Reconstructing early sponge relationships by using the Burgess Shale fossil Eiffelia globosa, Walcott

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The relationships of the sponge classes are controversial, particularly between the calcareous and siliceous sponges. Specimens of the putative calcarean Eiffelia globosa Walcott from the Burgess Shale show the presence of diagnostic hexactinellid spicules integrated into the skeletal mesh. The arrangement of these spicules in Eiffelia is shown to be precisely equivalent to that of early proaspidoporoid hexactinellids, and sponge growth occurred through an identical pattern to produce identical skeletal body morphology. The difference in spicule composition of the classes is interpreted through the observation of taphonomic features of Eiffelia that suggest the presence of at least two mineralologically distinct layers within the spicules. These results support molecular analyses that identify the calcarean-silicisponge transition as the earliest major sponge branch and suggest that the heteractinids were paraphyletic with respect to the Hexactinellida.

Materials and Methods
We examined all 30 catalogued specimens of Burgess Shale Eiffelia in the U.S. National Museum (USNM), Washington, D.C., and two previously unstudied specimens housed at the Royal Ontario Museum (ROM), Toronto. This study is based on the two ROM specimens (ROM 57022 and 57023) and six USNM specimens, including the type specimen (USNM 66523, 200648, 200653, 200638, 200654, and 200656). There is no stratigraphic information available for any of the USNM specimens nor for ROM 57023, which was collected from scree; ROM 57022 was collected 210 cm below the base of the “Phyllopod Bed” within the Walcott Quarry Shale Member of the Burgess Shale Formation (21).

All of the selected fossils were studied by using a binocular stereoscope with camera lucida attachment. ROM 57022 was also analyzed by using backscattered scanning electron microscopy and electron-dispersive Fourier analysis, with parts studied in a petrographic thin section or treated locally with concentrated hydrofluoric acid (HF). In addition to documenting the morphology of the constituent spicules, we consider the geometry of spicule arrangement in Eiffelia and develop a model of mineralogical transition in early sponge evolution.

Description. In life, Eiffelia was globose, up to 6 cm in diameter, with a lattice of spicules loosely arranged into a single layer (16, 17). Spicules occur in multiple size orders with the largest (first-order) spicules defining the overall geometry and smaller order spicules progressively filling the intervening spaces. Both Walcott (16) and Rigby (17, 22) identified the spicules as hexaradiate, but our reexamination reveals the additional presence of tetraradiate, including hexactine, spicules. As with most nonmineralized and lightly mineralized structures in the Burgess Shale, Eiffelia typically occurs flattened on bedding planes, the individual elements defined by reflective films with very slight relief.

ROM 57023. ROM 57023 (Fig. 1) is an articulated but incomplete Eiffelia with an estimated body diameter of ∼25 mm. Spicules...
vary widely in size and appear to fall into five distinct size orders, although, on the assumption of an incremental size-order ratio of 1.3–1.4, we infer a total of seven. The largest measured spicules have a ray length of >4.5 mm, a central disk diameter of 0.65 mm, and a basal ray diameter of 0.18 mm; the smallest have ray lengths of just 0.2–0.3 mm.

All of the first-order spicules in this specimen are hexaradiate and appear to lack perpendicular rays. Second-order spicules are also predominantly hexaradiate; however, the smaller size orders are increasingly dominated by tetraradiates. In the smallest size class, for example, tetraradiates outnumber hexaradiates by a ratio of 10:1. The largest certain tetraradiate has rays 3.5 mm long, equivalent to the second-order hexaradiates.

As the name implies, adjacent rays of tetraradiate spicules in ROM 57023 tend to diverge at angles of 90°, although this value can vary by up to 10° (Fig. 1B). At least some also preserve evidence of a compressed ray (or rays) oriented perpendicular to the plane of the other four (Fig. 1D). By contrast, the hexaradiate spicules exhibit generally regular 60° radial symmetry and show no evidence of perpendicular rays (ref. 16, p. 324). The tetraradiates also differ from adjacent, similarly sized hexaradiates in having a smaller basal disk (see Fig. 1B and D).

ROM 57022. ROM 57022 (Fig. 2) was collected as a small fragment exposing five articulated hexaradiate spicules and comprising just three size orders (maximum ray length 5 mm). Preservation of this specimen is excellent, however, and reveals a number of significant features. The spicules show slight relief, and the surface of each spicule is defined by a thin (<1 μm), HF-resistant, reflective carbonaceous film. The interior of the spicules (i.e., the material lying beneath the carbonaceous film and responsible for the slight topographic relief) is composed of diagenetic aluminosilicates, readily distinguished from the surrounding shale by its distinct cation composition (unpublished results) and differential response to HF.

