



Evidence for spicule homology in calcareous and siliceous sponges: biminerallic spicules in *Lenica* sp. from the Early Cambrian of South China

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The relationships of the extant sponge classes, and the nature of the last common ancestor of all sponges, are currently unclear. Early sponges preserved in the fossil record differ greatly from extant taxa, and therefore information from the fossil record is critical for testing hypotheses of sponge phylogenetic relationships that are based on modern taxa. New specimens of the enigmatic sponge *Lenica* sp., from the Early Cambrian Hetang Biota of South China, exhibit an unusual spicule structure. Each spicule consists of a siliceous core with an axial canal, an organic outer layer and a middle layer interpreted to have been originally calcium carbonate. This finding confirms previous work suggesting the existence of biminerallic spicules in early sponges. Combined with data from other early sponges, the new findings imply that the two fundamental spicule structures of modern sponges were derived from a compound, biminerallic precursor. Spicules are therefore homologous structures in Calcarea and Silicea, and if sponges are paraphyletic with respect to Eumetazoa, then spicules may also have been a primitive feature of Metazoa. □ *Calcarea*, *Early Cambrian*, *Hetang Biota*, *phylogeny*, *Silicea*, *taphonomy*.

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Sponges (phylum Porifera) are widely thought to be the most primitive of the extant metazoan phyla (e.g. Philippe *et al.* 2009 and references therein), and as such attract research interest in the hope of elucidating the early history of animals. However, the earliest sponges known from the fossil record are very different from the majority of extant taxa. Most early sponges, whether hexactinellids, demosponges or the extinct heteractinids or protomonaxonids were thin-walled, globose and solitary. In contrast, most living sponges are morphologically complex, thick walled and clonal.

Most sponges have a skeleton of mineralized spicules, of either siliceous or calcareous composition (Bergquist 1978). Although their mineralized spicules have a high preservation potential under certain circumstances, the fact that most early sponges did not have their skeletal spicules fused meant that they disarticulated easily upon death, so complete fossils of non-lithistid sponges are rare. Their fossil record extends back to the Late Precambrian, although most

of these reports are ambiguous (Pisera 2006). Sponges became widespread during the ‘Cambrian Explosion’, the interval covering the first appearance and radiation of skeletonized animals, although the reasons for the diversification are unclear (reviewed by Marshall 2006).

The relationships of the major groups of early sponges (heteractinids, protomonaxonids, tetractinellid and ceractinomorph demosponges and various hexactinellids) are still uncertain; despite apparent progress in molecular phylogenetics (e.g. Philippe *et al.* 2009 and references therein), there is still no consensus on even the basic relationships of the classes. In palaeontology, the recognition of previously unknown complexity in the structure of early spicules (Botting & Butterfield 2005; Bengtson & Vinther 2006), and the presence of a calcarean-like external sheath in hexactinellid spicules (Harvey 2010), suggests that the problems stem from unrecognized complexity in early sponge evolution. These findings show that features of the structure and composition of the

spicules found only in Calcarea or Silicea among extant faunas were not mutually exclusive in the Cambrian. Even the exclusivity of spicule composition among modern taxa has recently been overturned, with the presence of calcite regions within the spines of hexactinellid spicules (Ehrlich *et al.* 2011). A possible implication is that calcareous and siliceous spicules are homologous, but the data are presently so limited that it has not been possible to rule out convergent silica or carbonate biomineralization in Calcarea and Silicea, or convergent development of a robust spicule sheath, as suggested by Harvey (2010). Recognizing the taxonomic distribution of these features in the fossil record is hindered by taphonomic modification of original mineralogy, and the discontinuous record of early sponges has rendered the relationships of the various groups largely obscure. To resolve these relationships and clarify the nature of the poriferan last common ancestor more information is needed on spicule structure and composition in a range of early sponges. This article provides such information, and strongly supports the hypothesis that spicules are homologous in all modern sponge groups.

Spicule structure in modern and fossil sponges

The spicules of extant sponges are either siliceous (opal-A) or calcareous (high-magnesium calcite and sometimes amorphous calcium carbonate) (Uriz 2006). There are also some spicule-like organic structures, but although the organic spiculoids of the extant ceractinomorph demosponge *Darwinella* and other Aplysinnellidae show an internal core, there is no abrupt or mineralogical contrast between textural layers (Bergquist & Cook 2002), and they are not considered further here.

Siliceous spicules are found among all hexactinellids, many demosponges and most homoscleromorphs (Bergquist 1978). The centre of these spicules is composed of an axial organic filament (or an amorphous organic centre in some Homoscleromorphs; Uriz 2006) surrounded by many fine laminae of opal-A lepispheres, and with a variable component of organic matter within and between the laminae. Although the outermost laminae may be more resistant to diagenetic dissolution than the inner parts (e.g. Botting 2005), there is no structural boundary within most of these spicules except for the axial filament edge. However, in anchoring spicules of the modern hexactinellid *Monorhaphis*, which are the largest known biosilica structures, the central portion (up to a quarter) of the spicule is composed of a

massive core of homogeneous silica (surrounding a narrow filament), in contrast to the concentrically layered outer section of the same composition (Müller *et al.* 2007). Such a structural difference has the potential to be affected differently by dissolution under diagenetic conditions. This is also true of lithistid desmas in which the central part of the spicule is composed of relatively porous, granular silica (Pisera 2003) that may be more easily dissolved than the exterior and the centre of calcarean spicules also dissolves more easily than the exterior parts due to differences in organic content (Ledger & Jones 1991). However, in all these cases, any axial dissolution structure should be somewhat irregular.

The symmetry of axial filaments is distinct in the two classes. All demosponge filaments yet examined are either triangular or hexagonal in cross-section, whereas all known hexactinellid filaments are square (except possible circular canals illustrated by Sandford 2003); filament symmetry also corresponds to spicule symmetry, with the number of axes related to the sides of the filament, suggesting that spicule form is defined by axial filament form (Reiswig 1971). However, lithistid desmas in at least some cases have short filaments that cannot template the ray termini (Pisera 2003), and the axial organic structures of tetractine homosclerophorid spicules are broad and irregular (Uriz 2006). It has also been shown (Croce *et al.* 2003) that the packing organization of protein units differs between the demosponges and hexactinellids, suggesting that the symmetry difference reflects an aspect of spicule secretion that is independent of spicule symmetry, and results directly from a deep evolutionary transition in protein packing. Although these differences are significant, the presence of axial organic structures in all silicisponges implies a deep shared ancestry; the amorphous form of homosclerophorid axial structures may represent the primitive condition, which became condensed into the discrete filaments seen in modern Silicea. Clear axial canals with recognizable symmetry are rarely preserved in early sponges, and until they are better understood, it is important to appreciate that filaments in the earliest siliceous spicules may have been somewhat different to those of extant taxa.

