & Butterfield (2005), and previous reports based on ribosomal evidence. Further work is required to test this topology, to provide more concrete evidence for the placement of placozoans and hexactinellids, and to elucidate the internal topology of demosponges and homoscleromorphs. Nevertheless, basal metazoan phylogeny is becoming clearer, and the paraphyly of sponges allows not only for the polarization of character states and an enhanced understanding of the sequence of character acquisition, but sheds much light on the phylogenetic affinities of long-extinct taxa, and provides new insights into global oceanic oxygenation. Finally, the fact that one modern clade of demosponges, the cladorhizids, lost the WCS and feeds macrophagously upon mesozooplankton using a derived mode of extracellular digestion (Vacelet & Boury-Esnault 1995) may shed light on the analogous loss of the WCS in early eumetazoans (Vacelet & Duport 2004). The concordance between the observations that



cladorhizids live in oligotrophic environments, and that eumetazoans arose on the heels of the Marinoan 'snowball Earth', may be of some significance.

We thank S. Nichols for the gift of *Oscarella carmela* total RNA, and E. Dunn, C. Fiove, A. Evans and V. Moy for technical assistance. We also thank G. Love, D. Rothman, L. Buss, D.E.G. Briggs and R. Summons for discussion, and A. Collins and G. Narbonne for formal reviews. KJP is supported by the National Science Foundation, NASA-Ames, and Dartmouth College. EAS was supported by a NASA Planetary Biology Internship grant.

References

ADAMS, C. L., MCINERNEY, J. O. & KELLY, M. 1999. Indications of relationships between poriferan classes using full-length 18S rRNA gene sequences. *Memoirs of the Queensland Museum*, **44**, 33–43.

AMANO, S. & HORI, I. 2001. Metamorphosis of coeloblastula performed by multipotential larval flagellated cells in the calcareous sponge *Leucosolenia laxa*. *Biological Bulletin*, **200**, 20–32.

ANBAR, A. D. & KNOLL, A. H. 2002. Proterozoic ocean chemistry and evolution: a bioinorganic bridge. *Science*, **297**, 1137–1142.

AX, P. 1996. *Multicellular Animals, Vol. 1. A New Approach to Phylogenetic Order in Nature*. Springer-Verlag, Berlin.

BENGTSON, S. 2005. Mineralized skeletons and early animal evolution. *In*: BRIGGS, D. E. G. (ed.) *Evolving Form and Function: Fossils and Development*. Yale Peabody Museum Special Publication.

BENGTSON, S. & HOU, X. 2001. The integument of Cambrian chancelloriids. *Acta Palaeontological Polonica*, **46**, 1–22.

BENGTSON, S. & MISSARZHEVSKY, V. V. 1981. Coeloscleritophora—a major group of enigmatic Cambrian metazoans. *U.S. Geological Survey Open-file Report*, 81–743, 19–21.

BENUS, A. P. 1988. Sedimentological context of a deep-water Ediacaran fauna (Mistaken Point Formation, Avalon Zone, eastern Newfoundland). *In*: LANDING, E., NARBONNE, G. M. & MYROW, P. M. (eds) *Trace Fossils, Small Shelly Fossils and the Precambrian/Cambrian Boundary*. Bulletin of the New York State Museum, Albany, **463**, 8–9.

BORCHIELLINI, C., MANUEL, M., ALIVON, E., BOURY-ESNAULT, N., VACELET, J. & LE PARCO, Y. 2001. Sponge paraphyly and the origin of Metazoa. *Journal of Evolutionary Biology*, **14**, 171–179.

BORCHIELLINI, C., CHOMBARD, C., MANUEL, M., ALIVON, E., VACELET, J. & BOURY-ESNAULT, N. 2004. Molecular phylogeny of Demospongiae: implications for classification and scenarios for character evolution. *Molecular Phylogenetics and Evolution*, **32**, 823–837.

