

Peer Review File

Manuscript Title: A late Ediacaran crown-group sponge animal

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

Summary: A very clean, straightforward paper presenting morphologically easy-to-describe, important fossils of undisputed Ediacaran age that contribute very substantially to the exceedingly sparse and controversial fossil record of the early evolution of animals. I find nothing significant to comment on, suggest, or criticise, and recommend publication in Nature.

This paper is centred on a straightforward, nicely written, logically ordered description of startlingly recognisable bedding-plane impressions of large sponges from the latest Ediacaran in China. The evidence presented is straightforward, simple macromorphology of several reasonably well-preserved samples representing a single taxon, and is neither subtle nor difficult to describe and understand. The late Ediacaran age of the material is undisputed, because it comes from a well-known stratigraphic succession that has been subjected to a variety of dating methods at different levels (presented in an appendix), fairly tightly bracketing depositional age of the unit from which the specimens came. The fossils appear superficially similar to some fossils from the contemporaneous, classic Ediacaran fauna in both preservation and structure: they are bedding-plane impressions (although in carbonate rather than siltstone/sandstone) of fractal objects expressing multiple scales of the same microtopographic geometric feature (a rectangle), which the authors liken to a specific type of fractal (Cantor set). Unlike many of the fractally organised Ediacaran organisms, however, these fossils strongly resemble a known, uncontroversial analogue in the fossil record: hexactinellid sponges. The Ediacaran sponges have the same skeletal geometry as some Paleozoic spiculate hexactinellids, but with an organic rather than biomineralised skeleton - as one might predict.

Given that the subject matter is morphological, the figures are critical. Figure 1 presents side-by-side (a) photographic and (b) heat-mapped microtopography of the best specimen (it might be nice if the scale on the heat map were in even increments of mm) as well as (c) a cross-section to demonstrate that the object originally had three dimensions and that its interior was preserved at least in part by a carbonate cement that differed from the composition of the host rock. The heat map is a useful way of depicting the dimensions of the microtopography. Figure 2 is devoted to demonstrating the fractal organisation of the fossil impression; this is a helpful, interesting figure with no extraneous images. Not sure figure 3 (photos of more fossils) is really needed; could be demoted to the appendices if necessary. Figure 4 consists of a reconstruction of the fossil and the outcome of the Bayesian phylogenetic analysis. In short, the figures are necessary (except perhaps for fig 3) and well constructed for the needs of the reader.

The description of the simple fossils is brief and easy, and the bulk of the paper is given to discussion. This section predictably centres on (a) determining whether the fossils could have an explanation among established Ediacaran organism types (all of which have different type of body plan, so no); (b) exploring other organism types that might express similar fractal-like morphological characteristics (no success); and (c) determining whether the fossils belong to a specific known sponge group, mainly through the use of a Bayesian phylogenetic analysis. This is a well-rounded discussion that efficiently dispenses with the major questions that might arise in the minds of both specialist and general readers.

The scope of the background required for this paper means that a wide range of supporting publications must be cited. The authors seem to have done justice to the main publications in sponge paleobiology and present a competent review of Ediacaran and other material required for comparison.

The abstract is succinct, efficiently covering the fossil characteristics, their interpretation, and their implications.

With the subject matter consisting of simple, large macrofossils having a very distinctive, familiar morphology and a well-constrained depositional age, there is little to quibble over in this material, its description and interpretation, or its importance to the evolutionary history of animals.

Finding readily identifiable crown-group sponges in late Ediacaran strata is stupendous, but at the same time almost predictable. The existence of crown-group (probably) non-biomineralised sponges at that time implies a substantial evolutionary pre-history, which the authors (like others) suggest probably took place in aspiculate, non-biomineralised sponges. This adds another very valuable waypoint in the early fossil record of sponges, regarded by many as the most basal animal type. This is a fraught topic with an abundance of controversy and opinions, but virtually no physical evidence, so every scrap of possibly relevant fossil material is exquisitely precious. The broad importance of this material and this paper merit publication in *Nature*.