Calcareous spicules are in some cases mineralogically more complex than siliceous spicules, involving a core region of calcite, a thick layer of amorphous calcium carbonate (ACC), and a thin outer layer of calcite (Aizenberg *et al.* 2003). In most calcareans, however, the ACC region is lacking, and the spicule behaves as a single crystal of high-Mg calcite (Sethmann & Wörheide 2008), but consists of numerous aligned micro-crystalline domains. It is possible that the ACC represents a precursor mineralogy during

spicule secretion, rather than a typical component of a complete spicule.

An outer organic layer is present in extant Calcarea, and is robust enough to be isolated through acid digestion (Jones 1967), but no substantial organic sheath is known from extant Silicea. However, there are examples of hexactinellid spicule sheaths (including hexactines with pinnate rays) extracted by HF maceration of Cambrian shales (Harvey 2010), and a reflective film is present over the surfaces of the large spicules of the heteractinid calcarean *Eiffelia globosa* Walcott 1920 and the protomonaxonid *Pirania muricata* Walcott 1920 (Botting & Butterfield 2005) from the Burgess Shale. *Eiffelia* is particularly relevant to discussions of spicule structure, as it was shown by Botting & Butterfield (2005) to possess features of both hexactinellids and heteractinid calcareans, including probable compound spicules of silica and calcium carbonate. This dual mineralogy in *Eiffelia* spicules was confirmed by Bengtson & Vinther (2006), where they showed that the core of phosphatized *Eiffelia* spicules from South Australia was composed of silica, and the external part was preserved in a manner consistent with skeletal carbonate in that deposit.

Axial structures in fossil sponge spicules have been widely misinterpreted and treated over-simplistically. An organic axial filament (or axial canal following decay of organic material) is widely stated to be a sufficient character for the recognition of siliceous sponge spicules (e.g. Yin *et al.* 2001), and to differentiate spicules from inorganic crystals or non-sponge biological structures. Although a useful discriminant for certain spicule types, this is somewhat oversimplified, and the concept has also been widely misused (e.g. Zhang *et al.* 1998). Axial structures can form in a variety of inorganic and organic situations, and recognizing a true axial canal is not necessarily straightforward, with completely unambiguous determination dependent on preservation of aspects such as cross-sectional symmetry, which are almost invariably lost. Most importantly for palaeontologists, the axial filament in modern sponges is typically around 1–2 μm or less in diameter (e.g. Simpson *et al.* 1985), even for the largest known spicules (Müller *et al.* 2007), which is far smaller than the structures usually reported as being axial canals in early sponges (e.g. Ivantsov *et al.* 2005; Mergl & Duršpek 2006). Examples of true axial canals in the Early Palaeozoic include those from the lithistid *Multispongia* (Carrera 2007), in which the canals are visible as fine lines under high magnification light microscopy, but such structures are rarely preserved. Other putative canals (e.g. in *Coniculospongia*; Rigby & Clement 1995) are ambiguous, with axial canal diameters of 10–15 μm , but no discussion of any preserved symmetry.

Alleged axial canals in early spicules are frequently a significant fraction of the spicule ray in diameter, often approaching 20% of the ray diameter, and may reach or exceed 20 μm (e.g. Yuan *et al.* 2002). Hexactine and pentactine spicules of the Early Cambrian of North Wales have axial structures around 30 μm in diameter (Greenly 1919; Bassett *et al.* 1976), around one-third of the ray diameter. In at least some cases these structures may represent axial canals that have been enlarged through dissolution; axial dissolution is common in certain localities (e.g. Botting 2005), in some cases leaving only the external laminae. Such patterns of dissolution have also been seen in modern spicules from shallow-water environments (Land 1976) and confirmed experimentally in modern sponges (Rutzler & Macintyre 1978).

It is possible that the axial canals in early hexactinellids were much wider than in recent examples, in which case it should be possible to recognize this through preserved canal symmetry; this has never been described for the Early Palaeozoic. The clear preservation of regular symmetry would be a sufficient feature for recognition of genuine axial canals, but any secondary dissolution of the margins of the canal can obscure the form. Even where polygonal symmetry is obvious (e.g. Mergl & Duršpek 2006; fig. 3T), it is important to rule out an origin through pyrite cube growth, as appears to have been responsible for apparent canals in the spicules described by Hu *et al.* (2002) from the Hetang Biota.

Axial canals and expansion of canals by dissolution are not the only possible explanations for axial structures in sponge spicules, as even some modern species have more complex spicule structures (discussed further below). The precise determination of which features are represented in fossils is becoming critical to interpreting skeletal homology and phylogenetic relationships of the sponge classes.

Lenica from the Sinsk Biota

Lenica unica was erected by Goryanskii (1977) to accommodate a large, coarse-spiculed sponge from the Early Cambrian Sinsk Biota of Siberia that at least superficially resembles members of the protomonaxonid family Choiidae (see Finks & Rigby 2004), sponges with discoidal bodies with radiating (longitudinal) monaxonid spicules. A second species, *L. rigbyi* Leguta *in* Ivantsov *et al.* 2005; was erected from the same biota and the authors also tentatively assigned *Choia hindei* Dawson 1896 to the genus. The genus was included under open nomenclature among Hexactinellida, although *Choia* was regarded as a demosponge (Ivantsov *et al.* 2005). The hexactinellid attribution

was made because of the apparent presence of a triangular axial canal; however, a triangular axial canal indicates Demospongea rather than Hexactinellida, and in any case the structure is ambiguous based on their illustration, being preserved as an unclear shape marked by a distorted and partially detached internal spicule layer.

Although fundamental characters such as discoidal body with radiating spicules are shared with all Choidae, unlike the other genera in this family, the spicules of *Lenica* are not clearly divided into disc and coronal spicules. The spicules also show a unique ornament of longitudinal to helical ridges, and specimens from the Sinsk Biota appear to have possessed a broad axial canal. However, the taphonomy of these spicules is unclear, with illustrations of Ivantsov *et al.* (2005) generally showing a thick outer layer bearing external ridges, a partially separated layer inside it, and a hollow centre. The central cavity is extremely wide (typically comprising at least 95% of the spicule diameter according to the description), and broadly circular in cross-section, rather than polygonal. It is therefore unlikely to represent an axial canal, and probably reflects constructional layers within the spicule, although it is not known how much of each spicule has been recrystallized or dissolved, nor the original composition of each layer.

