Evolutionary Origins of Animal Skeletal Biomineralization

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Abstract

The evolutionary history of biomineralization in animals is crucial to our understanding of modern mineralized tissues. Traditional methods of unravelling this history have aimed to derive a theory of the development of biomineralization through evolution by the comparison of mineralized systems in model organisms. This has led to the recognition of the 'biomineralization toolkit' and raised the question of the homology of mineralized tissues versus convergent or parallel evolution. The 'new animal phylogeny' reveals that many of the groups known to biomineralize sit among close relatives that do not, and it favours an interpretation of convergent or parallel evolution for biomineralization in animals. In addition, the fossil record of the earliest mineralized skeletons presents a rapid proliferation of biomineralization across a range of animal phyla with fossil representatives of many modern biomineralizing phyla. A synthesis of molecular, developmental, phylogenetic and fossil evidence demonstrates the convergent or parallel evolution of biomineralization in animals at the phylum level. The fossil record of the Cambrian explosion not only provides vital evidence for the evolution of animal mineralized tissues but also suggests a mechanism for its rapid and synchronous convergent origin.

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Introduction

Biomineralogy has long been the focus of materials scientists, chemists and palaeontologists, but it has only recently captured the interest of developmental biologists [Wilt, 2005] who in short order have begun to address some of the fundamental questions in biomineralogy. Foremost is the question of whether disparate animal lineages evolved their mineralized skeletons independently or the ability to develop a mineralized skeleton is inherited from a common ancestor. The prospect of a skeletonized universal ancestor of animals has been revived in light of the discovery that orthologous genes and their encoded proteins are implicated in skeletal development in organisms as diverse as vertebrates, echinoderms, molluscs and sponges [Ettensohn et al., 2003; Livingston et al., 2006; Jackson et al., 2007]. Whether this 'biomineralization toolkit' of genes reflects a parallel co-option of a common suite of genes or the inheritance of a skeletogenic gene regulatory network from a biomineralizing common ancestor remains an open debate [Livingston et al., 2006; Jackson et al., 2007]. Reconciling these conflicting interpretations of the data requires knowledge of the phylogenetic distribution of the molecular and skeletal characters in question. These have changed significantly in recent years in light of molecular phylogenetics, which has radically changed our understanding of the evolutionary relationships among animal phyla.

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Biomineralization and the 'New Animal Phylogeny'

Mineralized skeletons are found in all of the major divisions of the animal tree in an astounding range of grades of organization, body plan and function. Outside of the context of evolutionary history the interpretations of relationships between these structures can be misleading. It is therefore prudent to examine the phylogenetic context of animal skeletons. The 'new animal phylogeny' reveals that many of the groups known to biomineralize are intercalated among relatives that do not. An exemplar case study is the change in our understanding of deuterostome phylogeny. The traditional synthesis resolved deuterostomes as a clade rich in animals that biomineralize, with several candidates for the invertebrate non-chordate origin of the vertebrate skeleton and the assumption that the range of skeletal systems exhibited was derived from a common biomineralizing ancestor [Moss, 1964]. However, the lophophorate phyla (brachiopods, phoronids and bryozoa) are united with molluscs, annelids and their allies to form the Lophotrochozoa in the modern synthesis, whilst the chaetognaths and nemerteans are allied with the other major division of protostomes, i.e. Ecdysozoa. The remaining mineralized clades, the calcareous Echinodermata and jawed vertebrates which possess apatite skeletons, have divergences intercalated by the jawless vertebrates, cephalochordates, tunicates and hemichordates, all of which primitively lack skeletal tissues. The primitive vertebrate skeleton originated as a cartilaginous endoskeleton associated with the pharynx, which is known to have mineralized in an extinct group of jawless fish (the conodonts) as part of an oropharyngeal feeding apparatus [Donoghue and Sansom, 2002]. The first true mineralized vertebrate skeletons evolved in ostracoderms, a group of stem gnathostomes, as a dermal skeleton independently of the echinoderm skeleton [Donoghue and Sansom, 2002].

