

The ‘biomineralization toolkit’ and the origin of animal skeletons

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ABSTRACT

Biomineralized skeletons are widespread in animals, and their origins can be traced to the latest Ediacaran or early Cambrian fossil record, in virtually all animal groups. The origin of animal skeletons is inextricably linked with the diversification of animal body plans and the dramatic changes in ecology and geosphere–biosphere interactions across the Ediacaran–Cambrian transition. This apparent independent acquisition of skeletons across diverse animal clades has been proposed to have been driven by co-option of a conserved ancestral genetic toolkit in different lineages at the same time. This ‘biomineralization toolkit’ hypothesis makes predictions of the early evolution of the skeleton, predictions tested herein through a critical review of the evidence from both the fossil record and development of skeletons in extant organisms. Furthermore, the distribution of skeletons is here plotted against a time-calibrated animal phylogeny, and the nature of the deep ancestors of biomineralizing animals interpolated using ancestral state reconstruction. All these lines of evidence point towards multiple instances of the evolution of biomineralization through the co-option of an inherited organic skeleton and genetic toolkit followed by the stepwise acquisition of more complex skeletal tissues under tighter biological control. This not only supports the ‘biomineralization toolkit’ hypothesis but also provides a model for describing the evolution of complex biological systems across the Ediacaran–Cambrian transition.

Key words: Ediacaran, Cambrian, skeletons, biomineralization, metazoan toolkit

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I. INTRODUCTION

In modern ecosystems, animal skeletons are hugely diverse in terms of morphology, ecology, function, and mineralogy,

and are found across all the major divisions of Metazoa (Knoll, 2003). Latest Ediacaran and early Cambrian rocks also preserve a staggering diversity of animal skeletons, largely in the form of small shelly fossils (Bengtson, 2004).

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Although many remain enigmatic, they are increasingly being recognised as representatives of modern skeleton-building animal groups (Murdock & Donoghue, 2011). They also provide evidence of a huge ecological shift across the Ediacaran–Cambrian transition. In fact, the origin of biomineralization is an integral component of the complex interaction of factors responsible for the explosion in animal diversity (Smith & Harper, 2013). Developmental genetics, following the identification of deep homology in regulatory genes, provides an alternative, independent, record for unravelling early animal evolution. Regulatory genes associated with a range of bilaterian-specific developmental programs have homologues in sponges and cnidarians (Marshall & Valentine, 2010), and it seems that the vast majority of regulatory genes are not novel to specific eumetazoan groups, but were pre-adapted in their unicellular ancestors. The discovery of this developmental 'toolkit' has generated hypotheses that can, in part, explain the rapid diversification of the Metazoa during the Cambrian (Erwin *et al.*, 2011). The establishment of this conserved toolkit is thought to have been followed by successive 'canalization' since the Cambrian, that precluded the evolution of novel high-level morphological traits after the evolution of the majority of animal phyla (Marshall, 2006). First-appearance data from fossil evidence of animal phyla and classes, coupled with molecular clock divergence dates, indicate that (with the exception of groups with very low preservation potential) all major phyla originate in the Ediacaran–Cambrian transition, and all bilaterian classes are in place by the end of the Cambrian, with virtually no innovation at higher taxonomic levels in the subsequent 500 million years (Erwin *et al.*, 2011). One of the key innovations during the Cambrian Explosion is the widespread use of mineralized skeletons, and there is a growing body of evidence (reviewed herein) that a pre-adapted 'biomineralization toolkit' played a crucial role. This hypothesis makes a number of predictions of the fossil record of the earliest skeletons: (i) mineralized tissues should appear independently in different biomineralizing lineages; (ii) biomineralized taxa should be pre-dated by soft-bodied representatives; (iii) the first skeletal tissues should show greater disparity than their descendants, being subject to looser biological control prior to canalization. These predictions are here tested by compiling what is known of the earliest fossil examples of each of the major groups of biomineralizing animals, alongside evidence from the skeletogenesis of their extant counterparts.

II. THE EVIDENCE FOR THE EARLY EVOLUTION OF ANIMAL SKELETONS

(1) Tube world: cnidarians and sponges

The earliest evidence for widespread and relatively large-scale (i.e. >1 mm) mineralized skeletons with possible animal affinity is around 550 million years old (Grant, 1990), in the terminal Ediacaran. This suite of largely tubular organisms

dominate the fossil record of biomineralized organisms up until midway through the Fortunian, around 536 million years ago (Ma) (e.g. Budd & Jackson, 2016). The affinity of these (and the majority of) organisms of Ediacaran age remains controversial. However, when interpreted as metazoans, they are generally regarded as cnidarian- or poriferan-grade animals, but see Schiffbauer *et al.* (2020) for an alternative interpretation of some cloudinomorphic fossils. The fossils of this period exhibit a range of mineralogies in their skeletons. *Sinotubulites* (Fig. 1A) is testament to aragonitic biomineralization in a complex organism in the terminal Ediacaran (Chen *et al.*, 2008). *Cloudina*-type fossils (Fig. 1B) may represent the earliest example of cnidarian biomineralization, in the form of stacked cones composed of high-Mg calcite (Wood, 2011). Wood, Ivantsov, & Zhuravlev (2017) note that many of these Ediacaran macrobiota exhibit microgranular or fibrous microstructures with a non-hierarchical organization, suggestive of biomineralization mechanisms under loose control. Further to this, a number of entirely organic contemporary counterparts to Ediacaran skeletal fossils have been described (Wood *et al.*, 2017), including for *Cloudina* (*Conotubus*) and *Sinotubulites* (*Corumbella*). These are reconstructed as representing the independent acquisition of biomineralized tissue utilising common calcification processes and pre-existing organic scaffolds.

Anabaritids have their origin in the terminal Ediacaran (Cai *et al.*, 2019), and are one of the most abundant components of the earliest small shelly faunas. These triradially symmetrical tubes made up of growth lamellae consisting of bundles of fibres (Fig. 1D) are interpreted to have been originally aragonitic (Kouchinsky & Bengtson, 2002). Their affinity remains enigmatic, but they are usually regarded as closely related to cnidarians. Their fibrous aragonitic microstructure is consistent with a pattern of early loose control over biomineralization, but without a better constrained phylogenetic hypothesis it is difficult to establish a pattern. Similarly, first appearing through the earliest three stages of the Cambrian, the aragonitic Coleoloida and phosphatic Hyolithemintida and Byroniida, are tubular problematica with possible cnidarian affinity (Kouchinsky *et al.*, 2011). These groups would increase the diversity of modes of biomineralization in the early evolution of the cnidarian skeleton. The earliest putative anthozoans (corals) are a suite of fossils known as 'coralomorphs' including the Khasaktiidae (high-Mg calcite), the Hydroconozoa (low-Mg calcite) and the Tabulaconida, with both aragonitic and high-Mg calcite (Zhuravlev, Debrenne, & Lafuste, 1993). The 'coralomorphs' are not a natural group, but a grade of taxa best regarded as closely related to corals (Landing *et al.*, 2018). Medusozoans ('jellyfish') are in most cases entirely soft-bodied, however, the phosphatic conulariids are generally regarded as an extinct group of scyphozoans, with possible origins in the latest Ediacaran.

Recent evidence supports a poriferan affinity for the Ediacaran skeletal macrofossil *Namapoikia* (Fig. 2E), with a likely originally aragonitic skeleton formed by the calcification of a pre-existing organic framework comparable to the extant

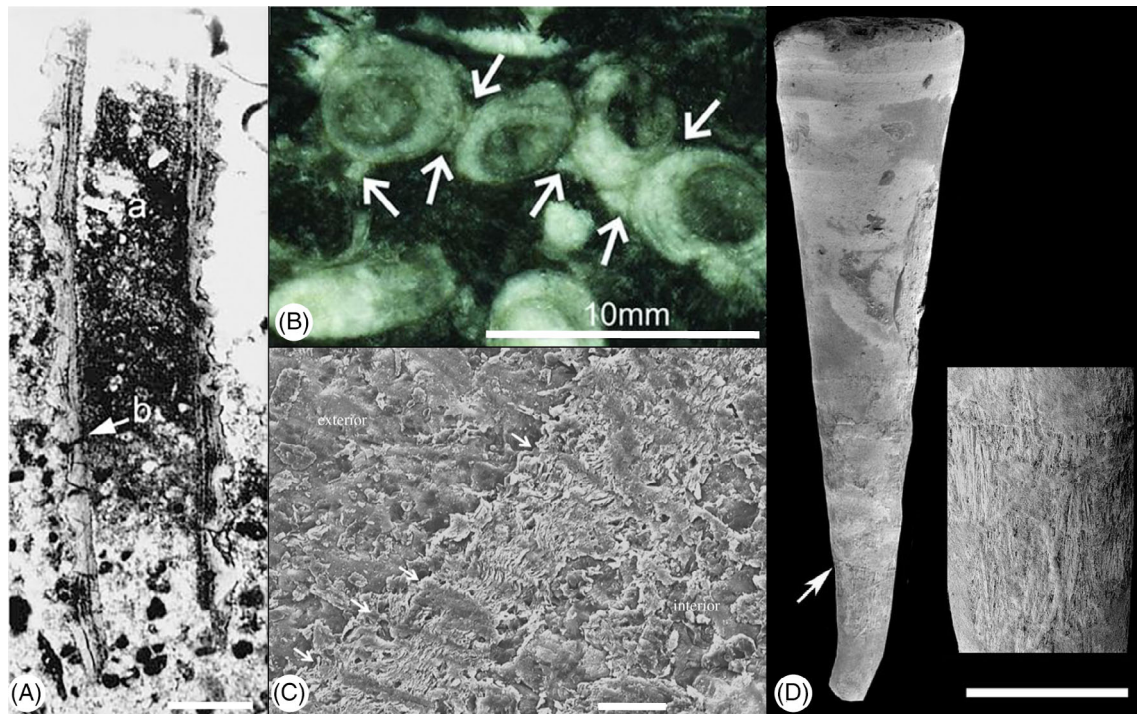


Fig 1 Microstructural diversity in the first biomineralizing animals. (A) The ‘tube-in-tube’ structure of *Sinotubulites* in longitudinal section exemplified at point ‘a’, arrow at ‘b’ indicates point of fracture (from Chen *et al.*, 2008). (B) Transverse sections through several *Cloudina* showing ‘funnel-in-funnel’ structure and calcite joining neighbouring individuals indicated by arrows (from Penny *et al.*, 2014). (C) Tripartite organisation of the skeleton of *Namacalathus*, columnar microlamellar inflections indicated by arrow (from Zhuravlev, Wood, & Penny, 2015). (D) *Anabarites* with close-up of apical end showing fibrous microtexture, at the point indicated by the arrow (from Kouchinsky *et al.*, 2009). Scale bars: A, 800 μm ; B, 10 mm; C, 100 μm ; D, 500 μm .

calcifying demosponge *Vaceletia* (Wood & Penny, 2018), although it has also been interpreted as a calcifying microbe (Antcliffe, Callow, & Brasier, 2014). If the sponge-affinity of *Namapoikia* is supported, it may represent the earliest evidence of co-option of a biocalcification toolkit inherited from the last common ancestor of metazoans for building skeletons (Germer *et al.*, 2015; Wood & Penny, 2018).