The original composition of *Lenica* spicules is unclear based on the Sinsk material, with Ivantsov *et al.* (2005) stating that many spicules had been replaced by calcite, from an originally siliceous (perhaps assumed) composition. From their SEM illustrations, the material comprising the outer parts of the spicules appears to be homogeneous and shows no evidence of crystallinity. In some specimens, the 'axial canal' was infilled by calcite, suggesting two stages of replacement. Given the carbonate-rich environment, calcitic layers would be more stable than siliceous layers, any siliceous component being liable to dissolve (Land 1976). The apparently calcitized outer part of the spicule may therefore have been originally calcareous.

Lenica from the Hetang Biota

The Hetang Biota (together with the lowest sponge faunules of the age-equivalent Niutitang Biota, e.g. Zhao *et al.* 1999) is probably the earliest known fauna of articulated spiculate sponges, and as such is critical to our understanding of their Cambrian diversification. Recent re-collection of the fauna has revealed significant unrecognized species diversity, and yielded new material of known taxa. A species of *Lenica* was described from the Hetang Biota as *Choia? striata* Xiao *et al.* 2005. The sponge is among the most abundant

species in the deposit, and closely resembles *L. unica*; it is possible that they should be synonymized, but a taxonomic revision is required to assess that, and the sponge is here referred to as *Lenica* sp.

Sponge spicules in the Hetang Biota are largely replaced by granular or blocky pyrite (personal observation, all authors), but in *Lenica* specimens a diverse range of preservational modes have been observed. These fossils demonstrate a more complex structure than those of co-occurring sponges, and despite limitations of the preservation, add substantially to our knowledge of early sponge biomineralization.

Materials and methods

Twenty new specimens of *Lenica* sp., both articulated specimens and disarticulated spicules, were collected from black mudstone blocks in the disused brick pit (29°52' N; 118°03' E; more precise locality information is deposited with specimens) that forms the main locality for the Hetang Biota sponge fauna. A detailed description of the Hetang Formation is reported in Xiao *et al.* (2005). Much of the exposure is inaccessible due to flooding of the pit, but the loose blocks are thought to have come from the middle to upper parts of the exposed sequence, and were collected along a major track on the northern side of the quarry. Isolated spicules were also found in outcrop in these beds. Specimens were initially examined by light microscopy, then with a Gemini Leo 1530VP Environmental Scanning Electron Microscope, operating primarily in backscattered electron mode, with EDX analysis performed by using Inca X-sight device made by Oxford Instruments. Operating voltage in backscattered mode was 10–15 kV. Specimens are deposited at the Nanjing Institute of Geology and Palaeontology, Nanjing, China (specimen numbers NIGP154160 – 154179).

Description

Morphology

Lenica is a large (up to 50 cm diameter) sponge with a radiating array of long, broad spicules. The spicules are of irregular length, but include some that appear to occupy the entire radius, radiating from the centre. No large, entirely complete specimens are yet known, leading to a diagnosis as a fan-shaped or conical skeleton, but some examples (Xiao *et al.* 2005) show what appears to be the centre of a radial array. Based on the preservation of the broad, flattened spicules, it is possible that the specimen named as *Choia utahensis*

(Xiao *et al.* 2005; fig. 2) is a juvenile *Lenica* sp., which would confirm a typically choiid-like, radial growth form. Many of the spicules, particularly the larger ones, are marked by longitudinal to slightly oblique ridges (Figs 1C, 2A), a feature also seen on some well preserved small spicules. Between the largest spicules is a dense array of very fine spicules (Fig. 2D). The basal parts of the spicules are often weakly preserved (Fig. 2C), but in a few specimens they are seen to be rounded bluntly.

Spicule structure and taphonomy

The taphonomy of the specimens is varied, and includes fully and partially pyritized spicules (Fig. 1D), and others partially replaced by clay

minerals (possibly illite) and accessory phases (Fig. 2B) such as a magnesium aluminium silicate. Different structures are preserved in different specimens, and it is by combining the information from the different taphonomic modes that the complete spicule structure has been reconstructed.

Spicules of other sponges in the same beds do not show any of the structural divisions shown here, being either external moulds with the spicule dissolved, or homogeneous replacements by pyrite, or sometimes void-filling aluminosilicates. This includes large monaxons from *Choia* and large root tuft spicules from hexactinellids. Pyritized spicules include specimens where the pyrite has infilled a mould and the exterior is smooth, and others where growth of blocky crystals has extended into the surrounding sediment. In no