If we examine the distribution of biominerals across a modern phylum level tree of animals (fig. 1), although silica as a biomineral is restricted to silica sponges, calcareous and phosphatic biomineralization occur in all of the major divisions of the tree. At the simplest level, two competing hypotheses to explain this distribution can be erected: either mineralized animal skeletons evolved once or independently in each case. A strict parsimony approach cannot distinguish between these hypotheses: either a single origin with 13 losses (or changes in mineral system) or 14 independent gains. If changes between mineral systems (i.e. between calcium carbonate and phosphate) can be achieved with relative 'ease' on the grounds that the same biochemical machinery can be co-opted into either role [Knoll, 2003], then a single origin is marginally favoured, but this is too simplistic. Rather it has been suggested that, at least between calcium carbonate polymorphs, once a lineage has adapted to using a particular system, changes are rare [Porter, 2010]. Molecular dissection of biomineralization indicates otherwise: orthologous genes have been co-opted and diversified in parallel among vertebrates and echinoderms [Livingston et al., 2006], molluscs [Jackson et al., 2010] and bilaterians [Jackson et al., 2010].
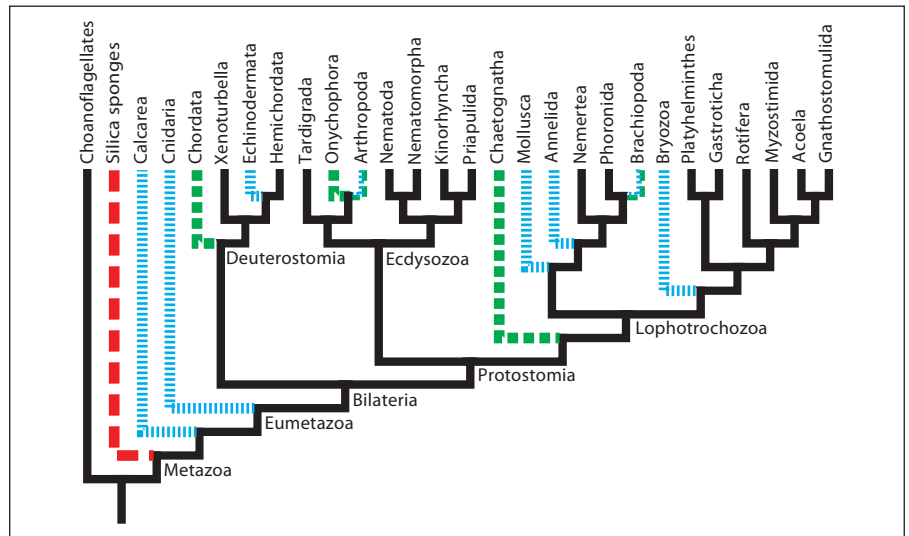
However, since mineralized skeletons of extant organisms, and the molecular machinery involved in producing them, have undergone a significant amount of evolutionary change since their divergences, they may not be representative of the ancestral condition. It is therefore necessary to utilize the evidence of the earliest animal skeletons afforded to us by the fossil record, representing a condition much closer to the deep divergences of animals.