Stem (and possibly crown) representative of three of the extant sponge classes are also found in the Cambrian, but the pattern of acquisition of sponge skeletons is far from clear. Essentially the problem can be couched as either favouring convergence of spiculation in different lineages or plesiomorphy of the poriferan skeleton (Fig. 2). The fossil record is not decisive on either competing hypothesis, but there is palaeontological evidence for homology of the siliceous spicules of demosponges and hexactinellids (Botting, Cárdenas, & Peel, 2014; Botting, Zhang, & Muir, 2017). Furthermore, some early sponge fossils possess a mosaic of characters seen in different extant groups, such as bimineralic sclerites of *Lenica* (Fig. 2C) that may represent the primitive sponge skeleton (Botting *et al.*, 2012); originally siliceous spicules with hexactinellid-type morphology and calcarean-type organic sheaths (Harvey, 2010); and *Eiffelia* (Fig. 2B) from the Burgess Shale that exhibits calcarean and hexactinellid-like symmetries and organic sheaths (Botting & Butterfield, 2005). Some

authors argue these lines of evidence support bimineralic biomineralization in the last common ancestor of modern sponges, implying loss of silica in the Calcarea, and loss of calcite in the other classes (Botting & Butterfield, 2005; Botting *et al.*, 2012). However, an analysis by Harvey (2010) suggests a more parsimonious solution is convergent evolution of sheaths and hexactine-like spicules, and that the early sponge skeleton exhibited high initial disparity perhaps followed by increasing constraint and canalization, entirely consistent with the biomineralization toolkit hypothesis. The recently described *Vasispongia delicata* (Fig. 2F), interpreted as a stem-silicean or stem-hexactinellid sponge, possessed spicules with an organic axial filament surrounded by a weakly silicified layer (Tang *et al.*, 2019). In the preferred scenario for sponge character evolution presented here (Fig. 2D), the spicules of *V. delicata* represent another example of early independent evolution of biomineralization under weak biological control.

The enigmatic sac-like cancelloriids (part of the ‘Coeloscleritophora’, see also see Section II.2) are problematic because although their overall morphology, inferred mode of life (e.g. Bengtson & Collins, 2015), and growth (Cong *et al.*, 2018) are very sponge-like, their sclerite microstructure is closely comparable to that of halkieriids, sachtids and siphononuchitids (Porter, 2008) which have a molluscan

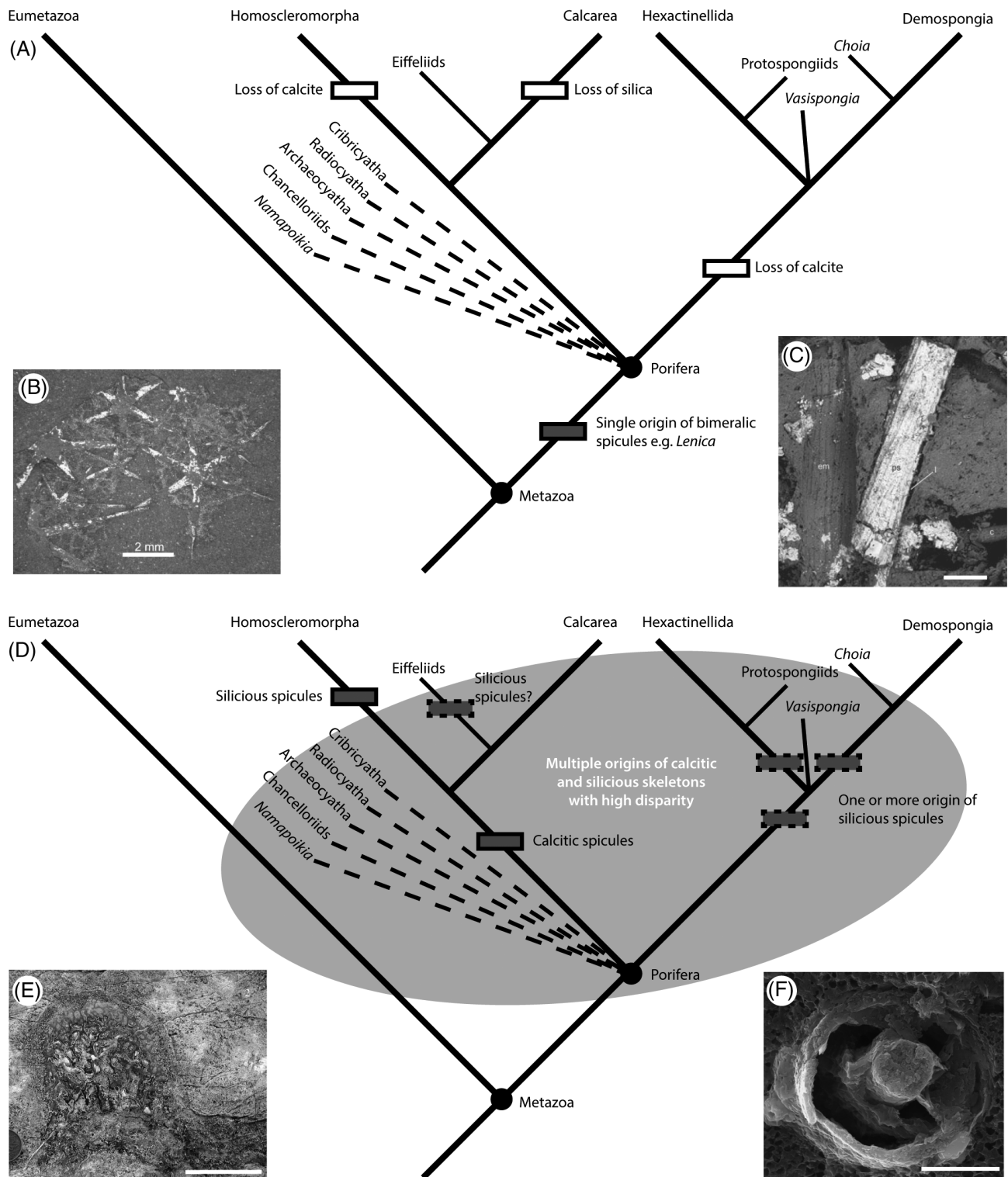


Fig 2 Alternative hypothesis for the origin of the poriferan skeleton (A,D) with examples of early sponge skeletons (B,C,E,F). (A) A single origin of bimineralic spicules (exemplified by those of *Lenica*) with subsequent reduction and modification in each of the extant sponge groups. (D) Alternatively, there were multiple origins of calcareous and siliceous spicules, supported by the high diversity of spicule types in Cambrian Stages 2 and 3. This is reflected by a range of taxa with disparate skeletal forms near the base of the Porifera, encompassed by the dark ellipse. The relationships between extinct sponge groups, and other taxa with proposed sponge affinities is unclear – dashed lines reflect this uncertainty. (B) *Eiffelia globosa*, a likely stem-calcarean (from (Figure legend continues on next page.)

affinity. Porter (2008) argued that commonalities of micro-structure are indicative of homology of the sclerites of coeloscleritophorans; in order to reconcile this with their proposed affinities, their sclerites must be plesiomorphic for Eumetazoa, and Coeloscleritophora must be paraphyletic (Bengtson & Collins, 2015). Alternatively, the common features of the skeletons of coeloscleritophorans are independently acquired but perhaps by co-option of the same molecular processes under similar selective pressures. This would predict the existence of a chancelloriid with an entirely organic precursor skeleton. Intriguingly, such a 'naked' chancelloriid was recently described (Cong *et al.*, 2018), although its affinities are as yet not entirely resolved.

Two studies (Jackson *et al.*, 2007; Germer *et al.*, 2015) described the molecular toolkit for biocalcification of the demosponges *Astrosclera willeyana* and *Vaceletia* sp., finding deeply conserved genes with a key role in biomineralization, which, in combination with the distribution of α -carbonic anhydrases (α -CAs) across Metazoa, suggests a single ancestral α -CA common to all metazoans that was subsequently duplicated and diversified in separate lineages. Furthermore Voigt *et al.* (2014) find an even more complex repertoire of α -CAs in two calcarean sponges, and reconstruct eight α -CAs in the last common ancestor of Porifera, with different and independent histories of duplication and loss in each sponge lineage. This is further elaborated by the acquisition of spicule-type-specific genes in individual lineages, such as the calcareous sponge *Sycon ciliatum* (Voigt *et al.*, 2017), demonstrating the independent evolution of a skeleton in each lineage.

This evidence for a 'biomineralization toolkit' in the first animals to biomineralize provides a mechanism, but what was the control on the timing of this evolutionary shift? Wood *et al.* (2017) propose that initial calcification of skeletons by animals in the terminal Ediacaran was a non-selective response triggered by environmental change, but by the early Cambrian increasing predation pressure drove an escalating defensive response, and tighter developmental control. This is supported by a shift into clastic environments and the decoupling of skeletal mineralogy from ambient seawater chemistry seen through the early Cambrian (Wood *et al.*, 2017). Thus, a combination of pre-adaptation in the genomes of soft-bodied ancestors of biomineralizing taxa, a changing environment and ecological escalation can explain the role of the skeleton in the Ediacaran–Cambrian transition.

(2) The lophotrochozoan common ancestor

Lophotrochozoan skeletons are hugely diverse, numerous and disparate in both extant taxa and throughout the Phanerozoic.