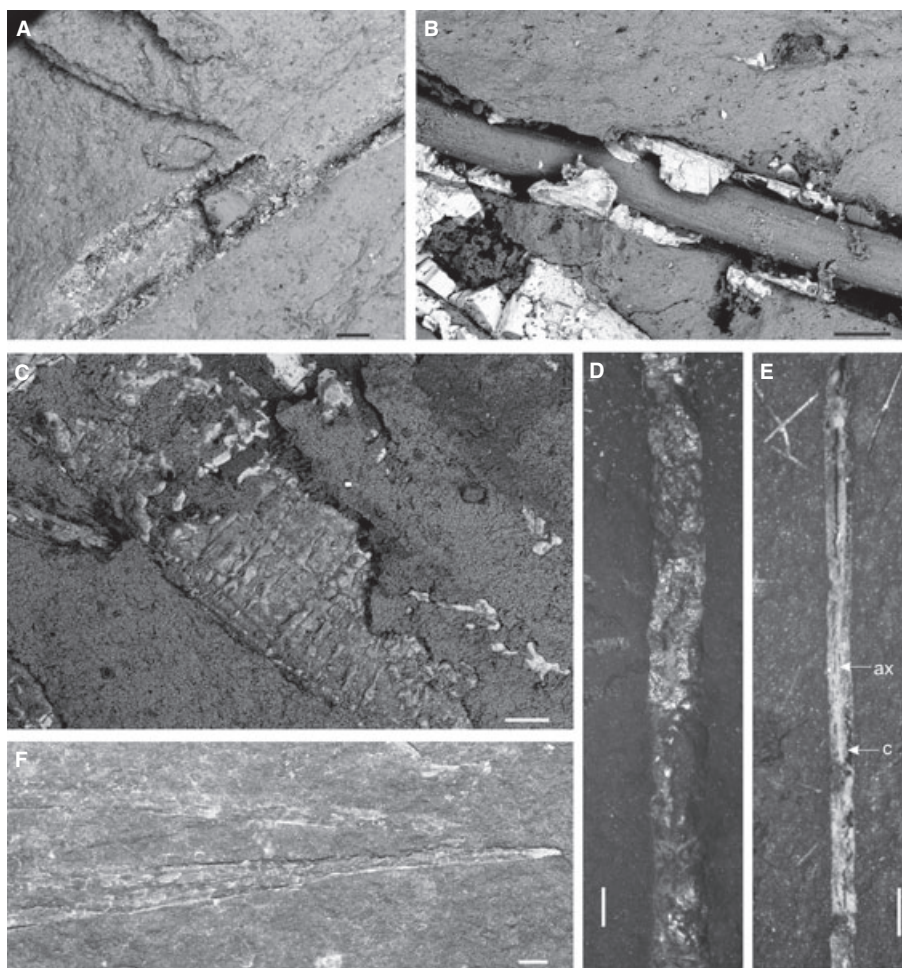


Fig. 1. Differences in preservational styles of *Lenica* sp. spicules, from the Early Cambrian Hetang Biota, China. A, backscattered electron microscope (BEM) image of NIGP154163, showing chalcedonic siliceous core with weathered iron oxides replacing outer layer (see also Fig. 4); B, BEM image of NIGP154160, showing aluminosilicate-replaced core, middle layer with void-filling pyrite (bright) and mould of outer lamina; C, BEM image of NIGP154162, with pyrite replacement of external sheath, flattened and showing numerous transverse fractures; D, light photograph of NIGP154165, isolated spicule showing block replacement by pyrite with crystalline quartz in centre (see also Fig. 3); E, light photograph of NIGP154164, isolated spicule, showing preservation of both core (c) and axis (ax) (see also Fig. 5); F, light photograph of NIGP154161, articulated partial specimen with moulds of flattened spicules showing brittle fracture of outer lamina but little pyrite replacement. Scale bars: A–B: 200 μ m; C: 100 μ m; D–F: 1 mm.

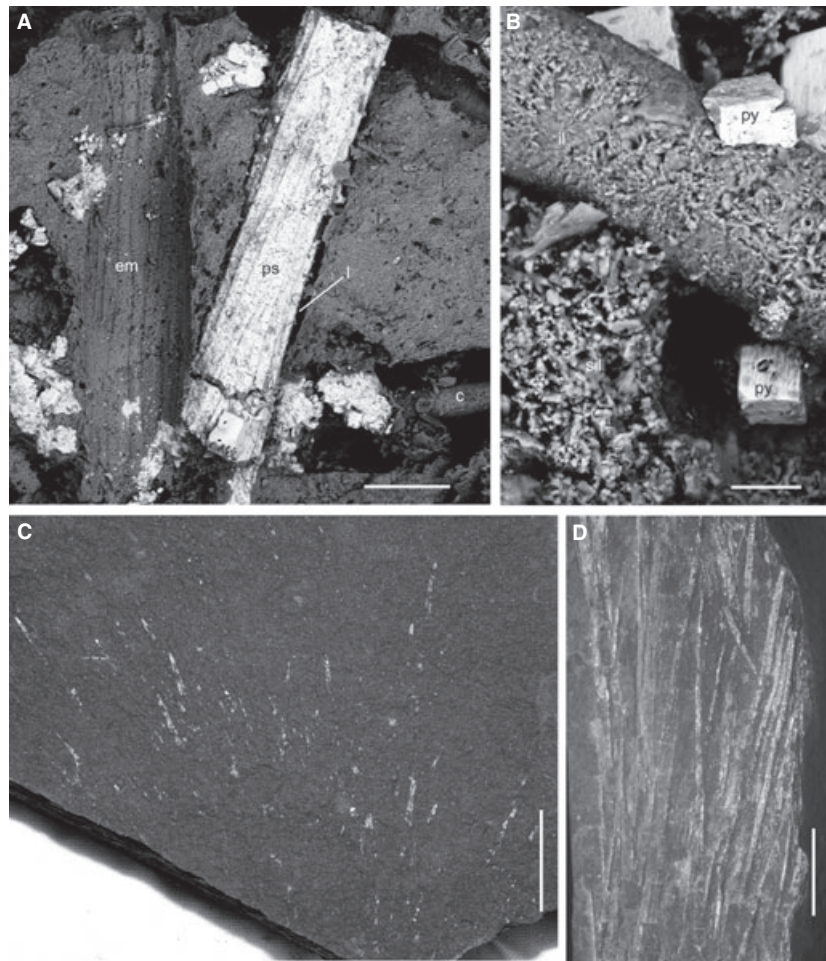


Fig. 2. *Lenica* sp. from the Early Cambrian Hetang Biota, China. A, backscattered electron microscope image of a variety of preservational styles in three-dimensionally preserved spicules in specimen NIGP154160, including external mould (em), pyrite steinkern of middle layer (ps), with external lamina (l) subsequently removed and core (c) altered to aluminosilicates, with middle layer dissolved. Scale bar 200 μ m. B, detail of spicule core replaced by illite-like aluminosilicate (il) and late-stage pyrite cubes (py) and with region of dissolved middle layer partly infilled by silica with aluminosilicates (sil); scale bar 20 μ m. C, light photograph of partial articulated specimen (NIGP154177), with spicules well preserved but flattened distally, and weak or unpreserved proximally; scale bar 10 mm. D, light photograph of partial articulated specimen (NIGP154162) with pyritized outer lamina, often compressed and with brittle fracture; scale bar 5 mm.

sponges other than *Lenica* have we observed flattening, a discrete outer lamina, or a discrete core. This implies that the structure of *Lenica* spicules genuinely differed from that of other taxa in the same beds, although the large size of its spicules would also have been more conducive to preservation of structural details.

Little direct comparison can be made with the taphonomy of other biomineralized material in the deposit, as sponges are almost the only biomineralized organisms yet known from the biota. The only exceptions are conical structures believed to be orthothecid hyoliths (Yuan *et al.* 2002); these would originally have been partly calcareous, but are invariably preserved as flattened organic or aluminosilicate films, with no trace of calcareous material remaining.

The *Lenica* spicules were composed of four primary layers, which are discussed in sequence below.

Outer lamina. – A very thin outer lamina, which often has the longitudinal external ridges typical of *Lenica unica* from the Sinsk Biota; in many spicules, particularly the smallest ones, no ridges are visible. This lamina is normally pyritized where preserved (Fig. 2D), with ornament of fine ridges on both external and internal surfaces (Fig. 2A), and also in some cases on the boundary between the core and middle layer (Fig. 1B). In some cases, the ornament is visible but the outer lamina is preserved only as a void, implying diagenetically late destruction. In other cases, a pyrite void-fill shows the inner surface of the lamina, but without the exterior void (Fig. 1B). In this case, the pyrite crystal arrangement demonstrates inwardly directed growth that is inconsistent with replacement of materials *in situ*, where nucleation should have been more evenly distributed; the void fill must therefore have occurred either with the outer lamina

present to act as a template, or against an external mould of the spicule in lithified sediment. In other specimens the spicules are flattened tubes consisting only of the outer lamina, which shows compression fractures; these often have an irregularly arcuate or branching arrangement (Fig. 1F). Where interiors are not preserved, the spicules are usually almost entirely flattened (except towards the tips), although in some cases the ornament is visible as external moulds on 3-D voids (Fig. 2A).