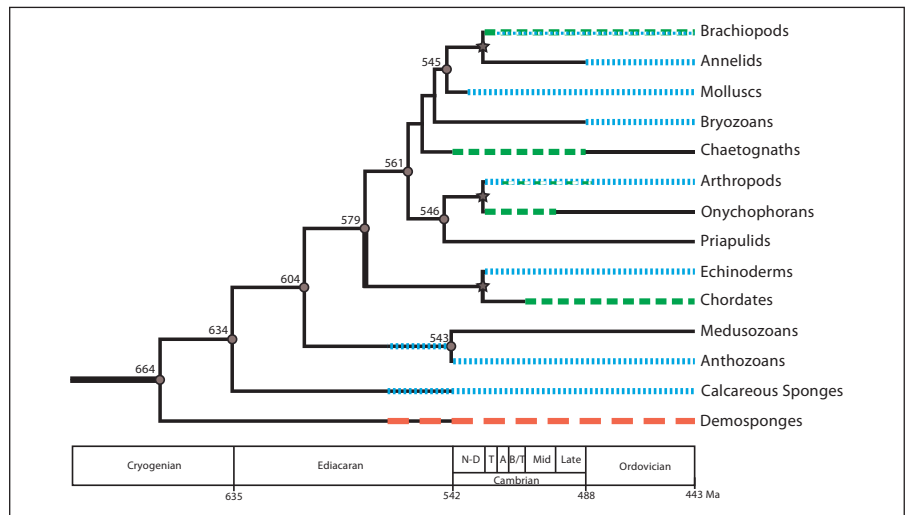## Lessons from the Fossil Record

The preservation potential of mineralized tissues is relatively good; therefore, were the latest common ancestor of extant mineralized groups to have been biomineralizing, we might expect to see remains of these structures in the fossil record. By contrast, multiple origins for biomineralizing animals would produce a different record, with the fossilized remains of animal skeletons appearing after the divergence of their respective groups. The earliest record of animal skeletons are the calcareous fossils of the 660-Ma Trezona Formation [Maloof et al., 2010] followed, in the latest Ediacaran period, by the weakly mineralized *Cloudina* (fig. 3b), *Namacalathus* and similar forms [Grotzinger et al., 2000], which have been allied with various cnidarian, poriferan and annelid groups. In the Early Cambrian the diversity and abundance of shelly fossils dramatically increased, and the fossil record was dominated by the 'small shelly faunas' until the end of the Middle Cambrian [Porter, 2004]. Amongst these are stem (a sister relative outside the living clade) or crown (a clade of living members of a group and all descendants of their common ancestor) representatives of all the modern biomineralizing animal phyla, and many orders, as well as many taxa whose biological affinity within the animal tree is yet to be constrained [Bengtson, 2005]. This rapid proliferation of fossil diversity, i.e. the Cambrian explosion, is one of the most intriguing problems in palaeontology, and the nature of this record is hotly debated [Runnegar, 1982]. Is it an explosion of animal diversity or the explosion in fossil diversity expected in the parallel evolution of biomineralized animal skeletons?

Fig. 1. Distribution of biomineralization across a phylum level animal tree. Terminal branches are coded to reflect known fossil or extant representatives with known mineralized tissues; red dashes are for silica, blue dashes for calcium carbonate and green dashes for calcium phosphate. The tree topology is modified from Dunn et al. [2008] to account for Poriferan polyphyly [Peterson and Eernisse, 2001]. The distribution of minerals is based on Lowenstam and Weiner [1989] and Knoll [2003], with additions.