BOURY-ESNAULT, N. & JAMIESON, B. G. M. 1999. Porifera. *In*: JAMIESON, B. G. M. (ed.) *Progress in Male Gamete Biology*. IBH Publishing Co., New Delhi, 1–20.

BOURY-ESNAULT, N., DE VOS, L., DONADEY, C. & VACELET, J. 1984. Comparative study of the choanosome of Porifera: 1. The Homoscleromorpha. *Journal of Morphology*, **180**, 3–17.

BOURY-ESNAULT, N., ERESKOVSKY, A., BÉZAC, C. & TOKINA, D. 2003. Larval development in the Homoscleromorpha (Porifera, Demospongiae). *Invertebrate Biology*, **122**, 187–202.

BOUTE, N., EXPOSITO, J.-Y., BOURY-ESNAULT, N., VACELET, J., NORO, N., MIYAZAKI, K., YOSHIZATO, K. & GARRONE, R. 1996. Type IV collagen in sponges, the missing link in basement membrane ubiquity. *Biological Cell*, **88**, 37–44.

BOWRING, S., MYROW, P., LANDING, E., RAMEZANI, J. & GROTZINGER, J. 2003. Geochronological constraints on terminal Neoproterozoic events and the rise of metazoans. *Geophysical Research Abstracts*, **5**, 13219.

BRUSCA, R. C. & BRUSCA, G. J. 2002. *Invertebrates* (2nd edn) Sinauer Associates Inc., Sunderland.

BUSS, L. W. & SEILACHER, A. 1994. The Phylum Vendobionta: a sister group of the Eumetazoa? *Paleobiology*, **20**, 1–4.

BUTTERFIELD, N. J. 1994. Burgess Shale-type fossils from a Lower Cambrian shallow-shelf sequence in northwestern Canada. *Nature*, **369**, 477–479.

BUTTERFIELD, N. J. 1997. Plankton ecology and the Proterozoic–Phanerozoic transition. *Paleobiology*, **23**, 247–262.

Fig. 3. A biological explanation for the Shuram excursion. Bold arrows or font indicate a relatively more important contribution from that source. SL, sea level; CE, carbonate equilibria (CO_2 equilibrates with seawater and combines with ocean Ca^{2+} to form carbonates). (**a**) In the early Neoproterozoic, the isotopic composition of marine carbonates ($\delta^{13}C_{carb}$) is equal to that of mantle-derived CO_2 (δw; generally interpreted to be *c.* $-6^{0/00}$ throughout Earth history) minus the fraction converted to organic carbon during photosynthesis (*f* org), multiplied by the fractionation introduced during the Rubisco cycle ($\Delta\delta$). Unlike the Phanerozoic, where *f* org $\cong$ sedimentary organic matter (OM), a significant percentage of primary production is not buried in the sediment but is partly degraded in the water column and accumulates as dissolved organic carbon (DOC). (**b**) By the early- to mid-Ediacaran, feeding by sponges and rangeomorphs on DOC led to volumetrically-significant heterotrophic respiration on this DOC pool for the first time in Earth history. Unlike in photosynthesis there is no isotopic fractionation during respiration (oxidation of the DOC), thereby producing large quantities of isotopically-light (e.g. $-27^{0/00}$) CO_2 that equilibrated with the water and eventually contributed to a long-lived isotopic excursion below the canonical mantle value. (**c**) By the Phanerozoic, the carbon isotopic system had achieved its present-day steady state, with a small DOC pool and an isotopic value of marine carbonates controlled primarily by the fraction of carbon buried as organic matter. The evolution of macrozooplankton at the base of the Cambrian finally allowed for the direct processing of algal products and their transport to seafloor as faecal pellets without a DOC intermediate.

BUTTERFIELD, N. J. & NICHOLAS, C. J. 1996. Burgess Shale-type preservation of both non-mineralizing and 'shelly' Cambrian organisms from the Mackenzie Mountains, northwestern Canada. *Journal of Paleontology*, **70**, 893–899.