This outer lamina was normally either pyritized or lost, but pyritization appears to have been most common in large spicules. The smaller spicules generally either lost the outer layer more easily than larger ones, or typically lacked the ornamental ridges. In some spicules, the outer lamina is preserved entirely flattened (e.g. Fig. 2C), indicating that it must have survived the loss of both calcium carbonate (middle layer, discussed below) and silica (core, discussed below) through dissolution, although it may have been pyritized by that stage. In others (Fig. 2A) the lamina is preserved as a void surrounding a pyrite void-fill of the middle layer; in these, the lamina survived intact until after the loss of the middle layer by dissolution, but was removed during later diagenesis. As the core in this type of material is replaced by densely packed aluminosilicates but maintains a sharp margin, replacement of the core must have also predated loss of the middle layer and outer lamina. Such recalcitrant behaviour during chemical alteration of adjacent mineral phases is consistent with organic

matter forming the outer layer, but is difficult to reconcile with a mineral phase. The different taphonomic behaviour of the material shows that it cannot have been composed of either silica or calcium carbonate, and no other minerals form a major component of known sponge skeletons.

In some specimens with heavily pyritized middle layers (e.g. Fig. 1B), irregular blebs of carbon-rich material within the outer part of the pyrite include substantial quantities of chlorine and sodium (Fig. 3). The restricted distribution of these elements, with significant concentrations coinciding precisely with the pyrite void-fill and the included blebs, indicates that they cannot be surface contaminants. Dispersed carbonaceous and chlorine-bearing material is consistent with the remains of an outer organic unit that has been kerogenized and migrated during diagenesis (behaviour of organic materials under diagenesis is discussed by Gupta & Briggs 2011).

Middle layer. – This constitutes the bulk of the spicule. This is the least well preserved layer, normally seen as a void (Figs 2A, B, 4) or replaced by massive crystalline pyrite that has grown inwards from the outer lamina (Figs 1B, 2A). In some cases, organic-rich blebs of material are incorporated into the outer part, and may have originated in the outer lamina (Fig. 3; see discussion of outer lamina, above). Where only the pyritized infill is preserved, the outer surface is irregularly lumpy (Fig. 1D). Such spicules are always isolated, rather than part of an articulated

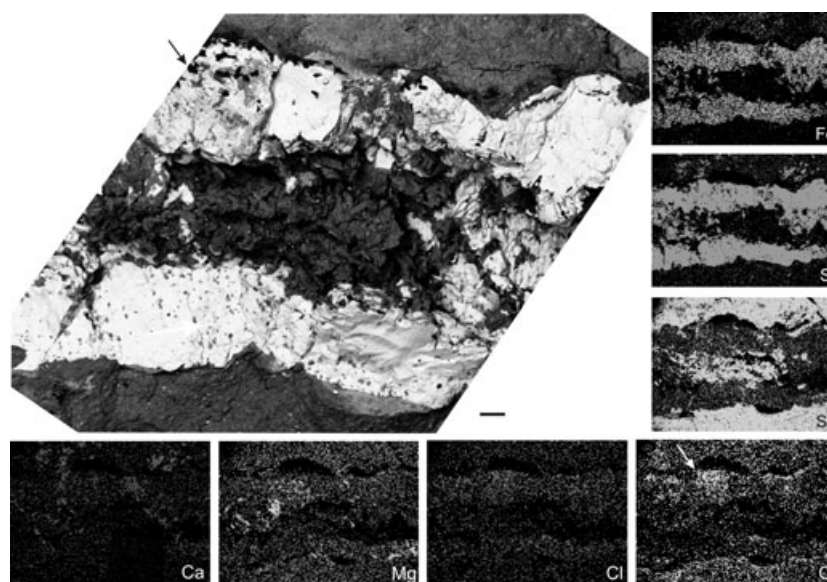


Fig. 3. Backscattered SEM image of *Lenica* spicule NIGP154165 (Early Cambrian Hetang Biota, China) with incomplete void-fill of blocky pyrite, and axial area infilled by micro-crystalline quartz, with elemental maps of same area. Arrows in main image and carbon plot mark electron-light patches of organic- and chlorine-rich material filling spaces between pyrite crystals, concentrated near the spicule surface. Patchy distribution of magnesium and calcium, the latter not recorded elsewhere except in specimen NIGP154164. Scale bar 100 μ m for main image.

sponge, and show the coarsest preservation; axial voids in these specimens are poorly preserved, with crystalline quartz (Fig. 3) representing incomplete replacement of the void or recrystallization of original mineralogy (see discussion of core, below). In some specimens there are partial void-fills of rubbly looking silica (Fig. 2B), an illite-like aluminosilicate (white- or grey-bladed crystallites, composition determined by EDX as aluminosilicate with magnesium and potassium), and a magnesium aluminium silicate phase.

In one specimen that preserves unique detail of the core and axis (Figs 1E, 5), there is a strong calcium signal from the middle region. A large part of the signal is recorded from substantial fans of bladed crystals that appear to be calcium sulphate (Fig. 5), a mineral otherwise seen in the samples only as small crystals formed by weathering of pyrite. This was restricted to the middle layer, and was not present in the partly hollow core region, implying that although the calcium-bearing phase is secondary, the origin of the calcium was local to the middle spicule layer.

The dissolution of this middle layer occurred, at least sometimes, after replacement or infill of the core region, but in other cases the layer has been removed without any significant loss of the silica from the core region (e.g. Fig. 4). It is highly unlikely for this

difference in the behaviour of the core and middle layers to reflect structural differences of the same composition, as one structural form should be consistently more soluble than the other, independent of the ambient chemistry. Although the core is sometimes preserved as *in situ* recrystallized silica (discussed below), there is never original mineralogy preserved in the middle layer. Such high solubility is typical of carbonates, and a calcium-bearing phase is consistent with the calcium signal detected in the middle layer of one specimen, discussed above.

