Carbonaceous preservation of Cambrian hexactinellid sponge spicules

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Early fossil sponges offer a direct window onto the evolutionary emergence of animals, but insights are limited by the paucity of characters preserved in the conventional fossil record. Here, a new preservational mode for sponge spicules is reported from the lower Cambrian Forteau Formation (Newfoundland, Canada), prompting a re-examination of proposed homologies and sponge inter-relationships. The spicules occur as wholly carbonaceous films, and are interpreted as the remains of robust organic spicule sheaths. Comparable sheaths are restricted among living taxa to calcarean sponges, although the symmetries of the fossil spicules are characteristic of hexactinellid sponges. A similar extinct character combination has been documented in the Burgess Shale fossil *Eiffelia*. Interpreting the shared characters as homologous implies complex patterns of spicule evolution, but an alternative interpretation as convergent autapomorphies is more parsimonious. In light of the mutually exclusive distributions of these same characters among the crown groups, this result suggests that sponges exhibited an early episode of disparity expansion followed by comparatively constrained evolution, a pattern shared with many other metazoans but obscured by the conventional fossil record of sponges.

Keywords: Cambrian; hexactinellids; biomineralization; spiculogenesis; metazoan phylogeny

1. INTRODUCTION

Fossil sponges provide a crucial, direct view onto early animal evolution. Molecular phylogenetic hypotheses of metazoan relationships can be tested against extinct character combinations (Botting & Butterfield 2005) and insights from palaeontology and biochemistry are revealing details of spiculogenesis, with implications for evolutionary biology and biomimetics alike (Ehrlich *et al.* 2010). Key outstanding questions are the inter-relationships of major living sponge clades (Hexactinellida, Demospongiae, Calcareo and Homoscleromorpha) and whether the various systems of sponge biomineralization are homologous (Sperling *et al.* 2007, 2010). A hindrance to the phylogenetic and histological resolution of early fossil sponges, however,

is that preserved characters are generally limited to isolated, mineral elements of sponge skeletons. Here I describe an assemblage of early Cambrian sponge spicules that exhibit the carbonaceous composition of calcarean-type spicule sheaths, but hexactinellid-type morphology. This extinct character combination suggests a reconsideration of spicule homologies, with implications for broad-scale patterns in early metazoan evolution.

2. MATERIAL AND METHODS

The Forteau Formation of Newfoundland, Canada, contains undeformed mudstones suitable for carbonaceous microfossil extraction. Samples were collected from the Middle Shale Member (late early Cambrian (Stage 4), *Bonnia-Olenellus* biozone; ca 515 Ma) at the Mount St Margaret quarry, Northern Peninsula (50°99' N, 56°86' W; Knight 1991). Fossils were extracted using hydrofluoric acid at 40 per cent, picked from washed residues using a pipette and mounted on glass coverslips for transmitted light microscopy. Figured specimens are deposited with the Geological Survey of Canada, 601 Booth Street, Ottawa, Ontario.

3. RESULTS

Diverse assemblages of carbonaceous microfossils extracted from mudstones of the Forteau Formation include arthropod spines, *Wiwaxia* sclerites, cyanobacteria, coenobial algae and 31 specimens identified as sponge spicules (figure 1). The spicules measure between 100 and 460 µm wide, and exhibit 90° angles between well-preserved rays, indicating an original three-dimensional geometry of three orthogonal axes, although the specimens are almost entirely flattened. Most specimens are hexactines bearing six smooth rays (figure 1a–h), but eight specimens are pinular pentactines with four coplanar smooth rays and a fifth, perpendicular ray that is ornamented with thorns (figure 1i–n). The preservation of rays varies within individual spicules, with those on one of the three axes commonly exhibiting a greater degree of deformation, such as foreshortening and irregularity in outline (figure 1), and protrusion from the single horizontal plane that contains the well-preserved rays (figure 1p). This pattern is attributable to variation in the burial angle of spicules, ranging from examples with all three axes oblique to the direction of sedimentary compaction (figure 1a,c) to those with one axis nearly parallel to it (figure 1d,i,p). Together with the straightness of well-preserved rays, the pattern of deformation suggests that the spicules were stiff upon burial, but were able to deform plastically during sedimentary compaction, presumably requiring the dissolution by that stage of any mineral component. The appearance of the rays as tubes that have been flattened to essentially two-dimensional carbonaceous films, which is apparent at the nodes (figure 1o) and in occasional longitudinal folds (figure 1b,i,p), allows for the original presence of an enclosed biomineral, but suggests that no mineral component remained prior to acid extraction.