CANFIELD, D. E. 1998. A new model for Proterozoic ocean chemistry. *Nature*, **396**, 450–453.

CANFIELD, D. E., POULTON, S. W. & NARBONNE, G. M. 2006. Late-Neoproterozoic deep-ocean oxygenation and the rise of animal life. *Science*, **315**, 92–95.

CAVALIER-SMITH, T. 1998. A revised six-kingdom system of life. *Biological Reviews of the Cambridge Philosophical Society*, **73**, 203–266.

CAVALIER-SMITH, T. & CHAO, E. E.-Y. 2003. Phylogeny of Choanozoa, Apusozoa, and other Protozoa and early eukaryotic megaevolution. *Journal of Molecular Evolution*, **56**, 540–563.

CAVALIER-SMITH, T., ALLSOPP, M. T. E. P., CHAO, E. E.-Y., BOURY-ESNAULT, N. & VACELET, J. 1996. Sponge phylogeny, animal monophyly, and the origin of the nervous system: 18S rRNA evidence. *Canadian Journal of Zoology*, **74**, 2031–2045.

CHOMBARD, C., BOURY-ESNAULT, N., TILLIER, A. & VACELET, J. 1997. Polyphyly of 'sclerosponges' (Porifera, Demospongiae) supported by 28S sequences. *Biological Bulletin*, **193**, 359–367.

CLAPHAM, M. E. & NARBONNE, G. M. 2002. Ediacaran epifaunal tiering. *Geology*, **30**, 627–630.

CLAPHAM, M. E., NARBONNE, G. M. & GEHLING, J. G. 2003. Paleoecology of the oldest known animal communities: Ediacaran assemblages at Mistaken Point, Newfoundland. *Paleobiology*, **29**, 527–544.

COLLINS, A. G. 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: an analysis of 18S rRNA molecular evidence. *Proceedings of the National Academy of Sciences, USA*, **95**, 15458–15463.

CONDON, D., ZHU, M., BOWRING, S., WANG, W., YANG, A. & JIN, Y. 2005. U–Pb ages from the Neoproterozoic Doushantuo Formation, China. *Science*, **308**, 95–98.

CONWAY MORRIS, S. & CHAPMAN, A. J. 1997. Lower Cambrian Halkieriids and other Coeloscleritophorans from Aksu-Wushi, Xinjiang, China. *Journal of Paleontology*, **71**, 6–22.

DONOGHUE, M. J. 2005. Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. *Paleobiology*, **31**, Supplement, 77–93.

EERNISSE, D. J. & PETERSON, K. J. 2004. The history of animals. *In*: CRACRAFT, J. & DONOGHUE, M. J. (eds) *Assembling the Tree of Life*. Oxford University Press, Oxford, 197–208.

FARQUHAR, J., BAO, H. M. & THIEMENS, M. 2000. Atmospheric influence of Earth's earliest sulphur cycle. *Science*, **289**, 756–758.

FIKE, D., GROTZINGER, J., PRATT, L. & SUMMONS, R. 2006. Oxidation of the Ediacaran ocean. *Nature*, **444**, 744–747.

GEHLING, J. G. 1991. The case for Ediacaran fossil roots to the metazoan tree: *Geological Society of India Memoir*, **20**, 181–224.

GEHLING, J. G., NARBONNE, G. M. & ANDERSON, M. M. 2000. The first named Ediacaran body fossil, *Aspidella terranovica. Palaeontology*, **43**, 427–456.

HALANYCH, K. M. 2004. The new view of animal phylogeny. *Annual Review of Ecology and Systematics*, **35**, 229–256.

HALVERSON, G. P., HOFFMAN, P. F., SCHRAG, D. P., MALOOF, A. C. & RICE, A. H. N. 2005. Towards a Neoproterozoic composite carbon-isotope record. *Geological Society of America Bulletin*, **117**, 1181–1207.