The middle layer, therefore, although not preserved intact, appears to have been composed of calcium carbonate, as in modern *Calcarea*, and in *Eiffelia* (Bengtson & Vinther 2006). If not composed of calcium carbonate, it must have been a more exotic phase, not previously recognized among fossil or living sponges. A labile organic composition, such as the 'pith' of certain living demosponges (as discussed by Butterfield & Nicholas 1996), is ruled out by the lack of *in situ* pyrite replacement of this layer (pyrite being present only as void-fills), and the 3-D void preservation with axially preserved core (the spicules would have been crushed if this layer was removed pre-lithification, with the spicule core fallen to the base). A calcitic composition for this layer is also consistent with the

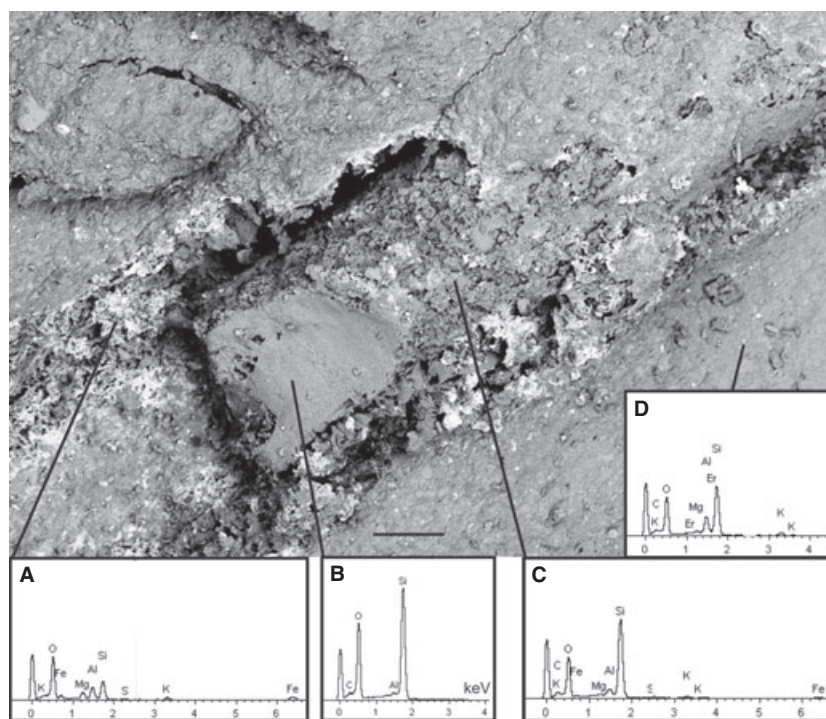


Fig. 4. Isolated *Lenica* sp. spicule NIGP154163 from the Early Cambrian Hetang Biota, China, with EDX analyses of various parts: A, middle and outer parts of spicule, replaced by secondary minerals including iron oxides and aluminosilicates; B, core region, composed of cryptocrystalline (chalcedonic) quartz with conchoidal fracture; C, coarse outer surface of core, with Mg-K aluminosilicates in addition to quartz signature; D, surrounding matrix, consisting dominantly of Mg-K aluminosilicates; the erbium peaks are likely to be an analytical error resulting from coincidence with minor peaks in the spectra of other elements in the analysis, although the Hetang Formation does yield high levels of a range of rare earth elements. Scale bar 100 μ m.

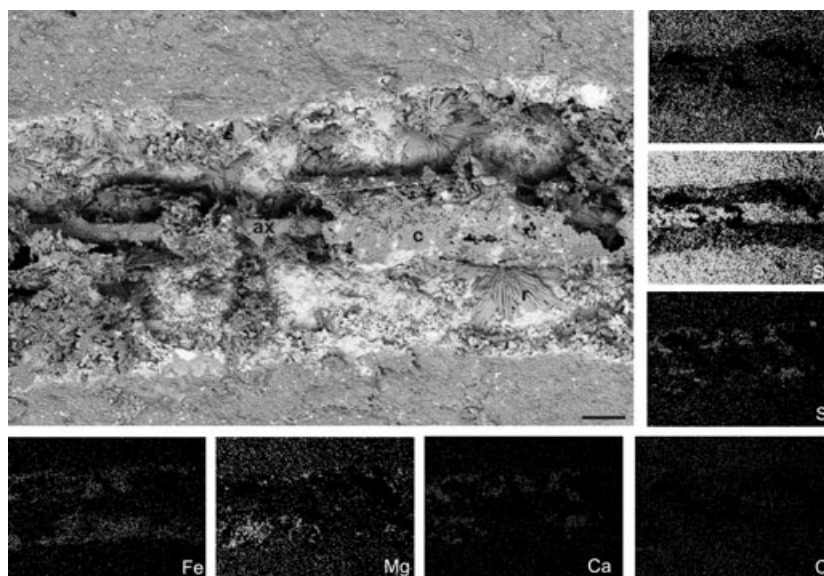


Fig. 5. Backscattered SEM image of *Lenica* sp. spicule NIGP154164 from the Early Cambrian Hetang Biota, China, showing mould of spicule with core (c) and central axis (ax); note also probable gypsum crystal rosettes infilling parts of middle layer (r). Elemental maps cover the main image area, and show that the core and axis are composed of silica, whereas the middle layer is replaced largely by magnesium, iron and calcium-bearing phases, including fans of probable calcium sulphate – probably a weathering product of pyrite in the presence of calcium. No other strong calcium signals or calcium-bearing phases have been observed in this study, except for patchy calcium recorded across the specimen and matrix in specimen NIGP154165. Scale bar 100 μ m for main image.

preservation of the outer part of the spicules as calcite ‘replacements’ in the carbonates of the Sinsk Biota (Ivantsov *et al.* 2005), as discussed above. The combination of data from these two taphonomic styles therefore strongly suggests that the original composition of the middle layer was calcium carbonate. If not calcitic, this layer must have consisted of an unknown, highly soluble mineral that is otherwise unique among known sponges; we therefore assume a calcium carbonate composition as the null hypothesis.

Core. – The core forms approximately $\frac{1}{4}$ – $\frac{1}{2}$ of spicule diameter in width. This part of the spicule is only preserved in a few specimens, either where spicules are largely flattened, or in some specimens with spicules preserved in three dimensions (Figs 1A, B, E, 2B, 4, 5). The structure is in the centre of the spicule (even within the 3-D spicules with middle layer missing, it has not fallen to the bottom of the spicule mould), circular in cross-section, and near-perfectly cylindrical. In most specimens analysed by EDX, it appears to be composed of a dense mass of bladed crystals of an aluminosilicate with K and Mg, perhaps illite (Fig. 2B). In two specimens, one of which preserves additional structural details of the axis (Fig. 5), the core is preserved as a partially hollow quartz cylinder. Quartz is also the dominant phase infilling partial pyritized void-fills following dissolution of the parts of the spicule within the outer lamina (Fig. 3).

One specimen of an isolated spicule is preserved as largely iron oxides replacing the outer, middle layer but the core is a smooth-edged, solid mass of silica with conchoidal fracture (Fig. 4). There is no axis preserved in this spicule, but the breakage pattern is typical of spicules preserved as chalcedony, following *in situ* recrystallization of original opal.