Fig. 2. Summary of the origin of animal skeletons showing the skeletal mineralogy, stratigraphic distribution, phylogenetic relationships and divergence time estimates for animals with a Cambrian fossil record. Branches are coded to represent the known fossil record and mineral system of skeletons from each phyla; red dashes are for silica, blue dashes for calcium carbonate and green dashes for calcium phosphate. The tree topology is as per figure 1. Nodes denoted with a circle have divergence time estimates taken from Peterson and Butterfield [2005], those denoted with a star are minimum estimates based on the fossil record and unmarked nodes have unknown divergence times. Stratigraphic and mineralogical data is modified from Lowenstam and Weiner [1989] and Knoll [2003], with additions. A = Atdabanian; B/T = Botomian/Toyonian; N-D = Nemakit-Daldynian; T = Tommotian.
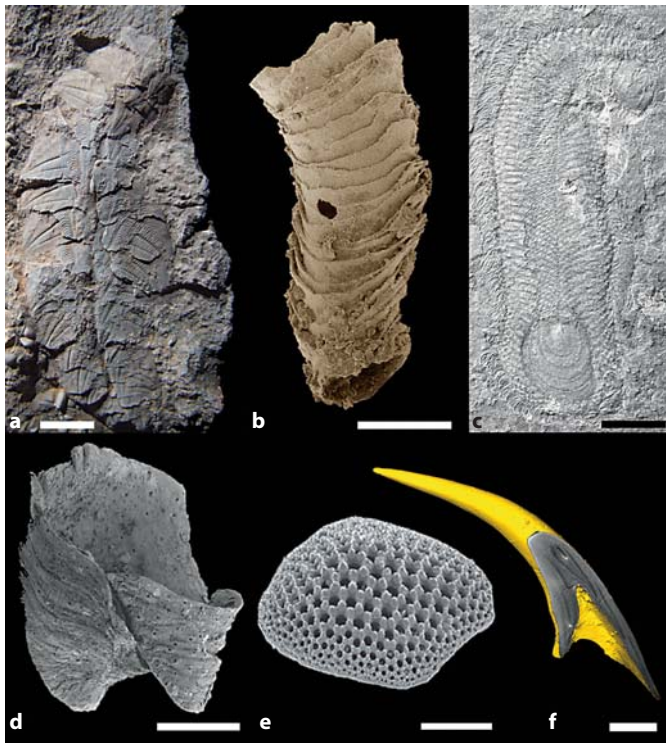

If we examine each of the animal groups that have representatives in the Ediacaran-Ordovician fossil record (fig. 2), the majority are biomineralizing. In addition, the first appearances of mineralized skeletons co-occurred within a geologically short interval from the latest Ediacaran to the Middle Cambrian. It is apparent, therefore, that biomineralization in animals post-dates the divergence of the major bilaterian clades, consistent with a parallel evolution of mineralized animal skeletons.

To support the hypothesis of multiple origins of biomineralization within animals, we would expect the small shelly faunas to be resolved as early members of extant phyla rather than stem representatives of larger clades such as Deuterostomia, Protostomia, Bilateria or even Metazoa. In many cases this can be demonstrated to be true. The coeloscleritophoran *Halkieria* (fig. 3c), original-

ly described as a stem brachiopod, provided evidence for the acquisition of a mineralized skeleton prior to the divergence of molluscs from the annelid-brachiopod clade, suggesting a single origin of biomineralization within the lophotrochozoa [Conway Morris and Peel, 1995]. However, revaluation of the fossil material has identified characters that place *Halkieria* within the molluscs [Vinther and Nielsen, 2005]. The origin of the brachiopod skeleton is believed to lie within a group of Cambrian fossils known as tommotiids (e.g. *Micrina;* fig. 3d) [summarized in Skovsted et al., 2009]. Crucially, this hypothesis invokes a single origin of mineralized shells *within* the brachiopod total group. Evidence for the origin of vertebrate mineralized tissues also exists from the Middle and Upper Cambrian [Smith et al., 2001], such as *Furnishina* (fig. 3f), and the controversial *Anatolepis*, including potential examples

Fig. 3. Representatives of the small shelly faunas with proposed affinities with modern phyla (see text for details). **a** *Plumulites tafennaensis,* a macheridian, is an armoured annelid [figured in Vinther et al., 2008]. **b** *Cloudina hartmannae,* a putative cnidarian or annelid [figured in Bengtson and Zhao, 1992]. **c** An articulated specimen of *Halkieria evangelista,* a stem mollusc. **d** Reconstructed scleritome of *Micrina etheridgei,* a stem brachiopod [figured in Holmer et al., 2008]. **e** *Microdictyon jinshaense,* a dorsal plate of an onychophoran [figured in Zhang and Aldridge, 2007]. **f** *Furnishina* sp., an early vertebrate feeding element with internal structure. Scale bars = 10 mm (**a**), 500 μm (**b–e**) and 100 μm (**f**).

of enamel, dentin and dermal bone [Donoghue and Sansom, 2002]. Some groups without modern mineralized taxa have fossil representatives with skeletons, such as the Onychophora (velvet worms) which have been allied with lobopodians possessing net-like plates, e.g. *Microdictyon* [Ramsköld and Hou, 1991]. Even annelids are recognized to have mineralized representatives in these faunas (fig. 3a) e.g. the macheridian *Plumulites* [Vinther et al., 2008]. Although the phylogenetic position of many Cambrian fossils remains intractable, new discoveries continue to allow biomineralizing taxa to be accommodated within the stems (if not the crown groups) of the modern animal phyla, rather than in the stems to aggregative clades.