Relationships between spiralian phyla, i.e. protostomes not included within Ecdysozoa (Giribet, 2002), are not well resolved. Within Spiralia, mineralized skeletons are restricted to the Lophotrochozoa (*sensu stricto*), as defined by Halanych *et al.* (1995), namely bryozoans (or ectoprocts), brachiopods, molluscs and annelids – calcareous structures are also known from nemerteans and flatworms (Kocot *et al.*, 2017). The most robustly supported hypotheses for the relative relationships of these biomineralizing phyla resolve Bryozoa as sister to a clade consisting of annelids, brachiopods and molluscs (along with non-biomineralizing nemerteans and phoronids), the Trochozoa (Fig. 3), and is the phylogenetic framework used here. Nevertheless, a number of alternative hypotheses exist for the interrelationships of the lophotrochozoan phyla (see e.g. Kocot *et al.*, 2017), with different resulting implications for the evolution of mineralized skeletons. Although rarely recovered by molecular phylogenetics, traditionally bryozoans were included in a clade with brachiopods and bryozoans, the Lophophorata, based on the presence of a horseshoe-shaped tentacular feeding apparatus. This could imply a common ancestry of bryozoan skeletons and those of brachiopods. Although they share several ultrastructural features (Vendrasco *et al.*, 2010a), there are none to the exclusion of molluscs. Furthermore, there is no evidence for such a common ancestor in the fossil record (but see below discussion of *Namacalathus*) despite several fossils of putative stem-brachiopods, discussed further herein. The relative relationships between annelids, molluscs and brachiopods are not well resolved, but studies that place molluscs as sister to brachiopods (plus phoronids) would be consistent with a single origin of external mineralized 'shells' within Trochozoa (and secondary loss in phoronids, see discussion see Section II.4). However, this topology is increasingly poorly supported, with a sister relationship between annelids and brachiopods recovered by a greater number of analyses (Kocot *et al.*, 2016). Furthermore, evidence from transcriptomic and proteomic studies (Isowa *et al.*, 2015; Jackson *et al.*, 2015; Luo *et al.*, 2015) comparing brachiopod and mollusc shell biomineralization is revealing significant differences in the genetic machinery involved. A sister-group relationship between annelids and brachiopods presents an intriguing hypothesis for the homology of chaetae across both phyla. A chaetae-like structure is exactly what would be expected of the organic precursor of a biomineralized skeleton. Furthermore, there are structural similarities between brachiopod chaetae and aculiferan mollusc sclerites (Vendrasco *et al.*, 2010a).

There have been a number of fossil candidates for the common ancestor of some, or all, of the lophotrochozoan phyla that bear mineralized skeletons (Fig. 3). The enigmatic

(Figure legend continued from previous page.)

Botting & Butterfield, 2005). (C) Different modes of preservation in spicules of *Lenica*: external mould (em), pyrite steinkern (ps), removed external laminae (l) and aluminosilicate replacement (c) (from Botting *et al.*, 2012). (E) The putative Ediacaran sponge *Namapoikia riotoogensis* (from Wood & Penny, 2018). (F) Cross section of the demineralized spicule of *Vasispongia delicata* showing inner core (from Tang *et al.*, 2019). Scale bars: B, 2 mm; C, 5 mm; E, 50 mm; F, 5 μ m.

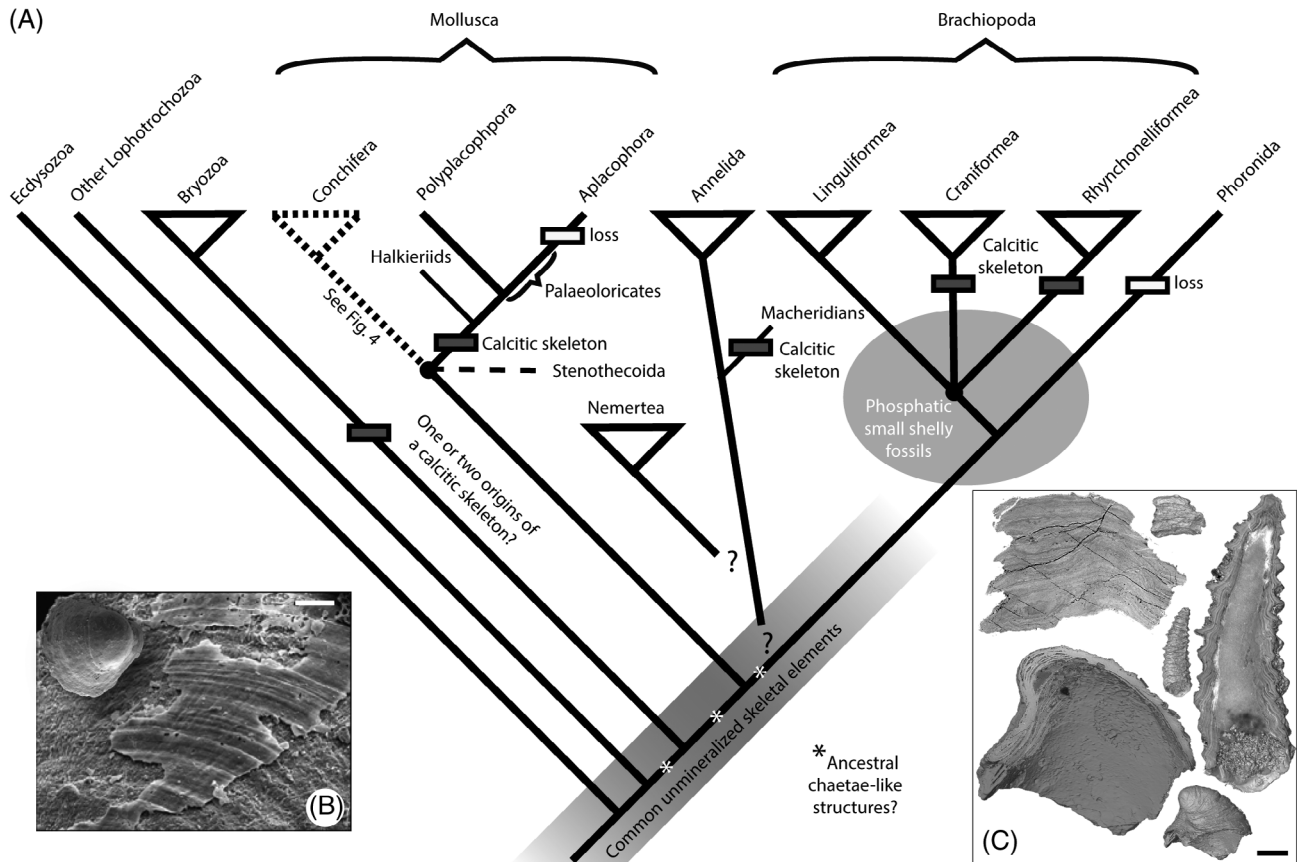


Fig 3 Summary of hypotheses for the origin of lophotrochozoan skeletons. (A) A common organic skeleton underwent mineralization in independent lineages numerous times during the Cambrian, with several subsequent instances of loss. In brachiopods, calcitic skeletons likely derived from the organo-phosphatic skeleton in different groups of small shelly fossils. The fossil evidence is currently unable to distinguish between a single origin of an organophosphatic skeleton for brachiopods+phoronids with subsequent loss or change of mineral system in a number of groups (Zhao *et al.*, 2017) or multiple origins. An ancestral chaetae-like structure may be the precursor organic skeleton for a putative clade containing annelids and brachiopods, all Trochozoa, or all biomineralizing lophotrochozoans. (B) The calcitic skeleton of the putative aculiferan mollusc *Ocruranus* showing lamello-fibrillar shell microstructure, example of complete valve inset (from Vendrasco *et al.*, 2010b). (C) Diversity of microstructure in the tomotioids, phosphatic stem-brachiopods. Virtual cross sections with renderings of entire valves inset: *Eccentrotheca* (top), *Lapworthella* (right), *Micrina* (bottom) (from Murdock *et al.*, 2012). Scale bars: B, 100 μ m; C, 100 μ m (top), 150 μ m (right), 100 μ m (bottom).

Ediacaran organism *Namacalathus* (Fig. 1C) exhibits features indicative of a possible lophotrochozoan affinity. These include asexual budding in a bilateral pattern and a skeleton with an organic-rich inner layer and a regular foliated ultrastructure with columnar microlamellar inflections, reminiscent of some brachiopod and bryozoan skeletons (Zhuravlev *et al.*, 2015). The common features suggest a similarity of mechanism for biomineralization between *Namacalathus* and lophophorate phyla, independently utilising a common genetic toolkit, given that the weight of evidence indicates that brachiopod and bryozoan skeletons are independently derived (see Section II.4). Nevertheless, the evidence for a lophophorate affinity of *Namacalathus* has been contested (e.g. Landing *et al.*, 2018) and has to remain equivocal given the uncertainty regarding the relationships between the extant taxa.

Aside from *Namacalathus*, perhaps the oldest fossil skeletons proposed to have lophotrochozoan (*sensu lato*) affinity are those of the protoconodont *Protohertzina*, appearing in the Fortunian (Khomentovsky & Karlova, 2005). Similarities in morphology, apparatus arrangement and internal structure, suggest protoconodonts represent the grasping spines of stem-chaetognaths (Szaniawski, 1982, 2002). Chaetognatha has proved to be one of the most problematic bilaterian phyla to resolve in the tree of life, but current consensus suggests they are protostomes more closely allied with spiralian than ecdysozoans (e.g. Marlétaz *et al.*, 2019). Grasping spines of extant chaetognaths are composed of chitin, whereas protoconodont elements are formed of primary calcium phosphate (Müller & Hinz-Schallreuter, 1998). Without better resolution of the relationships between chaetognaths and other biomineralizing groups it is difficult to explore this further, but

the current evidence is consistent with protoconodonts representing an independent origin of biomineralization that was either lost in subsequent lineages or was restricted to a now-extinct clade on the chaetognath stem.

Also among the small shelly fossils, along with rare examples preserving unmineralized and/or articulated skeletons, are the ‘coeloscleritophorans’ (Bengtson & Missarzhevsky, 1981). Although now not regarded as a natural group (e.g. Bengtson & Collins, 2015), largely through the discovery of exceptionally preserved fossils, various members of the ‘coeloscleritophora’ remain pertinent to this discussion. Sclerite-bearing animals known from Cambrian lagerstätten, *Halkieria*, *Wiwaxia*, and *Orthrozanclus*, along with similar disarticulated sclerites (e.g. siphononuchitids), have been interpreted to have a close affinity to molluscs, brachiopods and annelids (e.g. Conway Morris & Caron, 2007; Vinther *et al.*, 2017; Zhao *et al.*, 2017). The most widely supported consensus supports a molluscan affinity, Vinther *et al.* (2017) recover *Wiwaxia* as a stem-mollusc, suggesting a lack of a mineralized skeleton at the origin of crown-Mollusca, and support an independent Terreneuvian origin of biomineralization in conchiferans and aculiferans, including halkieriids and siphononuchitids as early biomineralized stem-acyliferans. Alternatively, a brachiopod affinity for halkieriids (Zhao *et al.*, 2017) would shift an independent origin of the aculiferan skeleton to the ‘palaeoloricates’ of the Furongian (Stinchcomb & Darrough, 1995), but has no direct bearing on the presence or absence of a mineralized skeleton in the last common ancestor of crown-molluscs (see also discussion on brachiopods in Section II.4). Similarly, alternative affinities of *Wiwaxia* as a stem-annelid or sister to a clade consisting of Annelida + Mollusca (Zhao *et al.*, 2017) are all consistent with an unmineralized last common trochozoan ancestor, and supportive of common ancestry of non-mineralized skeletal elements, such as sclerites and setae, being derived from serially repeated elements of an unmineralized scleritome (Fig. 4).