In some cases, the core was the first layer to be dissolved, and was replaced by an illite-like mineral, followed by dissolution of the middle unit, and then the outer lamina (e.g. Fig. 1B). The aluminosilicate may be a direct replacement of the opaline silica, or be authigenic, forming a cavity resulting from dissolution or decay of the core while the middle unit was still present.

Axial canal. – In one specimen (Figs 1E, 5), the core surrounds an internal cylindrical structure that is preserved as a coarsely recrystallized, silica tube or cylinder. The silica is micro-crystalline and irregular, and has not yielded any recognizable symmetry, but its position and narrowness are indicative of an axial canal, either infilled with crystalline quartz, or with only the margin preserved. The large size of the structure (around 40 μ m in external diameter), and the lack of preserved symmetry, make it impossible to show categorically that it is a true axial canal, but its relationship to the complex structure of the spicule makes alternative interpretations difficult. The canal shows consistent thickness over a long distance

(Fig. 1E), and therefore cannot represent a dissolution structure. Either it represents an axial canal homologous with that of modern Silicea, or it is a structure unknown in modern sponges.

Discussion

The taphonomy of these spicules is complex, but the combination of features indicates a biminerallic spicule with a core of silica, a middle layer of calcium carbonate and a robust outer organic layer (Fig. 6A); in effect a siliceous spicule inside a calcareous spicule. These layers reacted differently to different taphonomic conditions, and were often removed sequentially by decay, dissolution or recrystallization. The only clearly preserved original mineralogy in the Hengtang Biota is that of the core region, which was originally siliceous. The fracture pattern and presence of cryptocrystalline silica in the core (Fig. 4) indicate that the silica was not a secondary replacement; replacements or infillings by quartz are also seen, but these are micro-crystalline in texture (Fig. 5). The presence of an axial canal in one specimen is also characteristic of typical siliceous spicules, and the core region therefore closely resembles a normal hexactinellid or demosponge spicule. With the middle layer being originally calcareous (probably preserved intact in material from the Sinsk Biota; Ivantsov *et al.* 2005), and the robust outer layer originally organic, it is clear that the spicules of *Lenica* cannot represent an identical structure to that seen in any modern group. However, the fundamental structure agrees with observations of other anomalous early sponges (Botting & Butterfield 2005; Bengtson & Vinther 2006).

The presence of biminerallic spicules in a second group of Cambrian sponges shows that such structures were originally widespread in early sponge evolution. The recognition that *Eiffelia*, usually regarded as a heteractinid calcarean, possessed mineralogically compound spicules (Botting & Butterfield 2005; Bengtson & Vinther 2006) provided evidence linking the heteractinids to hexactinellids, and implied that spicules are homologous in Calcarea and Silicea. This assertion has been denied on the basis of the dissimilarity of the secretion mechanisms (e.g. Sperling *et al.* 2007), although there is now a consensus that spicules are largely homologous within Silicea, albeit probably exclusive of Homoscleromorpha (Philippe *et al.* 2009). This is a significant change from earlier views that spicules had independent origins even between Hexactinellida and Demospongiae (e.g. Reitner & Mehl 1996); the differences in secretion mechanisms were thought to be too great to be homologous. This understanding was based on very few studies, and better knowledge of the range of secretion in modern Silicea has shown that spicule secretion processes are more diverse, and therefore more evolutionarily flexible, than previously thought (Uriz 2006). Despite the well-known differences in secretion mechanisms, there is also now some recognition of similarities in secretion mechanisms between the Calcarea and Silicea (Sethmann & Wörheide 2008).

Biminerallic spicules would have grown by accretion of material onto the external surface of each growth stage, and the phases secreted in sequence. The growing spicules would initially have been siliceous with an axial canal, with the calcareous layer developing later beneath the external organic sheath. The appearance of biminerallic spicules might

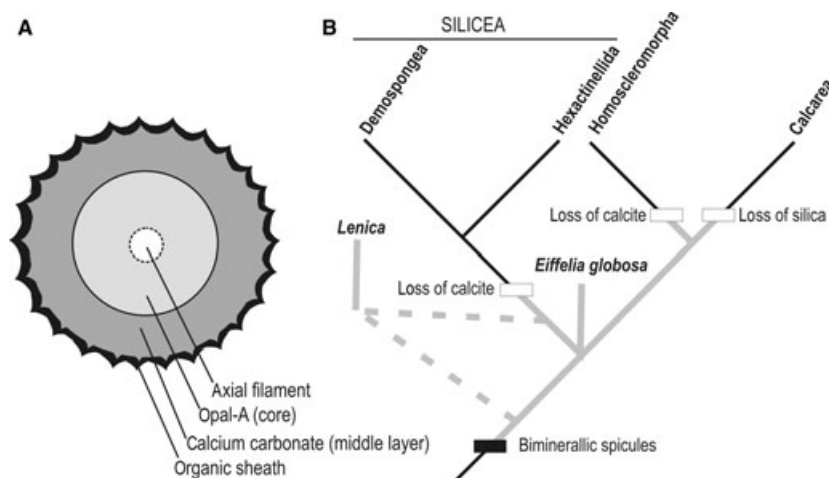


Fig. 6. Interpretation of *Lenica* based on the structures described in this paper. A, Reconstruction of cross-sectional structure of fully developed *Lenica* spicules; the symmetry of the axial filament is unknown. B, implications of biminerallic spicules in early sponges for the presence of spicules in poriferan phylogeny. Thick grey line represents biminerallic spicules; primitive spicules may have been siliceous, but no evidence is currently available regarding this. Basic phylogeny after dominant topology recovered from recent molecular analyses (e.g. Dohrmann *et al.* 2008). The position of *Eiffelia* close to the ancestor of Silicea and Calcarea follows Botting & Butterfield (2005).

therefore occur only in the larger spicules within an individual, and may only be obvious in fossil taxa that possessed unusually large spicules, such as *Lenica*. The evolutionary loss of the calcite layer would also be a likely result of evolutionary pathways in which large spicules were excluded in favour of dense arrays of small spicules – a form of spicular heterochrony. This would need to be tested through ontogenetic studies of large populations of *Lenica* preserving some elements of spicule mineralogy, combined with wider scale studies within the Choiidae to see the extent and evolution of biminerallic spicule development. The required data are not currently available, but investigations of this aspect may be possible in future.

Derivation of calcareous spicules from biminerallic ones could also potentially be achievable, assuming that the calcareous layer was deposited intercellularly, and onto the siliceous proto-spicule which was secreted intracellularly before being transported to the intercellular mesohyl. If this mineralization was induced by proteins such as aspartates (Uriz 2006), crystal growth could have progressed around concentrations of molecules in the intercellular medium, without necessarily a physical basement structure, and would then have been constrained largely by the pseudo-hexagonal crystal symmetry (Jones 1970). Such mediation without a precise physical template is likely to occur in, for example, Homosclerophorida, where the axial filament structure is irregular and diffuse (Uriz 2006).