Referee #2 (Remarks to the Author):

Review of "A late Ediacaran crown-group sponge animal" by Wang et al.

The authors describe a new genus and species, *Helicolocellus cantori*, from upper Ediacaran strata of China, based on seven specimens. Part of the body of *Helicolocellus cantori* in some specimens shows a gridded structure interpreted as an organic skeleton composed of spicule-like elements. They interpret the specimen as a crown-group sponge, related to the extant class Hexactinellida, on the basis of this inferred spicule-like framework.

The mystery of why there are no Ediacaran sponges, despite the long Precambrian range for phylum Porifera indicated by molecular-clock analyses, is an important issue in present-day palaeontology. Even a single Ediacaran crown-group sponge would be enormously informative. Unfortunately, there

are several major flaws in the interpretation of *H. cantori* as a hexactinellid, or even as a sponge. Firstly, the supposed sponge-like features of the organism are not convincing, mostly for taphonomic reasons. Secondly, the organism exhibits features that argue against a sponge affinity. Thirdly, there are problems with the cladistic analysis on which the conclusion of a hexactinellid affinity is based.

Supposed sponge-like features are unconvincing

The interpretation of the fossil as a crown-group sponge is not tenable on taphonomic grounds. All specimens are preserved in relief, i.e. not flattened. This relief preservation means that the organism would have been either very robust or very thick-bodied, or both. The organism (if a sponge) must have had a fused skeleton (as in the late Palaeozoic *Hydnoceras* and *Clathrospongia*) to have been preserved in this way. Cambrian (and early Palaeozoic in general) spiculate sponges, which did not have fused skeletons, are preserved flattened (see for example the Burgess Shale and Hetang sponges).

However, the regular grid pattern is only present in parts of some specimens: it cannot be seen in the basal part of any specimen, or at all in NIGP176536. NIGP176532 and 176533 show only small portions of regular mesh. This lack of regularity is attributed to partial disarticulation of the grid during degradation or compaction; in this case, the presumed spicules must have been unfused.

Thus, there is a contradiction: the skeletal grid must have been fused (to be preserved in relief) and unfused (to have disarticulated). The interpretation of the grid as a sponge-like skeleton therefore does not work.

In addition, the authors interpret the gridded structure as “an organic skeleton comprising orthogonally arranged cruciform elements” (abstract; they say elsewhere in the manuscript that the grid may have been lightly mineralised). However, no traces of the presumed spicules are preserved. This is surprising, given that the elements forming the grid structure must have been present when the rock was lithified (or the grid structure would not have been preserved at all). In the holotype, the first-order rectangles are separated by “grooves that are 1.1 mm wide and approximately 0.4 mm deep”. The first-order rectangles are 12 mm long and ca. 9 mm wide. Assuming that each ray of the largest “cruciform elements” extended over half a grid square (as in the Palaeozoic reticulosan *Cyathophycus*; see Botting 2003), the largest spicules should have had long rays 6 mm long, shorter rays 4.5 mm long, and been 0.4 to 0.5 mm wide. This would be quite large for Palaeozoic sponge spicules (which is not in itself a problem), but then one would expect to see imprints of the spicules in the sediment, because the largest spicules would be markedly larger than the grain size of the rock (see Fig. 2). No spicule imprints are visible in Fig. 2, suggesting that spicule-like elements were not present.

It is also relevant to mention that sponge taxa that are not hexactinellids can possess an approximately grid-like structure. See for example the Burgess Shale demosponge *Vauxia gracilenta* (<https://burgess-shale.rom.on.ca/fossils/vauxia-gracilenta/>, see ROM 53564 and USNM 66515) and the Cambrian ascosponge *Leptomitrus* and relatives (previously identified as protomonaxonid demosponges; see illustrations in the *Treatise on Invertebrate Paleontology*, part E (revised), vol. 3).