Biomineralization is not a common feature of modern annelids, being restricted to a few groups with much more recent origins (Parry, Tanner, & Vinther, 2014). However, throughout much of the Palaeozoic, an extinct group of annelids, machaeridians, bore a skeleton composed of calcitic shell plates (Vinther, Van Roy, & Briggs, 2008) that may represent another independent calcification of similar structures in a lophotrochozoan clade. Similarly mineralized tubes of vestimentiferan worms first occur much later in the Palaeozoic (Himmler *et al.*, 2008), with superficial similarities with various Ediacaran and Cambrian tubular fossils. An annelid affinity for some cloudinomorphic fossils has also been supported recently by putative soft tissues interpreted as guts (Schiffbauer *et al.*, 2020). However, given these cloudinomorphic fossils and vestimentiferan tubes have complex, different taphonomic histories and are peculiar to restricted environments (Haas *et al.*, 2009), a close affinity is not well supported. Nevertheless, should an annelid affinity for cloudinomorphs be verified, and given the known distribution of annelid skeletons, they would best be interpreted as an independent origin of biomineralization in the annelid stem.

Some nemerteans have been observed to bear calcified stylets. They appear to be formed of amorphous calcium phosphate (Stricker & Weiner, 1985), likely *via* calcification of a pre-existing organic scaffold (Stricker & Cloney, 1981; Stricker, 1984). The mechanisms of calcification in nemerteans is not well understood, nor is the evolution of these structures. The position of nemerteans as sister to annelids has been recovered in some molecular analyses, but not widely, and has few morphological characters to support it (Kocot *et al.*, 2017). Nemertean stylets, therefore, may also represent an independent evolution of biomineralization from a chaetae-like organic structure. However, without better resolution of the phylogenetic affinities of nemerteans and/or a fossil record of mineralized nemertean stylets, this remains equivocal.

Despite their skeletons being independently acquired and having an entirely soft-bodied common ancestor, molluscs, brachiopods and, to a lesser extent bryozoans, share some common skeletal features (Vendrasco *et al.*, 2010a), including shell pores, an organic-rich shell, a secretory mantle, a periostracum, a complex shell differentiated into layers, and similar shell microstructures (Vendrasco *et al.*, 2011b). Comparison of Hox gene clusters in brachiopods, molluscs and annelids supports the homology of chaetae across the Lophotrochozoa, and the homology of the chitin network associated with the shell fields of brachiopods and molluscs (Schiemann *et al.*, 2016). However, different Hox genes are deployed in the shell fields of brachiopods and molluscs, suggesting they do not share an ancestral role in specification of the shell-forming epithelium. Expression patterns of *engrailed* in larvae of molluscs and brachiopods show it is involved in shell formation in both phyla, but there are no conserved non-coding sequences and a comparison of gene synteny shows significant differences in the organization of their *engrailed* genes (Shimizu *et al.*, 2017), suggesting independent co-option of *engrailed* for shell formation (rather than by common ancestry) in brachiopods and molluscs, at least.

Based on the distribution of skeletons in molluscs and brachiopods, and the identification of commonalities in chitin synthases and bone morphogenetic protein (BMP) signalling, Luo *et al.* (2015) suggest three models for the evolution of common molluscan and brachiopod skeletons (supp. fig. 28 in Luo *et al.*, 2015). Two models propose a common ancestor with a mineralized skeleton (either phosphatic or calcareous) with subsequent loss or modification in different lineages. Given the evidence for multiple origins of skeletal tissues in molluscs, and the absence of such an ancestor in the fossil record, we can reject both these models. The third model proposes a common ancestor from which both phyla inherited a chitinous scaffold, mineralized with different phases in different lineages. This is consistent with all the evidence reviewed herein, and is consequently supported.

(3) Molluscs

Although the deep relationships of the extant mollusc groups are not fully resolved (Vinther, 2015), and the position of the

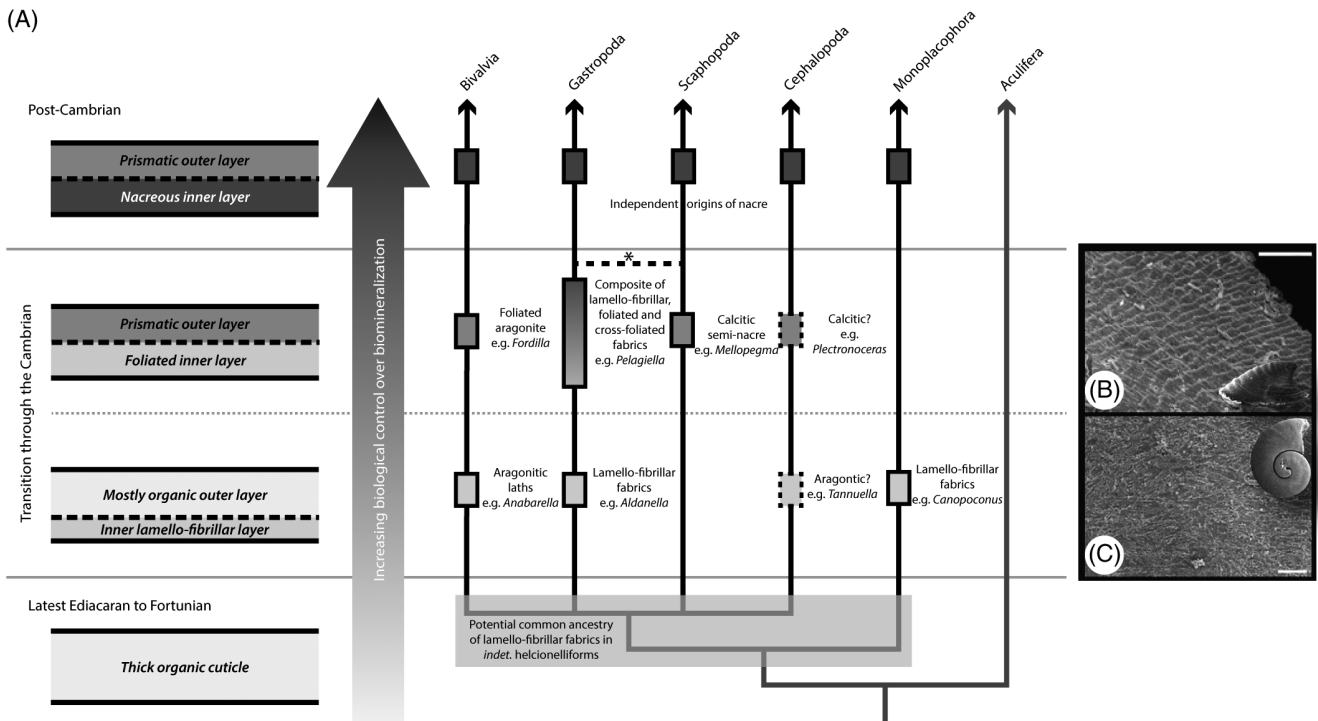


Fig 4 Evolution of the conchiferan skeleton. (A) The first conchiferan molluscs exhibit various loosely constrained lamello-fibrillar fabrics. From an ancestral organic scaffold, different mollusc groups first evolved this inner lamello-fibrillar mineralized layer in the Cambrian. These give rise in different groups, sometimes in different minerals, to laths and foliated textures, and ultimately nacre later in the lower Palaeozoic (after Vendrasco *et al.*, 2015). Crucially, this represents a general trend in increasing control over biomineralization in several lineages evolving in parallel. Uncertainty around the phylogenetic relationships of the extant classes, and affinities of some fossil taxa, leaves some ambiguity over the origin of the first lamello-fibrillar tissues. A recent analysis (Kocot *et al.*, 2020) suggests a sister relationship for Gastropoda and Scaphopoda and a later origin of the clade (the starred lineage), in which case the fossil taxa represented would be stem to this clade with a single common origin of a complex shell microstructure. (B) Prismatic microstructure of *Pojetaia* and (C) lamello-fibrillar fabrics of *Aldanella*; examples of entire shells inset (from Vendrasco *et al.*, 2010b). Scale bars: 100 μ m.

numerous fossil groups with proposed molluscan affinity is equally equivocal, molluscs are arguably the oldest bilaterian animals with a skeletal fossil record (with the possible exception of chaetognaths, see Section II.2). A division between those molluscs that bear a conch or shell (bivalves, gastropods, scaphopods, cephalopods and monoplacophorans), the Conchifera, and those that do not (chitons and aplousophorans), the Aculifera, is well supported (Vinther, 2015). The origin of Conchifera and the divergence and diversification of its constituent classes is estimated to have taken place across a very short window of time in the very latest Ediacaran to earliest Cambrian (dos Reis *et al.*, 2015; Vinther *et al.*, 2017; Kocot *et al.*, 2020), roughly coincident with the first appearance of their fossils. This, along with the diverse derived body plans of extant conchiferans, has made establishing their relationships challenging. Monoplacophora are generally regarded as sister to a clade containing the four other conchiferan classes, but virtually all other possible topologies have been suggested (Kocot *et al.*, 2011). The fossil record is also ambiguous, with representatives of all five conchiferan groups present by the end of the Fortunian, including stem and potentially crown taxa. They exhibit a range of

mineralogies, including aragonite, high- and low-Mg calcite and unknown or mixed calcareous forms (Wood & Zhuravlev, 2012). Among the earliest of these are the enigmatic 'helcionelliforms' many of which cannot be confidently placed within any one group. Many helcionelliforms, along with taxa for which there is better resolution of their affinity, exhibit various lamello-fibrillar [*Canopoconus*, stem-Monoplacophora; *Aldanella* (Fig. 4C), stem-Gastropoda] and crossed-bladed aragonitic (*Anabarella*, *Watsonella*, stem-Bivalvia) microstructures indicative of loose control over biomineralization (Vendrasco *et al.*, 2010a, 2010b, 2015). These gave rise in different groups, sometimes in different minerals, to laths and foliated textures: foliated aragonite in the stem-bivalves *Fordilla* and *Pojetaia* (Fig. 4B; Vendrasco, Checa, & Kouchinsky, 2011a) and the paragastropod *Yuwenia* (Kouchinsky, 2000a); a unique cross foliated aragonitic microstructure in the putative stem-gastropod *Pelagiella* (Li *et al.*, 2017); and calcitic semi-nacre in the probable stem-scaphopod *Mellopegma* (Vendrasco *et al.*, 2011b). Ultimately nacre evolved independently in each conchiferan group later in the lower Palaeozoic (Vendrasco *et al.*, 2011a). The fossil evidence suggests that from an