The exact phylogenetic implications of this understanding of *Lenica* are debatable, as it represents a group with uncertain links to other early sponges. It is likely that it is a choiid (Finks & Rigby 2004), a group of Protomonaxonida (Finks & Rigby 2004) that appears to have been very successful from the late Early Cambrian through the succeeding Ordovician and Silurian periods. If not a choiid, then the body form is strongly convergent. There is also some evidence in the crushed preservation of spicules of *Choia carteri* from the Marjum Limestone (Rigby 1983) for a similar construction to that of *Lenica*, which would imply that the similarity to *Choia* is not superficial. Despite the morphology of choiids being highly derived relative to other protomonaxonids, with a flattened discoidal body and greatly enlarged spicules, there is traditionally a general assumption that the Protomonaxonida are all closely related (Finks & Rigby 2004). However, the demosponge relationships of the Protomonaxonida have repeatedly been questioned (Mehl 1996; Debrenne & Reitner 2001; Botting 2003), and they may represent at least in part a group of stem or early crown hexactinellids. Although all protomonaxonid genera share a monaxon-based skeleton, some groups appear strikingly different to

others, and the monaxonid condition may be convergent in some cases. Some, such as the leptomitids, appear clearly to be derived reticulosan hexactinellids (Debrenne & Reitner 2001), but the relationships of other groups are less obvious. The Protomonaxonida also include *Pirania*, whose diagenetically flattened prosthalia also show an apparently organic surface layer similar to that of *Eiffelia* and *Lenica*, and may well have had a fundamentally similar structure.

Allowing for these uncertainties, *Lenica* was almost certainly either a stem hexactinellid or silicisponge (dependent on the phylogenetic origin of demosponges), or derived from a more basal sponge (Fig. 6B). If biminerallic spicules were present after the Hexactinellida-Demospongiaea split (e.g. in stem demosponges, as in traditional interpretations of the Protomonaxonida), an additional secondary loss of calcite is required in the stem Hexactinellida as well as in demosponges; although possible, it is not the most parsimonious solution. Alternatively, the Choiidae may theoretically represent a stem-group of Calcareae+Homoscleromorpha, which must also have been biminerallic at least basally. Spiculation within the heteractinids and calcareans appears to have been much more conservative than among silicisponges, however, and spicules rarely exceed a few millimeters. Also, most protomonaxonids (e.g. Leptomitidae) appear to have possessed slender, purely opaline spicules, which would require yet another convergent origin of silicisponges. We consider that the null hypothesis must be that *Lenica* represents a branch either from stem-Porifera or stem-Silicea, but this will become clearer with more information on the distribution of biminerallic spicules among early Porifera.

Resolution of the relationships of *Lenica* to traditional Choiidae requires information on the spicule structure and composition of a wide range of other taxa, and most particularly of early, presumed stem-siliceans such as the Protospongiidae, which share striking features of spicule morphology and arrangement with the biminerallic *Eiffelia* (Botting & Butterfield 2005).

Lenica is morphologically distant from *Eiffelia*, but we currently know very little about pre-eiffellid sponges. Hexactinellid-like spicules (or spicule-like pseudofossils) have been widely reported from the latest Precambrian (summarized by Pisera 2006), but their original mineralogy is invariably uncertain, and no unambiguous articulated Ediacaran sponges are known. There is increasing evidence that there was an extensive Precambrian history of sponges that remains hidden, perhaps due to a global taphonomic shift around the base of the Cambrian (Sperling *et al.* 2010). However, no large monaxons with robust

organic layers have yet been described from the Precambrian, suggesting that the group does not have an extremely deep ancestry. Assuming that the spicule structure is not convergent, the differences in spicule form, body form and skeletal architecture between *Lenica* and *Eiffelia* show that biomineralic spicules were widely distributed among Cambrian sponge taxa. It is unclear whether they were more prominent prior to the separation of Calcarea from Hexactinellida, or afterwards within the stem-silicean lineage, although *Eiffelia* shows that they were present close to the divergence point. In the first case, the common ancestor of the modern sponge classes possessed biomineralic spicules, which were reduced in each lineage by loss of one mineral phase and its template. The alternative requires calcareous spicules in the ancestral sponge, followed by development of the siliceous inner layer in the stem Silicea, and which later came to dominate. In either case, spicules are a primitive and homologous feature for all modern poriferan clades, and must have been present in the most recent common ancestor of all extant classes.

Whether extinct, possible sponge lineages such as the archaeocyathans (summarized by Debrenne 2006) were also originally spiculate is unclear, but if spicules originally had a role in body support rather than defence (as supported by Sperling *et al.* 2010), then it is likely that the erect poriferan Bauplan was initially dependent on a mineral skeleton. The origin of archaeocyathans is not likely to be Precambrian, as their robust skeletons should have been seen at least in the Ediacaran, in association with the earliest skeletal fossils. If they are sponges, it is likely that spicules were reduced or entirely lost following the development of the hypercalcified skeleton, and it is unclear to which lineage they are most closely related.

Conclusions

The complex spicules of *Lenica* are inferred to be bi-mineralic, supporting the homology of spicules in Calcarea and Silicea. Depending on phylogenetic topology, this homology may also include Homoscleromorpha, and if sponge paraphyly (Sperling *et al.* 2007) with respect to Eumetazoa is confirmed, then spicules would also be a primitive feature of all animals. Sponge paraphyly with respect to Eumetazoa is, however, currently uncertain, with monophyly regaining strong molecular support (Philippe *et al.* 2009). The presence of bi-mineralic spicules in the sponge ancestor could help to resolve conflicts between morphology and molecular studies, such as the molecular data supporting Homoscleromorpha being closer to Calcarea than to Silicea (e.g. Sperling *et al.* 2007;

Dohrmann *et al.* 2008). Under the arrangement here, spicules in Silicea and Homoscleromorpha would have resulted from parallel loss of the calcite component of the spicules, and Calcarea would result from loss of the siliceous component (Fig. 6B). The diffuse axial organic region in homoscleromorph sponges (Uriz 2006) may then be close to that of the earliest siliceous or compound spicules, consistent with the relatively broad putative canals seen in many early spicules.

The inferred bi-mineralic spicules in *Lenica* and *Eiffelia* illustrate how palaeontology can contribute unexpected insights into phylogenetic debates centred largely on molecular work. In this case, the insights are sufficient to stimulate reinterpretation of the nature of some of the earliest animals, because our interpretation implies that spicules as skeletal elements are homologous across all sponges, despite the differences in secretion processes seen in modern taxa, and that preservable spicules may have been present in the last common ancestor of Metazoa.

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