Helicolocellus possesses features that are not sponge-like

The stem plus basal disc of *Helicolocellus* are not sponge-like features; they are, however, very like those of other Ediacara Biota taxa, specifically fronds such as *Charnia*. The authors claim that the holdfast/basal disc represent a convergent adaptation to a soft substrate in several (unrelated) taxa living in the same environment. Convergence is certainly something that has occurred commonly in evolution, and should always be considered as a possibility. In this case, however, some sort of rigid framework could also have evolved convergently, meaning that *H. cantori* is not a sponge.

The presence of a marginal fringe implies that *H. cantori* is bilaterally symmetrical, rather than radially, as would be expected for a sponge. (There is evidence that early sponges were tetradially symmetric [Botting et al. 2014], but there is no sign of tetradial symmetry here.) If the organism is bilaterally symmetrical, then character 91 of the cladistic analysis is incorrectly coded (coded as radially symmetric in the analysis). This incorrect coding may have affected the results, because extant sponges are radially symmetric (or asymmetric); thus, incorrectly coding *H. cantori* as radially symmetric may have erroneously caused the organism to cluster with sponges.

The presence of a second fringe in the holotype is also not a sponge-like feature: the authors suggest that the second fringe may have resulted from contraction of the body. If the body has contracted, then the grid-like pattern cannot represent a spicule net, because the spicular framework of sponges supports the soft tissue and stops it moving. Allowing the soft tissue to contract would close the inhalant canals, meaning that the organism could not have filter-fed. The presence of the fringes is thus evidence against a sponge affinity.

Cladistic analysis

There are several issues with the cladistic analysis.

- Only extant taxa are included, meaning that the fossil must cluster with one of these taxa.
- As above, *Helicolocellus* appears to be bilaterally symmetrical; thus, character 91 is coded incorrectly.
- Character 176 in the cladistic analysis (highly regular hierarchical reticulate skeleton [quadrate mesh]) is stated to be present. However, the regular grid pattern is only present in parts of some specimens: it cannot be seen in the basal part of any specimen, or at all in NIGP176536. NIGP176532 and 176533 show only small portions of regular mesh.
- Character 182 in the cladistic analysis (skeletal organisation): *Helicolocellus* is coded as dictyonine-like, i.e., a rigid framework; however, a rigid framework would not show partial disarticulation, as is claimed for *Helicolocellus*. Lyssacine-like skeletal organisation is known in sponge taxa with regular grid patterns, such as *Protospongia* and *Cyathophycus*; thus, the presence of a regular grid is not sufficient to interpret the skeleton as being dictyonine.
- If *Helicolocellus* did have a spicule network, then the coding of all spicule types as being absent (characters 191 to 218), rather than unknown, would be problematic. The “spicules” (if they existed) would have had some morphology, which the authors say is cruciform.
- As far as I can make out, the stalk/basal disc and the fringe are not included in the cladistic analysis; thus, the analysis considers only some of the morphology of the organism.
- *Charnia* was included in the original dataset of Dunn, on which the cladistic analysis is based; however, the authors have chosen to exclude *Charnia*. I strongly suspect that if extinct taxa such as *Charnia* had been included, *H. cantori* would (on the basis of the stalk/basal disc) have been included in that group.

To summarise, given the lack of plausible sponge features, the presence of decided non-sponge morphology, and the issues with the cladistic analysis, the conclusions of the paper are not supported, and the paper is not suitable for publication.

Specific comments

Line 66: change “late Ediacaran Shibantan Member” to “upper Ediacaran Shibantan Member”.

Line 119: “In addition to the holotype, five other specimens are known”, but six additional specimens are listed (“Referred material” in line 65; NIGP176532, 33, 34, 35, 36, 37). Please clarify.

Line 120: NIGP176532 is referred to as a paratype, but in line 65 is part of “Referred material”, which would normally imply that it is not a paratype. Please specify whether specimens NIG176532–176537 are paratypes or not.

Line 218: Pontagrossia was not interpreted as a sponge by ref. 40.

References

- Botting, J.P., 2003. Growth patterns of Early Palaeozoic sponges. *Lethaia* 36, 41–52.
- Botting, J.P., Yuan, X. and Lin, J.P., 2014. Tetradial symmetry in early poriferans. *Chinese Science Bulletin*, 59, 639–644.