ancestral organic scaffold, different mollusc groups first evolved an inner lamello-fibrillar mineralized layer in the Terreneuvian, then foliated and prismatic layers, and ultimately multiple independent origins of nacre in different lineages (Vendrasco *et al.*, 2011a), with a combination of crossed lamellar microstructures and nacre being the dominant fabrics from the Ordovician (Li *et al.*, 2017). Crucially, this represents a general trend in increasing control over biomineralization in several lineages evolving in parallel (Fig. 4A). This hypothesis is compatible with the competing resolutions of their interrelationships and with the evidence from divergence time estimates which place the diversification of the conchiferan classes shortly after the origin of crown-Conchifera. The fossil record suggests either entirely soft-bodied ancestors to each of the conchiferan classes, or an inherited inner lamello-fibrillar calcified layer, independently elaborated in each lineage, perhaps represented by some helcionelliform fossil taxa. However, a recent study by Kocot *et al.* (2020) recovered a sister relationship of Gastropoda and Scaphopoda and estimated a maximum divergence time of this clade to around the base of Cambrian Stage 3 [95% highest posterior density (HPD) interval 429.25–525.494 Ma]. This would exclude older fossils as stem-gastropods (e.g. the earliest paragastropods, pelagiellids and aldanellids) or stem-scaphopods (e.g. *Mellopegma*), and implies the assembly of a complex multi-layered shell structure in the common ancestors of gastropods and scaphopods. More work needs to be done on the affinity of these fossils, and on biomineralization in scaphopods, to test this hypothesis.

The aculiferan molluscs today consist of the Polyplacophora (chitons) which possess a shell composed of eight aragonitic valves, and the so-called aplacophoran ‘worms’ (Aplacophora = Caudofovates + Solenogastres) that lack a mineralized skeleton entirely. The fossil record, however, reveals that aplacophoran ancestors bore similar (calcareous, possibly aragonitic) skeletons consisting of imbricated valves to those of chitons. The ‘palaeoloricates’ originated in the Furongian (Stinchcomb & Darrough, 1995), and are reconstructed as a paraphyletic assemblage of stem-aplacophorans (Sigwart & Sutton, 2007). The Polyplacophora have their immediate origins in the ‘multiplacophorans’ of the Ordovician (Sigwart & Sutton, 2007). However, the polyplacophoran skeleton may be present in the Fortunian; the small shelly fossils *Ocruranus* and *Eohalobia* are reconstructed as part of a multi-plated scleritome of a stem-polyplacophoran (Vendrasco *et al.*, 2009). They possess a lamello-fibrillar fabric (Fig. 3B), similar to other Cambrian molluscs among the small shelly fossils, and were likely originally aragonitic. The Paracarinachitidae and Cambroclavida are also lophotrochozoans with a possible stem-polyplacophoran affinity (Kouchinsky *et al.*, 2011; Sato *et al.*, 2014), although their scleritome reconstruction remains enigmatic. They bear a skeleton with an original aragonitic mineralogy and similar fibrous ultrastructures (Qian & Bengtson, 1989; Conway Morris & Chen, 1991). Whether or not the last common ancestor of all aculiferans had a mineralized skeleton, or if

biomineralization was independently derived in paleoloricates and polyplacophorans, remains unclear. However, the siphonoguchitids and halkieriids have been proposed as stem-auliferans (Vinther *et al.*, 2017; but see alternative affinities discussed in Section II.2) and possess mineralized (aragonitic) sclerites with some unique microstructural features consisting of an outer organic layer and inner layer comprised of aragonitic fibres (Porter, 2004, 2008). Although the affinity of siphonoguchitids and halkieriids is still under debate, if they do represent the plesiomorphic aculiferan skeleton, they may reveal an initially less well-constrained mode of shell secretion, followed by a lamello-fibrillar fabric evident in *Ocruranus/Eohalobia*, and ultimately the condition seen in modern polyplacophorans (Checa, Vendrasco, & Salas, 2017).

There is a great deal of evidence for the biomineralization toolkit in modern molluscan skeletons. Jackson *et al.* (2010) compared the skeleton-building gene sets of the abalone (*Haliotis asinina*) and the pearl oyster (*Pinctada maxima*), two molluscs that share a common ancestor in deep time, along with those expressed in embryonic skeletogenesis of the purple sea urchin (*Strongylocentrotus purpuratus*). They found very few commonalities in the transcriptome of the two molluscs, suggesting they independently evolved nacre since they diverged in the latest Pre-Cambrian. Critically, many of the genes shared by the abalone and the pearl oyster are also common to the sea urchin. This suggests that genes present in the (entirely soft) common ancestor of all bilaterians were exapted into a role of building skeletons. The independent origin of genes encoding mantle secretory proteins in gastropods and bivalves was further supported by subsequent studies (Aguilera *et al.*, 2017), and the common role of repetitive low-complexity domains in molluscan biomineralization is now well established (Kocot *et al.*, 2016; McDougall & Degnan, 2018). Luo *et al.* (2015) use comparative transcriptomics to confirm different origins of biomineralization-related genes in molluscs, brachiopods and vertebrates. Most of these genes can be found in both *Lingula* and humans, suggesting that they have general functions other than shell formation. The 30 genes shared by all selected genomes are mainly involved in cellular and metabolic processes and with other diverse functions not limited to biomineralization, suggesting that these genes may have been co-opted independently in each mollusc lineage. There is also new evidence from whole-genome and transcriptomic data for a much more recent co-option of the biomineralization toolkit in molluscs. The scaly-foot snail (*Chrysomallon squamiferum*) bears a complex skeleton of shells and plates reminiscent of certain Cambrian fossil taxa, but evolved in the Cenozoic (Sun *et al.*, 2020). These authors found that the transcription factors in the scale-secreting mantle differ to those of the shell-secreting mantle and are common not only to relatively distantly related molluscs (e.g. in aculiferan mantle) but also to a wider sample of lophotrochozoans (e.g. in brachiopod shells). This implies an ancient origin for these transcription factors as part of a biomineralization toolkit. Finally, the biomineralization toolkit hypothesis predicts a pre-existing

organic skeleton, for which there is good evidence in molluscs, chitin being a basic component of the nacre matrix (Du *et al.*, 2017).

(4) Brachiopods

Brachiopods comprise three clades, Linguliformea, Craniiformea, and Rhynchonelliformea, which likely diverged prior to the advent of mineralization in the brachiopod skeleton (Harper, Popov, & Holmer, 2017). If we examine the fossil record of brachiopod skeletons, there is growing evidence that each of the major brachiopod clades, along with their likely sister group the phoronids, can trace their origins to phosphatic microfossils in Cambrian Stages 2 and 3. The fossil group most closely associated with the origin of linguliform brachiopods are the tommotiids (Fig. 3C; Zhang *et al.*, 2013). Although the validity of some of the characters used to ally tommotiids with brachiopods has been challenged (Murdock *et al.*, 2014), and their precise relationships remain unresolved, the microstructural evidence for this affinity is robust (Balthasar *et al.*, 2009). The phosphatic microfossils *Salanygolina* and *Fengzuella* have been proposed as stem-rhynchonelliform and stem-craniiform brachiopods, respectively, based on clear similarities in morphology and microstructure (Holmer *et al.*, 2009) or shared characters (Williams *et al.*, 1996). Given the predominance of low-Mg calcite in these groups, this suggests the calcareous

brachiopods independently acquired calcite skeletons. Furthermore, given the lack of evidence for a stem-brachiopod with a skeleton, even phosphatic biomineralization could be independent in each group. The linguliform brachiopod *Eoobolus*, known from Cambrian Series 2, with a biminerallitic shell structure incorporating calcium carbonate and calcium phosphate, gives an indication of how this transition may have occurred (Balthasar, 2007). Nevertheless, more work needs to be done to establish the relationships between the extant clades and their fossil relatives. Furthermore, the taxa inferred to occupy these stem positions often exhibit similar microtextures within an organic-rich shell plus some considerable variation within and between individual sclerites (e.g. Murdock *et al.*, 2012; Topper, Holmer, & Skovsted, 2013). This is suggestive of loose control over biomineralization.

Phoronids are the closest living relatives of brachiopods, and have even been suggested as the sister group to linguliforms (i.e. within a paraphyletic 'Brachiopoda' or 'Brachiozoa') although the weight of molecular evidence supports brachiopod monophyly (Sperling, Pisani, & Peterson, 2011). The phoronid *Phoronis* possesses far fewer chitin synthase genes, known to play a key role in biomineralization, than the brachiopod *Lingula* (Luo *et al.*, 2015, 2017), including all of those with close orthologues in molluscs. Luo *et al.* (2017), therefore, attribute this to loss of these genes in the phoronid lineage. This is consistent with the hypothesis that crown-