An intriguing aspect of the early skeletal fossil record compared to subsequent biotas is the prolific use of calcium phosphate, particularly considering the high metabolic value and relative low abundance of phosphorus. The small shelly faunas, and in particular 'Örsten' deposits, preserve a large diversity of microfossils, often with exceptional fidelity and almost exclusively as calcium phosphate. This also coincides with a high abundance of sedimentary phosphate deposits [Brasier and Callow, 2007]. A similar, and perhaps more compelling, pattern has been identified in calcareous mineralizers. Porter [2010] reports a strong correlation between the origin of calcite versus aragonite skeletons and sea water chemistry. In addition, subsequent changes in the mineral system do not reflect the contemporaneous sea water chemistry; rather, they are evolutionarily constrained. Subsequent changes are more intrinsically driven based on the evolution of existing genetic and molecular mechanisms controlling biomineralization. These patterns not only provide a mechanism for the synchronous evolution of a particular biomineral but also evidences that the first appearance data of fossil skeletons faithfully reflects independent origins.

Discussion

By taking a holistic approach, integration of the evidence from molecular and developmental features of model organisms, the phylogenetic distribution in the 'new animal phylogeny' and the earliest fossilized remains of mineralized animal skeletons suggests independent origins of the skeleton at the phylum level. Similarities in the development of mineralized skeletons in extant animals reflect not a common ancestry but rather convergent, or parallel, evolution. Perhaps more significant than this, however, is the suggestion that a set of orthologous genes and their encoded proteins are implicated in the development of mineralized skeletons across the Bilateria, i.e. the 'biomineralization toolkit'. This may be derived from an unmineralized common ancestor to all modern bilaterians [Livingston et al., 2006; Jackson et al., 2010]. Minelli [2007] proposed mineralized tissues may derive from the mineralization of pre-existing skeletal substrates (e.g. 'cuticle'), the possession of a skeleton predating the advent of biomineralized structures. Perhaps the parallel evolution of biomineralization in the Cambrian not only co-opted pre-existing molecular machinery but also utilized unmineralized skeletons derived from a common ancestor as is shown to have occurred during the evolution of the vertebrate skeleton [Donoghue and Sansom, 2002]. In addition, a better appreciation of the mechanisms of the evolution of biomineralization in animals may also provide greater insight into biomimetic synthesis of biominerals by understanding the nature of the precursors to mineralized tissues.

Conclusions

Our knowledge of the distribution of biominerals across the metazoan tree has changed dramatically through advances in molecular phylogenetics. In particular, calcium carbonate and phosphate are known from all major divisions of the animal tree, and crucially biomineralizing groups are no longer allied; rather they are separated by unmineralized intermediates. The first appearance of mineralized skeletons of all of the major animal phyla co-occurred within a short window across the Ediacaran-Cambrian transition, and it suggests a rapid synchronous evolution of biomineralization in many phyla, i.e. the Cambrian explosion. Increasing studies of this record are continually resolving fossil taxa into stem (or even crown) positions within extant groups, not revealing a mineralized common ancestor. Recent evidence from molecular studies supports this interpretation of multiple origins rather than a single origin of the animal skeleton and further suggests that the developmental and molecular architecture may have evolved prior to the divergence of the metazoan phyla, providing a possible mechanism for synchronous multiple origins of biomineralization through exaptation of existing genes. The Cambrian fossil record, therefore, possesses a real biological signal and may itself be explained by such a mechanism.

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