Close examination of the margins of spicules in ROM 57022 shows a distinct bilayered construction, the outer region consistently 200–300 μm wide (Fig. 2B). Walcott (16) noted this finding in the type material (see Fig. 1C), and it appears to have been the basis of his interpretation that the spicules contained a central canal. Certainly the same double-walled spicules can be seen in the type material, but the original constitution has been obscured by pervasive diagenesis; we assume that Eiffelia spicules were originally solid, like those of later heteractinids (14). In a cross section, the outer layer of Eiffelia spicules is distinguished by thickening and the divergence of the otherwise collapsed, essentially coalesced, carbonaceous film (Fig. 2C).

Although there is no remnant of the original mineralogy, the conspicuously different responses of these two spicule layers to diagenesis points to marked differences in composition.

USNM-Type Material. Only six of 30 Eiffelia specimens in the USNM-type collection were sufficiently well preserved to allow detailed analysis of smaller spicules and sufficiently large to distinguish true absence of tetraradiate spicules from sampling error. Of these six, all except USNM 66523 include tetraradiate spicules, and in the case of USNM 200656, they comprise more than one-half of all spicules (Fig. 3E). In all instances, the largest spicules are hexaradiate, whereas most tetraradiates are third-order or smaller (Fig. 3A–E).

In USNM 200656, 25 of 38 visible spicules are tetraradiate and allow a determination of their larger-scale arrangement in the Eiffelia skeleton. Beginning with a camera lucida tracing of the specimen (Fig. 3E), we assumed a fixed orientation for the majority of spicules but replaced the few remaining hexaradiates with tetraradiates of equal size, orthogonal to the fixed orientation, or rotated to reflect the original orientation of each hexaradiate if this orientation differed from the fixed direction (Fig. 3F). The result is a pattern of irregular quadruling (quadruled is a rectangular grid subdivided by orthogonal spicules, with members of each smaller order positioned in the center of
spaces between the previous order; see Fig. 3G) identical to that of early protospongioids.

There are sufficient near-complete specimens of USNM Eiffelia, including those with poor preservation of small spicules, to follow Botting’s (23) approach to analyzing growth patterns. The results show a proportional increase in maximum spicule ray length with sponge diameter, indistinguishable from the pattern observed in primitive hexactinellids and demosponges, but subsequently modified in various ways in almost every sponge lineage (23).

Discussion

Skeletal Structure of Hexactinellids and Calcareae. Primitive, early Paleozoic hexactinellids (“Reticulosa”; refs. 12 and 23–27) possessed a broadly consistent body-wall spiculation composed of hexactine-based tetraradial spicules arranged in up to nine morphologically similar size orders; adjacent size orders typically differ by a factor of ≈1.3 but can vary significantly (23). Spicules grew throughout life, to a limit, with smaller orders appearing sequentially between larger spicules (23), eventually giving rise to the idealized “quadruling” arrangement of Protospongia sensu stricte (24). Most early members of this clade, however, express the irregularly quadruled pattern seen in Eiffelia, suggesting that this design was the ancestral architecture among hexactinellids (12, 28). By the Late Paleozoic, most hexactinellids possessed thickened body walls composed of multiple spicule layers and no longer exhibited the discrete size ordering of earlier forms (e.g., ref. 29). Differentiated hexactinellid microscleres are present by at least the Ordovician but are rarely preserved in situ (30).

Like derived hexactinellids, most extant calcareae are characterized by thickened body walls and irregularly arranged spicules. The simplest (“ascon”) forms, however, are structurally equivalent to the protospongioids, with axial symmetry and an outer wall composed of a single layer of spicules. Although morphologically distinct microscleres are absent, calcarean spicules often occur in two or more sizes, either of two distinct magnitudes (e.g., ref. 31) or varying over a range. In the latter case, the sequence can sometimes be separated into successive size orders, related by a constant geometric factor that is typically 1.1–1.5, often near 1.3 (J.P.B., unpublished data); such size orders
failed to resolve the relationships and polarity of the sponge classes, 
calcareae depends on accurate phylogenetic reconstruction of the 

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The triradial symmetry of extant Clathrinida spicules is poten-
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(e.g., ref. 32) and the similarity of heteractinid spicule structure to 
that of modern calcareans (14). The Paleozoic Eiffeliaeidae are 
widely regarded as the most basal heteractinids (29), and some 
authors have regarded the lineage as probable stem Calcarea (11).