Lucy Muir

Referee #3 (Remarks to the Author):

Wang and colleagues describe a new fossil - *Helicolocellus* – from the Ediacaran of China. They describe *Helicolocellus* as a sponge and, after phylogenetic analysis conclude that it represents a stem-group hexactinellid and therefore a crown-group sponge. They interpret *Helicolocellus* as having been either weakly or non- biomineralised and use this as a springboard to suggest that the living Classes of sponge may have biomineralised independently of each other. I found the description of the fossil interesting and compelling, but the manuscript falls down in the execution of the phylogenetic analysis (and subsequent interpretations), the inferences about the evolution of biomineralization and the discussion of other, relevant taxa. I hope my comments and suggestions are useful.

1. Other taxa: The discussion of other, relevant fossil sponges is limited to very specific comparisons with reference to obvious Ediacaran or Cambrian taxa cursory. Two obvious examples from the Ediacaran Period are *Gibbavasis* which shares many clear similarities to *Helicolocellus* and the potential sponge material described by Turner in *Nature* last year which – if interpreted correctly – would likely represent a much more ancient crown-group sponge and it also interpreted as non-mineralised. If this interpretation is correct it undermines the unique selling point of the current manuscript and therefore needs to be dealt with appropriately and in the main text. The regular cross-hatch pattern is reminiscent of the Palaeozoic reticulosans which are mentioned only (from my reading) through *Protospongia* and *Diagonella*, which is described confidently as a hexactinellid, when my reading of the literature is that affinities of *Protospongia* (and other reticulosans) are far less certain. In fact, the reticulosans share several characters with other non-reticulosan Cambrian sponges, including taxa like *Eifellia* which is commonly interpreted as a stem-group calcarerous sponge potentially demonstrating that these characters were much more widespread, plesiomorphically for sponges. The manuscript currently does not give enough time to considering the Ediacaran and early Palaeozoic sponge fauna. The other thing to note here is that when these taxa are discussed it is almost exclusively with reference to the differences between X and *Helicolocellus* and does not really reference shared derived similarities, of which there are plenty, and which underpins phylogenetic thinking today. I suggest the authors change the tone of the discussion to focus more on this.

I think the authors spend too much time arguing against a cnidarian affinity – certainly I would not think a cnidarian affinity likely from looking at the fossil, but the authors might consider devoting some text to *Paramackenzia*, recently described as sharing similarities to modular Ediacarans and which shows some similarity to *Helicolocellus*.

2. Phylogenetic analysis: The analysis presented is not appropriate for what the authors want to say or use it for. There are methodological problems: not enough information about parameters etc is presented in the methods and so far as I can see there are no sensitivity tests – the authors do not explain how they have dealt with ascertainment bias and why and do not run additional sensitivity tests, and despite their tree not resembling molecular trees of living sponges in e.g. the reciprocal monophyly of calcareans + homoscleromorphs and demosponges + hexactinellids, the authors have not presented a molecular backbone tree. These and other relevant tests need to be presented as so much relies on the phylogenetic position of *Helicolocellus* having been interpreted correctly. In my

view these are absolutely essential before this paper can be accepted in Nature, or any other journal. The dataset used is also not appropriate because it only really samples hexactinellids and includes no other sponge fossils. The Dunn et al. matrix only scored sponges at Class level, and only considered the living members of the group; polymorphisms are essentially treated as missing data in these analyses and therefore you are reducing the signal massively in groups which are clearly and obviously relevant for this study. The authors need to improve taxon sampling of living sponges and include additional relevant fossil sponges (including reticulosans and Eiffelia, for example). This expanded dataset would then be appropriate for downstream ancestral state reconstructions, directly testing hypotheses about the plesiomorphic nature of sponges.