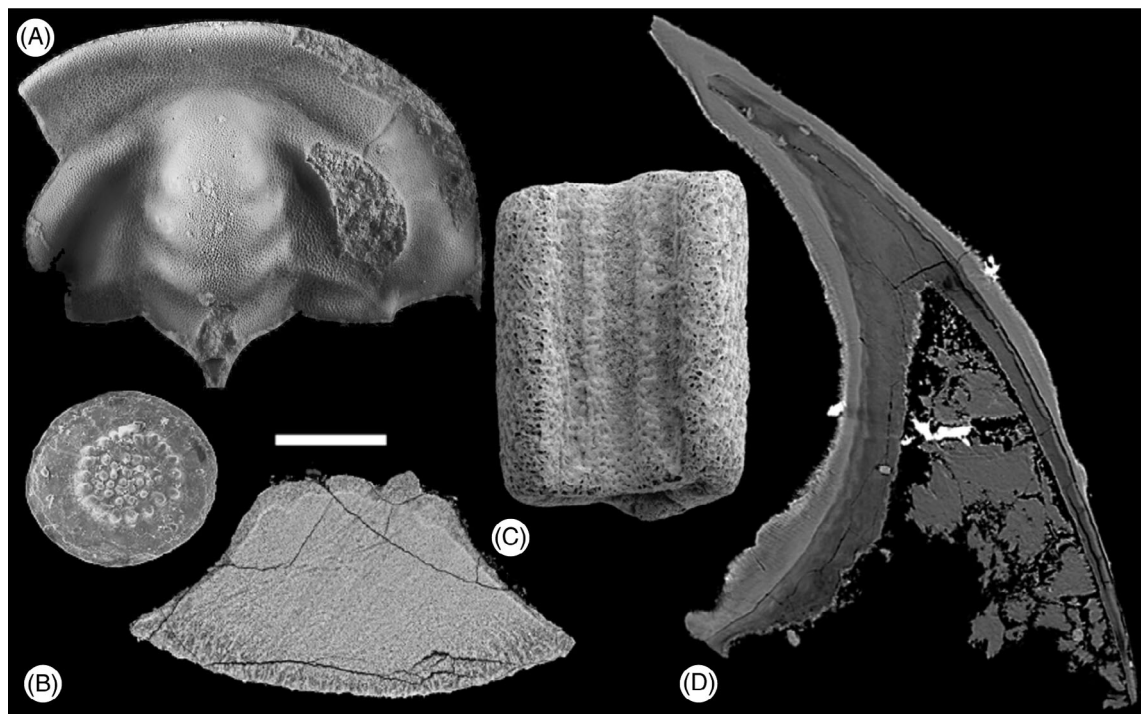


Fig 5 Examples of biomineralization in Cambrian ecdysozoans and deuterostomes. (A) Partial cephalon of one of the earliest trilobites, *Profallotaspis* (from Bushuev, Goryaeva, & Pereladov, 2014). (B) Dorsal view and cross section of mineralized cuticular plates of the palaeoscolecid *Hadimopanella* (from Harvey *et al.*, 2010). (C) Isolated Cambrian Series 2 echinoderm sclerite with characteristic stereom (from Zamora *et al.*, 2013). (D) Virtual cross section of the early euconodont *Proconodontus* (from Murdock *et al.*, 2013). Scale bar: A, 2.5 mm; B, 5 mm; C, 200 μ m; D, 100 μ m.

phoronids derive from a biomineralizing ancestor and with the reconstruction of *Eccentrotheca* as a stem-phoronid. The suite of shell-matrix proteins in the organophosphatic *Lingula anatina* (Linguliformea) are almost entirely different from those of the calcitic *Magellania venosa* (Rhynchonelliformea), with only five in common – which are all also found in the non-mineralizing *Phoronis australis* (Phoronida) and in other metazoans with functions not related to biomineralization (Luo *et al.*, 2017). This is, as the authors describe, suggestive that, ‘lineage-specific gene expansions, acquisition of novel genes and redeployment of extra-cellular matrix genes are involved in the evolution of lophophorate biomineralization’ (Luo *et al.*, 2017, p. 147). This supports multiple independent origins of mineralized skeletons in brachiopods, as in molluscs.

(5) Ecdysozoans

Biomineralization is not as widespread in extant ecdysozoans as in the other major divisions of the bilaterians, the calcified exoskeleton of several groups of crustaceans being a notable exception. Crustacean skeletons are also well represented in the fossil record, with stem-crustaceans bearing low-Mg calcite skeletons known from Miaolingian and Furongian ‘Orsten’-type deposits (Maas *et al.*, 2006). However, these are pre-dated by phosphatic groups (bradoriids and phosphatocopids) appearing in Stages 2 and 3, respectively (Kouchinsky *et al.*, 2011), that have been suggested to have crustacean affinity (Zhang, Dong, & Xiao, 2011). Further adding to the diversity of arthropod mineralized skeletons in the Cambrian are other phosphatic extinct taxa such as the aglaspidiids (Lerosey-Aubril *et al.*, 2013) and *Phytophilaspis* (Lin, Ivantsov, & Briggs, 2011) that may be more closely allied to chelicerates. The prevalence of both calcite and apatite as crustacean biominerals is not restricted to the Cambrian, with dual mineralization documented in a range of crustacean mandibles (Bentov *et al.*, 2016).

Perhaps the best known of all extinct arthropod groups, however, are the trilobites. Trilobite body fossils are first known from the beginning of Cambrian Series 2 (Fig. 5A) their skeletons predominantly composed of low-Mg calcite, but with some phosphatic examples (Dalingwater, Hutchinson, & Siveter, 1991), appearing relatively abruptly with high diversity, disparity, and established provincialism. A recent study has estimated that the true origin of trilobites pre-dates their fossil record, roughly coincident with the first *Rusophycus*-like trace fossils in the Fortunian, to which trilobites are often attributed as likely trace-makers (Paterson, Edgecombe, & Lee, 2019). These authors suggest this Terreneuvian trilobite gap could be indicative of a lack of biomineralization in the earliest trilobites, with an independent and synchronous origin of calcareous skeletons across multiple trilobite lineages. Trilobites also provide evidence for canalization of skeletal characters, a study of polymorphism in Palaeozoic trilobites shows a significant post-Cambrian reduction in morphological variability (Hughes,

Chapman, & Adrain, 1999; Hughes, 2007; Webster, 2007). There is, therefore, a consistent pattern in the fossil record of several arthropod groups independently deriving calcareous and phosphatic biomineralization.

In addition to arthropods, a host of other phosphatic microfossils believed to have ecdysozoan affinity appear in Cambrian Stages 2 and 3, some of which are also known from articulated or exceptionally preserved specimens. A number of lobopodian taxa bear phosphatic plates or spines. The lack of consensus over the affinity of the representatives in this grade of panarthropod taxa makes it difficult to assess the distribution of skeletal characters, nevertheless multiple independent origins (and subsequent losses) of phosphatic mineralization in stem-onychophorans (e.g. *Microdictyon* and *Collinsium*), and stem-tardigrades (e.g. *Onychodictyon ferox*) is supported by recent phylogenetic reconstructions (e.g. Yang *et al.*, 2015). The palaeoscolecsids (Fig. 5B) are cycloneuralian worms bearing small phosphatic plates proposed as stem-priapulids (Harvey *et al.*, 2010), but which have been allied to other groups such as nematomorphs (Hou & Bergström, 1994). As with lobopodians, and given the distribution of soft-bodied sister-groups, biomineralizing palaeoscolecsids most likely represent another example of an independent foray into hard skeletons in the early diversification of animals.

(6) Deuterostomes

In deuterostomes, the phosphatic vertebrate skeleton and the calcitic echinoderm skeleton (intercalated by the soft-bodied cephalochordates, tunicates and hemichordates) share cellular and molecular processes in their early skeletogenesis, yet the protein-coding genes essential to downstream biomineralization processes were acquired largely independently in both lineages (Livingston *et al.*, 2006). These observations support the hypothesis that there may have been a set of pre-adapted genes with elements that were independently utilised many times in the evolution of the skeleton, a ‘biomineralization toolkit’ (Rafiq *et al.*, 2014). The vertebrate endoskeleton is also highly likely to have evolved *via* the calcification of an organic scaffold (Hirasawa & Kuratani, 2015). This hypothesis has been strengthened by the presence of a collagenous skeleton in the cephalochordate *Branchiostoma floridae* (Yong & Yu, 2016). The generally accepted model for the origin of the vertebrate skeleton is that initial vertebrate tissue mineralization utilized collagen as a scaffold and SPARC (specific protein acidic and cysteine rich gene) as a critical mediator of mineral crystallization. Differentiation into different fibrillar collagens and a greater diversity of skeletal tissues followed a series of whole-genome or large segmental duplications, resulting in multiple independent origins of skeletal tissues (Kawasaki & Weiss, 2006).

The fossil evidence broadly supports this, with independent modifications to the plesiomorphic skeleton in every major skeletonizing vertebrate lineage and multiple times in different lineages (Keating & Donoghue, 2016). The oldest vertebrates with a mineralized skeleton are the conodonts

(including 'paraconodonts' and 'euconodonts'); these otherwise entirely soft-bodied jawless fish possessed a set of phosphatic tooth-like elements, with an abundant and widespread fossil record. Paraconodonts, with a dentin-like skeleton, are first known from the Guzhangian (Dong, 2004) followed by euconodonts after the evolution of the conodont crown, an enamel-like tissue unique to euconodonts (Fig. 5D). The first conodont skeletal tissues likely evolved *via* the calcification of cyclostome-like organic mouthparts and are largely organic, with hard parts apparently under relatively loose biological control. Subsequently we see the evolution of increasing basal body complexity, and ultimately the origin of the euconodont crown (Murdock *et al.*, 2013).

In parallel with vertebrates, echinoderms are a major biomineralizing phylum of deuterostomes. Echinoderms have a near-synchronous global origin around 525 million years ago (Smith, Zamora, & Alvaro, 2013; Zamora *et al.*, 2013), with a good Cambrian and Ordovician record of both articulated and isolated skeletal elements (Fig. 5C). While the five modern echinoderm body plans are present in the early Ordovician, the earliest (Cambrian) echinoderms belong to a number of strange groups, with a greater plasticity of construction (Zamora & Smith, 2012). The diagnostic structure of stereom, the calcium carbonate mineral that comprises the echinoderm skeleton, is common to all five modern classes of echinoderm, along with fossil representatives. However, the larval echinoderm skeleton is not so widespread, being either lost in asteroids and much reduced in holothurians or independently evolved in echinoids and ophiuroids (Raff & Byrne, 2006; Cary & Hinman, 2017; Morgulis *et al.*, 2019). Developmental evidence suggests that a common set of transcription factors are involved in adult skeletogenesis in all echinoderms, and some used in the developing larval skeleton evolved at the base of the phylum. Clade-specific parts of the regulatory network may have evolved in echinoids and ophiuroids during the independent evolution of the larval skeleton (Cary & Hinman, 2017; Morgulis *et al.*, 2019) however more recent evidence supports the loss of the asteroid larval skeleton rather than independent origins in other groups (Erkenbrack & Thompson, 2019). The rare aragonitic epidermal ossicles of hemichordates also appear to be independently derived from other deuterostome skeletons (Cameron & Bishop, 2012), with several unique biomineralization genes in the enteropneust *Saccoglossus kowalevskii*, while still sharing carbonic anhydrase involved in biomineralization with the sea urchin *Strongylocentrotus purpuratus*. Furthermore, the tunicates (phylogenetically intermediate between echinoderms and vertebrates) share the same ancient gene regulatory networks (Tiozzo *et al.*, 2008), but do not produce widespread biominerals, with the calcareous spicules seen in extant tunicates not known prior to the Upper Triassic (Wendt, 2018), providing further support for independent co-option for biomineralization in different deuterostome clades.