HARRISON, F. W. & DE VOS, L. 1991. Porifera. *In*: HARRISON, F. W. & WESTFALL, J. A. (eds) *Microscopic Anatomy of the Invertebrates. 2: Placozoa, Porifera, Cnidaria, and Ctenophora*. Wiley-Liss, New York, 29–89.

HOLLAND, H. D. & BEUKES, N. J. 1990. A paleoweathering profile from Griqualand West, South Africa: evidence for a dramatic rise in atmospheric oxygen between 2.2 and 1.8 bybp. *American Journal of Science*, **290–A**, 1–34.

HOOPER, J. N. A. & VAN SOEST, R. W. M. 2006. A new species of *Amphimedon* (Porifera, Demospongiae, Haplosclerida, Niphatidae) from the Capricorn-Bunker Group of Islands, Great Barrier Reef, Australia: target species for the 'sponge genome project', *Zootaxa*, **1314**, 31–39.

JANUSSEN, D., STEINER, M. & MAOYAN, Z. 2002. New well-preserved scleritomes of Chancelloridae from the Early Cambrian Yuanshan Formation (Chengjiang, China) and the Middle Cambrian Wheeler Shale (Utah, USA) and palaeobiological implications. *Journal of Paleontology*, **76**, 596–606.

JENNER, R. A. 2004. When molecules and morphology clash: reconciling conflicting phylogenies of the Metazoa by considering secondary character loss. *Evolution and Development*, **6**, 372–378.

KRUSE, M., LEYS, S. P., MÜLLER, I. M. & MÜLLER, W. E. G. 1998. Phylogenetic position of the Hexactinellida within the phylum Porifera based on the amino acid sequence of the protein kinase C from *Rhabdocalyptus dawsoni*. *Journal of Molecular Evolution*, **46**, 721–728.

KRUSE, P. D. & DEBRENNE, F. 1989. Review of archaeocyath microstructure. *Memoirs of the Association of Australasian Palaeontologists*, **8**, 133–141.

LE GUERROUE, E., ALLEN, P. A., COZZI, A., ETIENNE, J. L. & FANNING, M. 2006*a*. 50 Myr recover from the largest $\delta^{13}C$ excursion in the Ediacaran ocean. *Terra Nova*, **18**, 147–153.

LE GUERROUE, E., ALLEN, P. A. & COZZI, A. 2006*b*. Chemostratigraphic and sedimentological framework of the largest negative carbon isotope excursion in Earth history: The Neoproterozoic Shuram Formation (Nafun Group, Oman). *Precambrian Research*, **146**, 68–92.

LEYS, S. P. 2003. Comparative study of spiculogenesis in demosponge and hexactinellid larvae. *Microscopy Research and Technique*, **62**, 300–311.

LOGAN, G. A., HAYES, J. M., HIESHIMA, G. B. & SUMMONS, R. E. 1995. Terminal Proterozoic reorganization of biogeochemical cycles. *Nature*, **376**, 53–56.

LOVE, G. D. & FIKE, D. A. *ET AL*. 2006. Constraining the timing of basal metazoan radiation using molecular biomarkers and U–Pb isotope dating. *Geochimica et Cosmochimica Acta*, **70**, A371.

MALDONADO, M. 2004. Choanoflagellates, choanocytes, and animal multicellularity. *Invertebrate Biology*, **123**, 1–22.

MALOOF, A. C., SCHARG, D. P., CROWLEY, J. L. & BROWRING, S. A. 2005. An expanded record of Early Cambrian carbon cycling from the Anti-Atlas Margin, Morocco. *Canadian Journal of Earth Science*, **42**, 2195–2216.

MANUEL, M., BORCHIELLINI, C., ALIVON, E., LE PARCO, Y., VACELET, J. & BOURY-ESNAULT, N. 2003. Phylogeny and evolution of calcareous sponges: monophyly of Calcinea and Calcaronea, high level of morphological homoplasy, and the primitive nature of axial symmetry. *Systematic Biology*, **52**, 311–333.