There are no known extant calcareans with hexactine (rather than 
hexaradiate) spicules or their derivatives and only a single fossil 
species, the enigmatic Canistrumella rigbyi (17), also from the 
Burgess Shale. In the absence of data on microscleres or soft tissue, 
the only real distinctions between eiffeliids and protospongoidiids 
are spicule symmetry and mineralogy; our results show that even these 
differences do not hold in Eiffelia (Table 1).

Implications for Phylogeny. On the basis of its preserved morphology 
and growth patterns, Eiffelia could be regarded either as a peculiar 
hexactinellid with hexaradiate spicules or a peculiar calcarean with 
tetradrate spicules, although there is no compelling evidence to 
prefer one interpretation over the other (Table 1). Such mosaics of 
morphological features are common in the early fossil record and 
increasingly are being recognized, not as bizarre experiments in 
early evolution, but as the stem-group “intermediate” stages linking 
early higher-order taxa (9). In this light, Eiffelia is readily inter-
preted as intermediate between the heteractinid calcareans and the 
protospongoid hexactinellids.

Whether Eiffelia is a stem-group exactinellid or a stem-group 
calcarean depends on accurate phyllogenetic reconstruction of the 
estant crown groups. Unfortunately, molecular phylogenies have 
failed to resolve the relationships and polarity of the sponge classes, 
although two scenarios have received most of the support: (i) 
(Hexactinellida plus Demospongea) (Calcarea plus Eumetazoa) (5, 
6, 33–37) in which Silicispongea is monophyletic, and (ii) Hexacti-
nellida (Demospongea (Calcarea plus Eumetazoa)) (8, 38–41), 
which gives a paraphyletic Silicispongea. The balance of recent 
molecular phylogenies, based on a range of genes, is approximately 
equal (42), but with an increasing shift toward silicisponge mono-
phyly [albeit with Homoscleromorpha excluded from both Demo-
spongea (43) and Silicispongea (44)], particularly in light of cor-
roroborative biochemical (7), paleontological (45), and of course 
mineralogical data. Molecular analyses also consistently identify a 
close relationship between Calcarea and Ctenophora plus Cnidaria 
(e.g., ref. 8), usually interpreted as poriferan paraphyly with respect to 
Eumetazoa.

The discovery of an evolutionary intermediate between hexacti-
nellids and calcareans argues strongly against a basal Hexactinellida 
because this result would require repeated derivation of siliceous 
spicules secreted onto axial filaments, in hexactinellids and demo-
sponges, and perhaps also Homoscleromorpha. By providing evi-
dence of a direct link between Calcarea and Hexactinellida, it also 
categorically excludes some less well supported topologies such as 
that of Adams (5), in which Calcarea are nested among Cnidaria 
and Ctenophora. In contrast, the hypothesis of silicisponge mono-
phyly that is emerging prominently from neontological work is 
entirely consistent with our data.

Recognition of a probable (Hexactinellida plus Demospong-
ea) (Calcarea plus Eumetazoa) topology is interesting, but 
molecular studies do little to constrain character polarity and, 
thus, the nature of the common ancestral node. By contrast, our 
analysis of Eiffelia provides direct evidence for both the deriva-
tion of demosponges from total-group (probably stem) hexacti-
nellids (cf. ref. 45) and the derivation of Eumetazoa from 
(probably stem) calcareans (see Fig. 4). Unfortunately, calcar-
ean/hexactinellid polarity remains unresolved because of the 
lack of unequivocal outgroup comparisons, although we offer a 
calcarean-to-hexactinellid trajectory in light of the mineralogical 
transition suggested by Eiffelia (see below). Future fossil discov-
eryies of demonstrable stem-group poriferans with preserved or 
implied mineralogy and symmetry will be needed to disprove or 
corroborate this hypothesis.

Mineralogical Transition: An Hypothesis. The evolution of silici-
ponges from a primitive calcarean requires a number of fundamen-
tal shifts in spicule structure, including a transition from an 
external organic sheath to an internal filament (46, 47) and from a 
composite magnesium calcite/amorphous calcium carbonate 
(ACC) (48) mineralogy toopal. Given the consistent positioning 
and size-order relationships of spicules between Eiffelia and 
early hexactinellids, it appears that spicules underwent a trans-
formation rather than a loss and subsequent replacement.