III. THE PHYLOGENETIC DISTRIBUTION OF ANIMAL SKELETONS

Here, I compile records of the first fossil appearances of extant biomineralizing groups, at (predominantly) class level, for all animal phyla. These are combined with example taxa from the Ediacaran–Ordovician fossil record that are interpreted as their closest extinct sister taxa, plus fossil taxa placed as sister to more inclusive clades, and selected additional, often enigmatic, taxa to cover the range of biomineralizing animals through this interval. These fossil ranges, along with the mineralogy of the skeletons they possessed, are mapped onto a consensus animal phylogeny [based principally on dos Reis *et al.* (2015) and Vinther *et al.* (2017)], then time-calibrated. Along with the extant and fossil ranges, minimum node age estimates were applied to 17 nodes representing high-level crown clades using the minimum composite 95% HPD limits of posterior divergence times of dos Reis *et al.* (2015), except for crown-Brachiozoa (absent from that analysis) where the Sperling *et al.* (2011) minimum node age was used, see online Supporting Information, Appendix S1. These were chosen to reflect a conservative but realistic minimum age for the nodes for which the fossil ranges are uninformative. No minimum node age was used for Porifera, owing to the lack of consensus around the age and relationships within and immediately outside this clade. These data were plotted against the International Commission on Stratigraphy (ICS) timescale (Cohen *et al.*, 2014) using the *paleotree* package (Bapst, 2012) in R, employing a minimum branch length of 0.5 million years.

The distribution of different mineral systems across biomineralizing animals (particularly if the fossil record is included) favours multiple independent origins of skeletal tissues (Fig. 6) with successive waves of first appearances of biomineralizing groups throughout the latest Ediacaran and Cambrian. The timing of origin of biomineralizing classes and, to a large extent, phyla is coincident with their first appearance in the fossil record (with fossils pre-dating the minimum estimates in some cases), while the minimum dates for the more inclusive clades pre-date the fossil record of animal skeletons. This is precisely the pattern to be expected if biomineralized skeletons, and the increased preservation potential they afford, post-date the divergence of skeletonized clades, supporting the hypothesis that the common ancestor of deeply divergent biomineralizing clades was unmineralized. Fossils representing aragonitic, calcitic (high- and low-Mg), organophosphatic and siliceous taxa are all present by the end of Cambrian Stage 2, and with a range of interpreted ecologies (Wood & Zhuravlev, 2012). This rapid diversification of known skeletal fossils, as well as the fact that non-mineralizing groups are intercalated with carbonate-, phosphate- and silica-mineralizers (Murdock & Donoghue, 2011), does not support a single origin of the animal skeleton followed by modifications and radiations of mineral types, but instead also points to multiple independent origins of biomineralization from descendants of a last

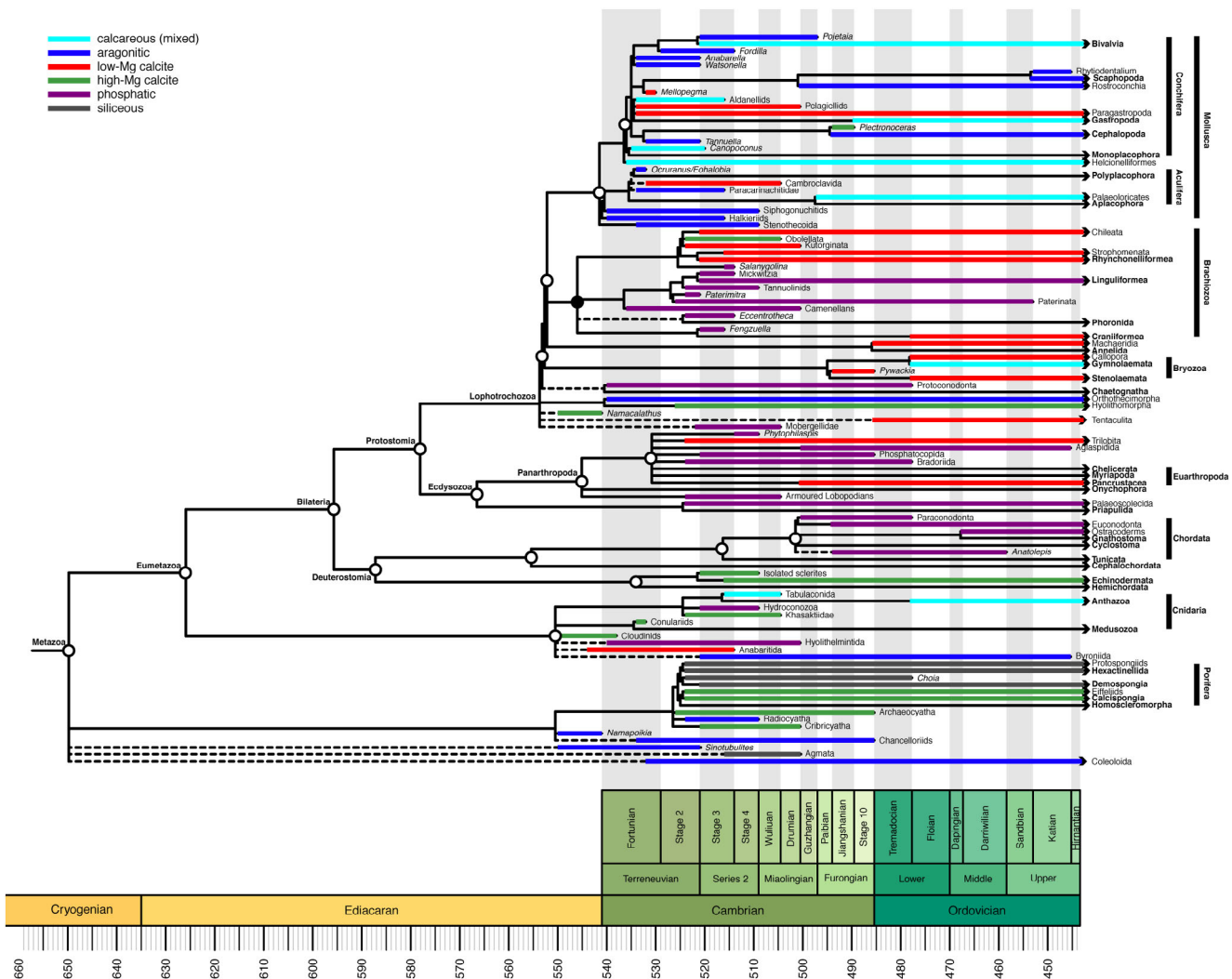


Fig 6 The phylogenetic and temporal distribution of the first animal skeletons. First appearance data for animal phyla and classes modified from Erwin *et al.* (2011), plotted on a consensus phylogeny [based principally on dos Reis *et al.* (2015) and Vinther *et al.* (2017)], with some clades collapsed to reflect disagreement in the literature, plotted against the ICS timescale (Cohen *et al.*, 2014). The bars represent stratigraphic ranges in stage level (or local stages for the Terreneuvian and Cambrian Series 2) time bins for the first appearances of their skeletons, colour-coded by their main mineralogy [data modified and expanded from Wood & Zhuravlev (2012)]. For full list of taxa, and references, see Appendix S1.

common metazoan ancestor that was entirely soft-bodied. Furthermore, this pattern of multiple origins of skeletal tissues extends within individual phyla (and in some cases, classes). For example: the first Cambrian molluscs, cnidarians and sponges show a greater degree of diversity of mineral system than their descendent clades, and calcitic skeletons likely evolved multiple times within brachiopods and arthropods as well as diverse phosphatic skeletons that are otherwise under-represented in modern assemblages.

From the compiled data represented in Fig. 6, I subsampled to retain only the extant lineages, their fossil ranges, and minimum node estimates. Fossils were excluded owing to the biased nature of the fossil sampling method, i.e. choosing representative taxa from a restricted temporal window. The

tree was time-calibrated using the method described above, to assign branch lengths, and, along with the dominant mineralogy of the extant biomineralizing representatives of the clade, was used to estimate the ancestral state of each node (Fig. 7). This was achieved using the *rerooting Method* (Yang, Kumar, & Nei, 1995) in the *R* package *phytools* (Revell, 2012), using an equal rates model. This maximum likelihood method estimates the marginal likelihood of each state of each character (in this case, biomineralization) for each internal node in the tree. An equal rates model, essentially an *Mk* model (Lewis, 2001), assumes an equal probability of changing between all pairs of states, across all branches in the tree. By using the character states at the tips, along with the branch lengths over which those states may change, the