The preserved carbonaceous films exhibit discrete spicule-shaped outlines, approximately regular thickness, and sharp rendering of external ornamentation (such as the pinular thorns), suggesting that they are derived from an organic sheath that was synthesized by the living sponge and was intimately, evenly and exclusively associated with the exterior surfaces of

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Figure 1. Carbonaceous Cambrian sponge spicules from the Forteau Formation. (a–h) Hexactines; (i–n) pinular pentactines; (o) details from (a); (p) details from (i), at two focal planes to reveal overlapping rays. (a–n): scale bar, 150 μm ; (o, p) scale bar, 40 μm . Note that the side-branch in (a) was reflexed during preparation, and that opaque bodies are crystals of diagenetic pyrite. Specimen numbers for (a–n) are GSC134282–GSC134295.

individual spicules. By contrast, equivalent regularity in outline and thickness could not be expected to result from the *post-mortem* templating of spicules by some extraneous organic material derived from decaying sponge tissues, microbial films or sedimentary organics. Furthermore, co-occurring carbonaceous microfossils, such as arthropod cuticles, *Wiwaxia* sclerites, cyanobacterial sheaths and algal cell walls, were originally composed of extracellular organic tissues of biomacromolecular composition, and it is likely that the spicule sheaths possessed similar qualities of physical and chemical robustness.

4. DISCUSSION

The carbonaceous sponge spicules from the Forteau Formation extend the geographical and phylogenetic range of organic preservation in the Cambrian, confirming the unique palaeobiological contribution of carbonaceous microfossils (Butterfield & Nicholas 1996). They also suggest that this taphonomic mode can circumvent a hypothesized bias against the preservation of siliceous spicules in the late Precambrian, the

projected time of earliest metazoan divergence (Sperling *et al.* 2010). However, it is their fresh perspective on Cambrian spicule structure that provides the most important insight.

The morphologies of the Forteau spicules, which include hexactines and pinular pentactines, are characteristic of hexactinellid sponges (Leys *et al.* 2007). The ornamented pentactines preclude close comparison with the spicule-like but non-biomineralized ‘spiculoids’ of some extant demosponges, including *Darwinella* (von Lendenfeld 1889), while the differing symmetry and absence of layered and fibrous microstructures distinguishes the Forteau fossils from the acid-extractable sclerites of cancelloriids, Cambrian animals of at least superficially sponge-like appearance (Butterfield & Nicholas 1996; Sperling *et al.* 2007). Where hexactine and pinular pentactine spicules are known from conventional mineral preservation in the early Cambrian fossil record, they exhibit evidence for original biomineralization in silica (Bengtson *et al.* 1990), consistent with the condition in living hexactinellid sponges. An originally siliceous composition for the Forteau spicules is therefore likely, and is

Figure 2. Two hypotheses for the phylogenetic significance of the Forteau Formation sponge spicules. The Forteau spicules and *Eiffelia* are positioned on the basis of spicule characters onto a constraining ‘consensus’ topology of crown groups (Hexactinellida, Demospongiae, Calcarea, Homoscleromorpha and Eumetazoa (with Placozoa)) (after Sperling *et al.* 2009). For details of these and alternative scenarios, including a constraint tree in which sponges are monophyletic (after Philippe *et al.* 2009), see the electronic supplementary material. (a) A scenario in which homology hypotheses arising from Botting & Butterfield (2005) are tested by enforcing single origins of spicule sheaths and orthogonal spicule symmetry, and a mineralogical transition between silicean and calcarean spicules. Character transformations: (1) origination of siliceous, sheathed, orthogonal-symmetry spicules; (2) addition of hexactines and pinular pentactines; (3) loss of robust spicule sheaths; (4) transition in spicule symmetry, or loss of hexactines/pentactines, or loss of spicules; (5) additional mineralization in calcite, and addition of tri/hexaradiate spicules; (6) loss of siliceous mineralization, and of orthogonal-symmetry spicules; (7) loss of spicules; (8) independent origination of siliceous spicules. (b) A scenario in which the extinct character combinations in the Forteau spicules and *Eiffelia* are attributed to autapomorphies rather than symplesiomorphies. Character transformations: (9 and 11) independent originations of siliceous spicules; (10) origination of sheathed, calcitic spicules of tri/hexaradiate symmetry; (12) independent origination of spicule sheaths; (13) independent origination of orthogonal-symmetry spicules and perhaps silica mineralization.

consistent with the inferred changes in their mechanical properties from stiff upon burial, to plastic during subsequent (syn- or post-dissolution) compaction.