3. Evolution of biomineralization: The authors do not have enough information to draw conclusions about the evolution of biomineralization. They are, after all, working with one data point which they concede may also be lightly biomineralised and so their argument is not compelling even in the absence of other relevant fossils which will have significant bearing on this. I suggest this is either removed or greatly reduced in the manuscript and the focus be shifted to encompass a more thorough discussion of contemporaneous sponges and discussion of the phylogenetic implications of this fossil.

Referee #4 (Remarks to the Author):

Review of “A late Ediacaran crown-group sponge animal” by Xiaopeng Wang and colleagues. These represent my personal views on the topic, and should the authors have any questions, they are most welcome to reach out for clarity. By Marc Laflamme.

This manuscript describes and names a new species of crown group sponge from the late Ediacaran Dengying Formation of southern China. Important Ediacaran fossils are not new from the Dengying, however *Helicolocellus cantori* might prove to be the most influential one. Understanding the Ediacaran-Cambrian transition has been extremely controversial; whether it be due to preservational biases associated with the fossilization of soft-tissues, or bonafide evolutionary innovations in response to large scale climatic or biological competition, fossil communities on either side of the boundary are extremely disparate. Although there have been some strong cases for Eumetazoa in the Ediacaran (many of which were interpreted by this authorship team), the search for sponges remains difficult in the absence of silicious or calcareous spicules that, according to molecular clock studies, should be present. With this said, *Helicolocellus cantori* represents an exceedingly rare instance where conclusive morphological evidence is present in a presumed Ediacaran sponge. In my opinion, this is the strongest case for Ediacaran sponges published to date. The diagnostic rectangular fractal pattern is so similar to *Diagoniella*/*Hydnoceras* that I would be hard pressed to think of an alternative to explain the morphological similarities. Furthermore, the apparent absence of a mineralized lattice strengthens the hypothesis that these structures were derived from organic precursors and evolved independently in Hexactinellida and Demospongiae. Honestly, *Helicolocellus* could represent a Rosetta Stone for our understanding of the evolution of Metazoa as a whole.

I do have a few comments that I hope will help the authorship team. The first thing that struck me was the apparent presence of a featureless smooth stem at the base of the presumably filter-filtering cup. I'm not a sponge expert but I find it peculiar that a sponge built from a modular fractal pattern to filter feed would forgo feeding from the lower tiers, and importantly construct a non-fractal morphological structure with little use other than elevating the feeding cup off the seafloor. We see a similar problem with many Ediacaran taxa (esp. the Rangeomorphs), which has been tentatively explained as a means of reducing competition with lower-tier feeders or alternatively as a means of elevating reproductive units to improve dispersal. I did try to investigate this problem with Ediacaran fronds back in 2008/2009, but never really got a good answer. Do the authors have a hypothesis for this? Why do modern sponges forgo a stem/stalk? I'm wondering if it would prove useful to run some Computational Fluid Dynamics (CFD) tests on the benefits of elevating the cup above the sediment/water interface? I'd recommend reaching out to Brandt Gibson (he's a postdoc here at UofT with me) to see if this is something that can be investigated. It might prove to be too much additional work for this manuscript specifically, but if not, I'd love to see some data to help interpret this weird phenomenon.

Another minor point would be to clarify a few comments from the text. For example, L52-54 *H. cantori* also suggests that biomineralised skeletons may have evolved independently among sponge classes and emphasizes biomineralization as the major innovation driving the Cambrian explosion. – Can the authors comment on why mineralization is viewed as a “driver” of the Cambrian explosion, rather than a “response” to other factors driving the diversification? The cause/effect implication here might be worth discussing.

Ediacaran fossils are notoriously difficult to photograph and given the similarity of *H. cantori* to these fossils, I wanted to make sure the images were described correctly. In Figure 2, 2b–d is described as “negative relief preserved on a bed sole (Fig. 2b–d)”, however they appear to be in positive relief in the photo. Is it just a case of lighting from a different angle, or are 2b–d from the same positive relief specimen as 2a (and not the counterpart)?

I would also suggest adding an image of the casting and molding of *Pontagrossia* from the cited Chahud & Fairchild paper (maybe within the Extended Data Fig. 3?) The similarities are striking and would help with their argument.