MCCAFFREY, M. A., MOLDOWAN, J. M., LIPTON, P. A., SUMMONS, R. E., PETERS, K. E., JEGANATHAN, A. & WATT, D. S. 1994. Paleoenvironmental implications of novel C30 steranes in Precambrian to Cenozoic age petroleum and bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.

MEDINA, M., COLLINS, A. G., SILBERMAN, J. D. & SOGIN, M. L. 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *Proceedings of the National Academy of Sciences, USA*, **98**, 9707–9712.

MEHL, D. 1996. Organization and microstructure of the chancellorid skeleton: implications for the biomineralization of the Chancelloridae. *Bulletin de l'Institut Oceanographique de Monaco*, **14**, 377–385.

MORRIS, P. J. 1993. The developmental role of the extracellular matrix suggests a monophyletic origin of the kingdom Animalia. *Evolution*, **47**, 152–165.

MURICY, G. & DIAZ, D. 2002. Order Homoscleromorpha Dendy, 1905, Family Plakinidae Schluze, 1880. *In*: HOOPER, J. N. A. & VAN SOEST, R. W. M. (eds) *Systema Porifera: A Guide to the Classification of Sponges*. Kluwer Academic/Plenum Publishers, New York, 71–82.

NARBONNE, G. M. 1998. The Ediacara-biota: a terminal Neoproterozoic experiment in the evolution of life. *Geological Society of America Today*, **8**, 1–6.

NARBONNE, G. M. 2004. Modular construction of early Ediacaran complex life forms. *Science*, **305**, 1141–1144.

NARBONNE, G. M. 2005. The Ediacara biota: Neoproterozoic origin of animals and their ecosystems. *Annual Review of Earth and Planetary Sciences*, **33**, 421–442.

NARBONNE, G. M. & GEHLING, J. G. 2003. Life after snowball: the oldest complex Ediacaran fossils. *Geology*, **31**, 27–30.

NICHOLS, S. A. 2005. An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I. *Molecular Phylogenetics and Evolution*, **34**, 81–96.

NIELSEN, C. 2001. *Animal Evolution: Interrelationships of the Living Phyla* (2nd edn). Oxford University Press, Oxford.

PAYNE, J. L., LEHRMANN, D. J., WEI, J., ORCHARD, M. J., SCHRAG, D. P. & KNOLL, A. H. 2004. Large perturbations of the carbon cycle during recovery from the end-Permian extinction. *Science*, **305**, 506–509.

PETERSON, K. J. & BUTTERFIELD, N. J. 2005. Origin of the Eumetazoa: Testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences, USA*, **102**, 9547–9552.

PETERSON, K. J. & EERNISSE, D. J. 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evolution and Development*, **3**, 170–205.

PETERSON, K. J., WAGGONER, B. & HAGADORN, J. W. 2003. A fungal analog for Newfoundland Ediacaran fossils. *Integrative and Comparative Biology*, **43**, 127–136.

PETERSON, K. J., LYONS, J. B., NOWAK, K. S., TAKACS, C. M., WARGO, M. J. & MCPEEK, M. A. 2004. Estimating metazoan divergence times with a molecular clock. *Proceedings of the National Academy of Sciences, USA*, **101**, 6536–6541.

PETERSON, K. J., MCPEEK, M. A. & EVANS, D. A. D. 2005. Tempo and mode of early animal evolution: inferences from rocks, *Hox*, and molecular clocks. *Paleobiology*, **31**, Supplement, 36–55.

RANDALL, R. D., LIEBERMAN, B. S., HASIOTIS, S. T. & POPE, M. C. 2005. New chancelloriids from the Early Cambrian Sekwi Formation with a comment on chancelloriid affinities. *Journal of Paleontology*, **79**, 987–996.

REITNER, J. & MEHL, D. 1996. Monophyly of the Porifera. *Verhandlungen des naturwissenschaftlichen Vereins in Hamburg (Neue Folgue)*, **36**, 5–32.