However, the presence of a robust organic sheath in the Forteau spicules is at odds with the condition in known hexactinellids. In living siliceous sponges, including hexactinellids, the organic component of the spicule is largely restricted to the axial filament, which unlike the Forteau fossils does not reflect external spicule morphology (e.g. Leys *et al.* 2007). Furthermore, the axial filament along with other intra-spicular organic material (whether dispersed particles, layers or scaffold) consists chiefly of silicateins (e.g. Müller *et al.* 2008; Wang *et al.* 2010), which are enzymatic proteins of presumably low preservation potential. In the few reported cases of more chemically robust organic components to siliceous spicules, whether chitin (e.g. Ehrlich *et al.* 2007) or collagen (e.g. Ehrlich *et al.* 2008; for reservations, see Wang *et al.* 2010), sheath-like arrangements are unknown. Similarly, the collagenous nets that coat the outsides of some hexactinellid spicules (Müller *et al.* 2007) do not form discrete, even sheaths that could account for the Forteau fossils.

On the other hand, the spicules of living calcarean sponges possess sheaths with all the properties inferred for the fossil structures (Jones 1967; Botting & Butterfield 2005; Sethmann & Wörheide 2008). In addition to being discrete, tubular structures that trace the external spicule morphology, the sheaths of calcareans are composed of collagen and can attain micron-scale thickness, and are known to be both chemically and physically robust, even when isolated from the mineral spicule (Jones 1967). As such, the Forteau spicules exhibit an extinct combination of

characters: the defining symmetry of hexactinellid spicules, but with calcarean-type organic sheaths. A comparable mosaic of characters has been documented in the Burgess Shale sponge *Eiffelia*, which in addition to bearing both calcarean (heteractinid) and hexactinellid-like (stauract) spicule symmetries (but not pinular pentactines or unambiguous hexactines), exhibits a robust, organic sheath-like component to its spicules (Botting & Butterfield 2005).

On the basis of the extinct character combination in *Eiffelia*, Botting & Butterfield (2005) argued that the spicules of extant Calcarea and Silicea (Hexactinellida plus Demospongiae) are homologous. To test whether the Forteau spicules support this hypothesis, I have plotted them on a ‘consensus’ tree of sponge relationships based on the well-sampled molecular phylogenetic analysis of Sperling *et al.* (2009), with homologies enforced among sheaths, and between the spicules of Calcarea and Silicea (figure 2a; for details and alternative topologies, see the electronic supplementary material). Under this experimental scenario, sheathed, siliceous spicules of orthogonal symmetry resolve as present in the common ancestor of sponges and other animals—a metazoan plesiomorphy. Also implied, however, is a suite of character reversals, transitions and losses higher in the tree (figure 2a), meaning that although the homology statements accord well with the fossil data, they are not compelling overall. The alternative—that sheaths and hexactine-like spicules each have convergent evolutionary origins in hexactinellids and calcareans (figure 2b)—is more parsimonious, assuming equal character weighting, but surprising in view of the mutually exclusive distributions and marked conservation of these spicule characters among the sponge crown groups.

Considered in a different light, however, the Forteau spicules and *Eiffelia* suggest that sponges, like many other metazoans, exhibited an early episode of morphological innovation and disparity expansion that was unmatched later on, implying increasing ecological constraints and/or the canalization of developmental programmes (see Erwin 2007; Peterson *et al.* 2009). This pattern is suggested by the many unusual symmetries among isolated Cambrian mineral spicules (Bengtson *et al.* 1990) and the record of extinct sponge-grade animals (Sperling *et al.* 2007), although complementary taphonomic modes such as the carbonaceous preservation of the Burgess Shale and Forteau Formation are required to bring it into focus. It is becoming clear that Cambrian fossil sponges cannot be systematized using 'modern' rules. However, extinct character combinations might not record deep homologies so much as a period of exploding disparity across multiple lineages in the face of unprecedented ecological upheaval.

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