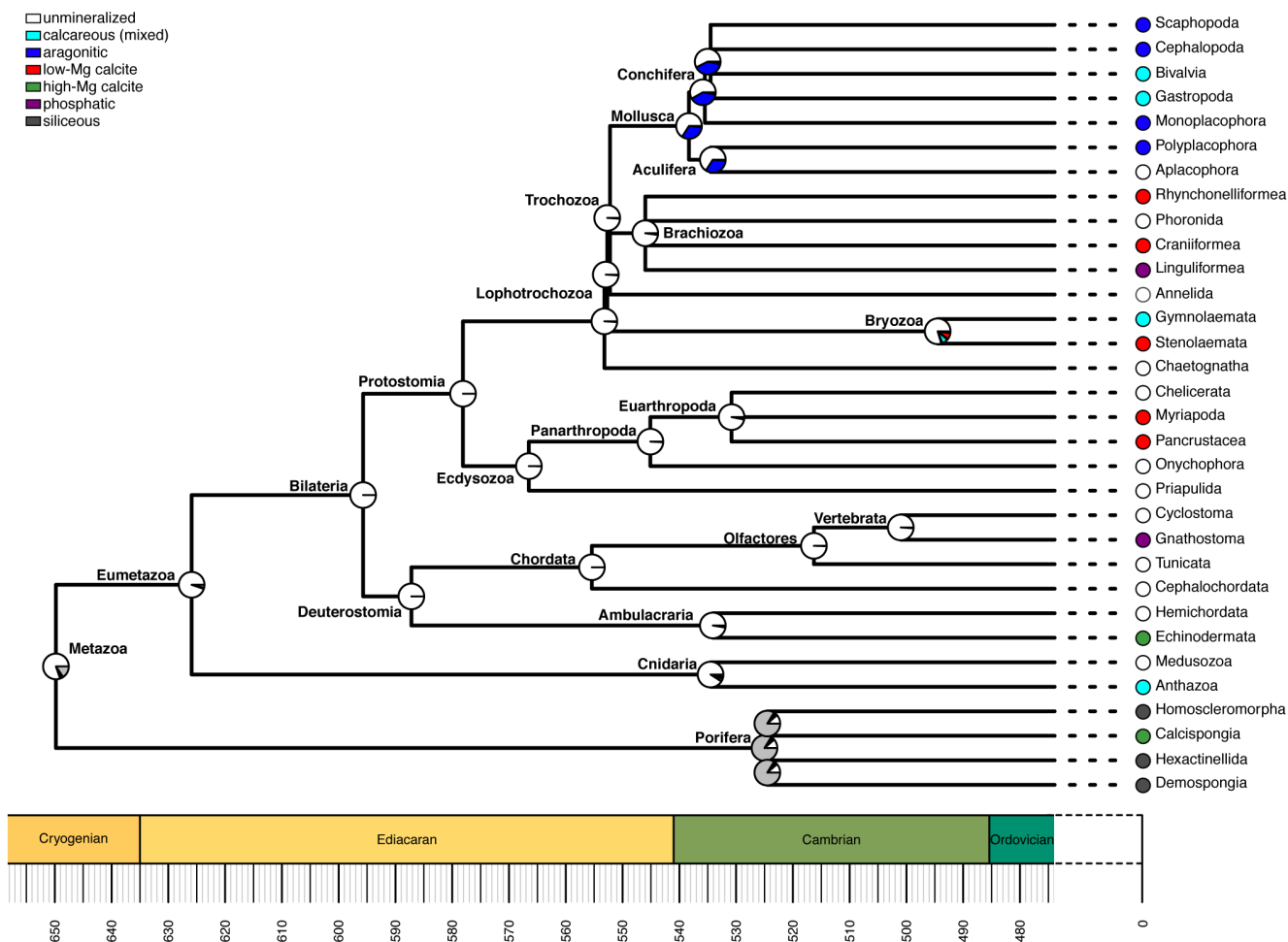


Fig 7 Ancestral state reconstruction of composition of animal skeletons. Nodes are colour-coded based on the marginal probability for each state at that node, estimated under a Mk model, represented by a pie chart. Colour-coding and topology are as in Fig. 6. Given the deep divergence times of these lineages, branch length is estimated from the fossil record of animal skeletons (summarised in Fig. 6). With the possible exception of the Porifera (but see explanation in text), the common ancestor of all phyla, and the higher taxa to which they belong, is reconstructed as entirely soft-bodied.

conditional probability of observing each character state, given the data, is first estimated for the root node. The tree is then progressively rerooted to estimate the marginal likelihoods for all internal nodes. The sum of the likelihoods for all possible states must equal one, thus the marginal likelihoods for each node are displayed as a pie chart with sum = 1 (Fig. 7). This methodology was chosen as it makes the fewest possible assumptions of character evolution, and can accommodate uncertainty of tree topology by randomly resolving polytomies with branches of zero length then matching nodes to derive the original topology.

This analysis strongly supports soft-bodied common ancestors to virtually all extant biomineralizing phyla and classes, which is consistent with the fossil and molecular evidence reviewed here. The exception are the sponges, which are reconstructed as likely ancestrally sharing a siliceous skeleton. This is supported by some fossil evidence (see

Section II.1 and Fig. 2), however, uncertainties in the results shed doubt on this conclusion. The branch lengths in the tree are estimated based on the fossil data (Fig. 6) and minimum composite 95% HPD limits of posterior divergence times of dos Reis *et al.* (2015). Due to uncertainty around the deep interrelationships of the poriferan classes, uncertainty of the relationship between sponges and the rest of the Metazoa, and a lack of a robust fossil calibration, the 95% HPD limits for this node are wide with a minimum divergence in the Carboniferous (dos Reis *et al.*, 2015). Therefore, the node age is derived from the oldest crown sponge fossil included (using the method above), which almost certainly represents a considerable underestimation of the true origin of the poriferan crown. With more fossils resolved as stem taxa to the crown-poriferan classes, and/or less uncertainty in the divergence time estimates, there would be much longer branch length between the origin of crown-Porifera and the origin

of its constituent classes, and therefore more time to accommodate the independent origin of sponge skeletons in each class, rather than inheriting an ancestral siliceous skeleton.

IV. SKELETONS AND THE ‘METAZOAN TOOLKIT’

The presence of a ‘metazoan toolkit’ of genes, and their associated networks of regulatory control and interactions, is now well established and its role in the origin of animal body plans is becoming clearer [see Erwin (2020) for a recent review]. In the context of the evolution of animal skeletons, can we establish which (if any) ancestral genomic regulatory elements were co-opted and if these are common to some, or all, biomineralizing animal lineages? In several cases gene sets and gene regulatory networks with a role in metabolism seem to have been co-opted for biomineralization. One of the best-supported candidates for the ancestral repertoire of genes co-opted for biomineralization is those involved in the calcium-regulated extracellular matrix system. Chitin synthase and bone morphogenetic protein signalling, along with calcium-binding and extracellular-matrix proteins [see Luo *et al.* (2015) for a full compilation] have been found to play key roles in biomineralization in molluscs (Shimizu *et al.*, 2011), vertebrates (Chen, Deng, & Li, 2012) and phosphatic brachiopods (Luo *et al.*, 2015). These observations are entirely compatible with the independent calcification of an inherited organic scaffold, utilising common metabolic pathways but employing different downstream biomineralization-related genes. There is compelling evidence that chitin, and therefore chitin synthase genes, is a crucial component of such a scaffold. A chitin network forms at the onset of brachiopod and mollusc shell fields (Schiemann *et al.*, 2016) and has been found to be expressed in epithelial cells of fishes and amphibians (Tang *et al.*, 2015). Brachiopods and molluscs have also possibly independently co-opted *engrailed* into shell formation (Shimizu *et al.*, 2017). Among the suite of 25 transcription factors found by Sun *et al.* (2020) are several involved in shell matrix protein production including *pif*, chitin-binding peritrophin-A domain gene, and chitin synthase. Carbonic anhydrases, which have a wide range of fundamental physiological roles, have also been shown to play a crucial role in biomineralization in calcareous sponges (Jackson *et al.*, 2007) and independently in different mollusc lineages (Jackson *et al.*, 2010), also playing a role in calcification of organic precursors. In vertebrates, an ancestral SPARC gene is thought to have given rise to a number of calcium-binding phosphoproteins *via* vertebrate-specific gene duplication (Kawasaki & Weiss, 2006), with potential paralogs in sea urchin SPARC/osteonectin genes and even in some lophotrochozoans (Jackson *et al.*, 2010). Cyclophilins, found to be integral to skeletogenic sea urchin primary mesenchyme cells (Livingston *et al.*, 2006), have similarities with proteins in mollusc shell-secreting cells (Jackson *et al.*, 2010). Finally, some shell matrix proteins (hephaestin and hemicentin) of the

brachiopod *Lingula* are also found in the coral skeletal organic matrix (Luo *et al.*, 2015), but have a role in metabolism in other metazoans. These lines of evidence all point towards independent co-option of similar gene sets involved in metabolism and, more specifically, calcium-regulation *via* calcification of an organic scaffold. This would be consistent with a physiological response to changing seawater chemistry (Wood *et al.*, 2017).

Intriguingly, the echinoderm skeleton may provide an alternative model for co-option playing a role in the origin of animal biomineralization, with its origin in nutrient or blood collection and transport. Morgulis *et al.* (2019) identify five transcription factors and three signalling pathways involved in vascular endothelial growth factor (VEGF) signalling that are common to biomineralization in echinoderms and vascular tubulogenesis in vertebrates. They find common cytoskeletal remodelling proteins essential for echinoderm spicule formation and the formation of vertebrate vascular tubes – structures with similar overall geometry but fundamentally different functions. This would represent a unique co-option of an ancestral tubulogenesis program for biomineralization in echinoderms. Although speculative, this may contribute to some of the unique properties of echinoderm skeletal tissues, such as the remarkable conservation of the microstructure of stereom and the apparent singular origin of echinoderm skeletons in contrast to multiple origins and progressive addition of complexity in virtually all other biomineralizing phyla.

V. CONCLUSIONS

(1) Mineralized tissues appear independently in different biomineralizing animal lineages. The numerous first appearances of skeletal tissues from the late Ediacaran to the early Ordovician are disparate in terms of mineralogy, phylogenetic distribution, functional ecology, and from across a range of time scales. This is also reflected in the modern distribution of biominerals suggestive of no common ancestry among skeletal taxa.

(2) Biomineralized taxa are pre-dated by soft-bodied representatives. Although soft-bodied counterparts of biomineralizing organisms are inherently less fossilizable, there is both direct and indirect evidence for them in the fossil record, such as in the terminal Ediacaran, a ‘naked’ chancelloriid, and the apparent gap in the early trilobite record filled with *Rusophycus*-type trace fossils.

(3) The first skeletal tissues in animals show greater disparity than their descendants, being subject to looser biological control prior to canalization. There is a consistent pattern in the fossil record of early animal skeletons of an organic scaffold inherited from a common ancestor, independent origins of similar laminar microstructures under loose control, followed by the evolution of more complex, more derived and increasingly entrenched microstructures. This is perhaps best documented in Cambrian molluscs, but also apparent

in, for example, anabaritids, *Namapoikia*, bimineralic sponge spicules, and paraconodonts.

(4) There is considerable evidence for the 'biomineralization toolkit' hypothesis whereby a common set (or sets) of ancestral genes were independently co-opted for building skeletons in many animal groups at the same time. The skeleton evolved many times, both across and within phyla (and even classes). But biomineralizing groups inherited an organic scaffold and a toolkit of genes from a common ancestor. Furthermore, there is a consistent pattern of increasing complexity and control in the early evolution of skeletons in different animal groups.

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VII. Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Appendix S1. Complete set of data used for analyses to construct Figs 6 and 7. ‘Tree Data’, compilation of known

stratigraphic ranges and inferred biomineral type of each taxon included in the analysis, principally compiled from Erwin *et al.* (2011), Wood & Zhuravlev (2012), Maloof *et al.* (2010) and references therein, with additional references cited in the table. ‘Node Ages’, age constraints applied to internal nodes of trees used in Figs. 6 and 7, taken from 95% HPD intervals of dos Reis *et al.* (2015), node numbers

refer to those in Figs S1 and S2. ‘Timescale calibrations’, diagrammatic explanation of the calibration of the source timescales onto the ICS timescale used to calibrate Figs 6 and 7.

Fig. S1. Tree used to construct Fig. 6 with node numbers labelled.

Fig. S2. Tree used to construct Fig. 7 with node numbers labelled.

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