The bilayered construction of Eiffelia spicules (Figs. 1C and 
2B) clearly identifies two components of distinctly different 
chemical and/or physical properties. Comparison might be made 
with the differentiated magnesium calcite/amorphous calcium 
carbonate composition of extant calcarean spicules (cf. ref. 48), 
but the similar diagenetic lability of these two carbonate phases 
is unlikely to account for the observed differences. Moreover, it 
is the outer layer that is more substantially preserved in the 
fossils, but in modern calcareans it is the core that is composed of 
the more stable magnesium carbonate. Insofar as a mineralogical 
transition is demanded in most transformational scenarios be-

### Table 1. Comparison of presence and absence of primary characteristics of siliceous sponges (Hexactinellida plus Demospongea), Calcarea, and Eiffelia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Silicispongea</th>
<th>Eiffelia</th>
<th>Calcarea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial organic filament</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hexactine spicules</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Opal-A- in spicules</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rhomboidal first-order grid</td>
<td>1*</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>Multiple size orders</td>
<td>1*</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>Axial symmetry</td>
<td>1*</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>ACC/Mg-calcite in spicules</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Organic spicule sheath</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hexaradiate spicules</td>
<td>0</td>
<td>1</td>
<td>1*</td>
</tr>
</tbody>
</table>

1, presence; 0, absence; 1*, present in ancestral forms but subsequently lost in at least some extant taxa; ? character state probably as given, based on our observations, but could not be categorized with the available material.
tween calcareans and silicisponges, and given the widespread
capacity for combined calcium carbonate and silica biomineral-
ization among extant and fossil sponges (49), we suggest that the
two layers of *Effelia* spicules represent distinct carbonate and
opaline silica phases. Assuming a Calcarea to Hexactinellida
polarity, the spicule core is likely to have been calcareous and
surrounded by a secondary layer of opaline silica.

The organic sheath surrounding modern calcarean spicules con-
sects of dense bands of collagen fibrils, each up to a maximum of 130
nm in diameter (50), the sheath with a total thickness of ~0.5 μm
(ref. 50; Fig. 1). This measurement is comparable with the carbonaceous
film associated with *Effelia* spicules (Figs. 1 and 2), although whether the two structures are
directly homologous remains to be seen. In *Effelia*, for example, the
sheath appears to envelope the whole of the spicule rather than just
the core. Moreover, the ability of extant demosponges to produce
unmineralized spicules (e.g., *Darwinella*) (51) and the presence of conspicuous,
apparently carbonaceous films around spicules of
otherwise unproblematic Burgess Shale demosponge *Pirania* (see ref. 17) and whether these films might be
homologous with the organic film surrounding the spicules of
*Effelia* and/or the spicules of extant Calcarea.

Chancelloriids are problematic Cambrian fossils with a
sponge-like form, including multilayered, lightly mineralized sele-
rites surrounded by a thin, reflective, carbonaceous film (51)
similar to that associated with *Effelia* and *Pirania* (but also most other organically preserved fossils). Unlike sponge spicules,
however, chancelloriid sclerites are “hollow” and constructed of
multiple elements, making it difficult [although not impossible (51)] to accommodate them within the poriferan bodyplan (55, 56). Even so, it may be worth considering their structure with reference to our model of mineralogical transition: in this case
the originally calcitic core may simply have abandoned
mineralization but retained the original form of the “calcarean”
organic sheath (rather than having it condense to an axial
film as we propose for the transition to Silicispongea).
In other words, the bipartite constitution of chancelloriid rays (the
hollow core and lightly mineralized wall) conceivably corre-
sponds to the bipartite constitution of *Effelia* spicules.

Although the polarity of the transition cannot be established
from the available molecular data, we suggest the possibility that
Calcarea are paraphyletic with respect to Silicispongea; the
definitive resolution of this issue depends on clarification of many aspects of basal metazoan phylogeny. However the rela-
tionships are eventually resolved, *Effelia* now joins *Propospongia*
(12), *Canistrumella* (17), and various other problematic Paleozo-
ic sponges in its exclusion from any class-level crown group. As
such, it offers new insights into the deep interrelationships of the
Porifera, particularly in light of the conflicting results arising from
recent molecular analyses.

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