RONQUIST, F. & HUELSENBECK, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

RONQUIST, F., HUELSENBECK, J. P. & VAN DER MARK, P. 2005. MrBayes. Distributed by the authors.

ROTHMAN, D. H., HAYES, J. M. & SUMMONS, R. E. 2003. Dynamics of the Neoproterozoic carbon cycle. *Proceedings of the National Academy of Sciences, USA*, **100**, 8124–8129.

ROWLAND, S. M. 2001. Archaeocyaths—a history of phylogenetic interpretation. *Journal of Paleontology*, **75**, 1065–1078.

RUNNEGAR, B. 1982. The Cambrian explosion: animals or fossils? *Journal of the Geological Society of Australia*, **29**, 395–411.

SAVARESE, M. 1992. Functional analysis of archaeocyathan skeletal morphology and its palaeobiological implications. *Paleobiology*, **18**, 464–480.

SEILACHER, A., GRAZHDANKI, D. & LEGOUTA, A. 2003. Ediacaran biota: The dawn of animal life in the shadow of giant protists. *Paleontological Research*, **7**, 43–54.

SEMPERE, L. F., COLE, C. N., MCPEEK, M. A. & PETERSON, K. J. 2006. The phylogenetic distribution of metazoan microRNAs: insights into evolutionary complexity and constraint. *Journal of Experimental Zoology (Mol Dev Evol)*, **306B**, 576–588.

VACELET, J. & BOURY-ESNAULT, N. 1995. Carnivorous sponges. *Nature*, **373**, 333–335.

VACELET, J. & DUPORT, E. 2004. Prey capture and digestion in the carnivorous sponge *Asbestopluma hypogea*

(Porifera: Demospongiae). *Zoomorphology*, **123**, 179–190.

WANG, X. & LAVROV, D. V. 2007. Mitochondrial genome of the homoscleromorph *Oscarella carmela* (Porifera, Demospongiae) reveals unexpected complexity in the common ancestor of sponges and other animals. *Molecular Biology and Evolution*, **24**, 363–373.

WALCOTT, C. D. 1920. Middle Cambrian Spongiae. *Smithsonian Miscellaneous Collections*, **67**, 261–364.

WALLBERG, A., THOLLESSON, M., FARRIS, J. S. & JONDELIUS, U. 2004. The phylogenetic position of the comb jellies (Ctenophora) and the importance of taxonomic sampling. *Cladistics*, **20**, 558–578.

WOOD, R. A., ZHURAVLEV, A. Y. & DEBRENNE, F. 1992. Functional biology and ecology of Archaeocyatha. *Palaios*, **7**, 131–156.

WOOD, D. A., DALRYMPLE, R. W., NARBONNE, G. M., GEHLING, J. G. & CLAPHAM, M. E. 2003. Paleoenvironmental analysis of the late Neoproterozoic Mistaken Point and Trepassey formations, southeastern Newfoundland. *Canadian Journal of Earth Sciences*, **40**, 1375–1391.

WOOLLACOTT, R. M. & PINTO, R. L. 1995. Flagellar basal apparatus and its utility in phylogenetic analyses of the Porifera. *Journal of Morphology*, **226**, 247–265.

YAHEL, G., SHARP, J. H., MARIE, D. & GENIN, A. 2003. *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: bulk DOC is the major source for carbon. *Limnology and Oceanography*, **48**, 141–149.

YUAN, X., XIAO, S., PARSLEY, R. L., ZHOU, C., CHEN, Z. & HU, J. 2002. Towering sponges in an Early Cambrian Lagerstatte: disparity between nonbilaterian and bilaterian epifaunal tierers at the Neoproterozoic-Cambrian transition. *Geology*, **30**, 363–366.

ZRZAVY, J., MIHULKA, S., KEPKA, P., BEZDEK, A. & TIETZ, D. 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics*, **14**, 249–285.