The “fringe” structures are interesting. The authors provide two alternatives to explain them, but I found the “contraction” argument to be less likely. I'd argue that the potential “contractions” of *Dickinsonia* and *Aspidella* is difficult to explain without musculature. I've also found it very hard to explain how you would capture two “phases” (pre and post contraction, one of which clearly being very short lived) via deathmask casting and molding (I have the same issues with true Ediacarans mind you). Again, this doesn't take away from the study, but it might not be necessary to include it at all.

Author Rebuttals to Initial Comments:

Reviewer comments in this document are provided in black, with our responses in red. We also provide a copy of the manuscript with changes tracked.

Referee #1

Summary: A very clean, straightforward paper presenting morphologically easy-to-describe, important fossils of undisputed Ediacaran age that contribute very substantially to the exceedingly sparse and controversial fossil record of the early evolution of animals. I find nothing significant to comment on, suggest, or criticise, and recommend publication in Nature.

This paper is centred on a straightforward, nicely written, logically ordered description of startlingly recognisable bedding-plane impressions of large sponges from the latest Ediacaran in China. The evidence presented is straightforward, simple macromorphology of several reasonably well-preserved samples representing a single taxon, and is neither subtle nor difficult to describe and understand. The late Ediacaran age of the material is undisputed, because it comes from a well-known stratigraphic succession that has been subjected to a variety of dating methods at different levels (presented in an appendix), fairly tightly bracketing depositional age of the unit from which the specimens came. The fossils appear superficially similar to some fossils from the contemporaneous, classic Ediacaran fauna in both preservation and structure: they are bedding-plane impressions (although in carbonate rather than siltstone/sandstone) of fractal objects expressing multiple scales of the same microtopographic geometric feature (a rectangle), which the authors liken to a specific type of fractal (Cantor set). Unlike many of the fractally organised Ediacaran organisms, however, these fossils strongly resemble a known, uncontroversial analogue in the fossil record: hexactinellid sponges. The Ediacaran sponges have the same skeletal geometry as some Paleozoic spiculate hexactinellids, but with an organic rather than biomineralised skeleton - as one might predict.

We thank the reviewer for their overwhelmingly positive comments.

Given that the subject matter is morphological, the figures are critical. Figure 1 presents side-by-side (a) photographic and (b) heat-mapped microtopography of the best specimen (it might be nice if the scale on the heat map were in even increments of mm) as well as (c) a cross-section to demonstrate that the object originally had three dimensions and that its interior was preserved at least in part by a carbonate cement that differed from the composition of the host rock. The heat map is a useful way of depicting the dimensions of the microtopography.

The heat-map scale in Figure 1b has been changed to include even increments of mm.

Figure 2 is devoted to demonstrating the fractal organisation of the fossil impression; this is a helpful, interesting figure with no extraneous images. Not sure figure 3 (photos

of more fossils) is really needed; could be demoted to the appendices if necessary. Figure 4 consists of a reconstruction of the fossil and the outcome of the Bayesian phylogenetic analysis. In short, the figures are necessary (except perhaps for fig 3) and well constructed for the needs of the reader.

We believe that including Figure 3 in the main body of the text enhances the overall understanding of the new species. Figure 3a illustrates a stem and a basal disc, which are not preserved in the holotype, and which would otherwise not be figured in the main paper. Additionally, it shows that the grid-pattern is not consistently arranged in all specimens. Since these features are discussed in the main text, we believe it is beneficial to retain Figure 3.

The description of the simple fossils is brief and easy, and the bulk of the paper is given to discussion. This section predictably centres on (a) determining whether the fossils could have an explanation among established Ediacaran organism types (all of which have different type of body plan, so no); (b) exploring other organism types that might express similar fractal-like morphological characteristics (no success); and (c) determining whether the fossils belong to a specific known sponge group, mainly through the use of a Bayesian phylogenetic analysis. This is a well-rounded discussion that efficiently dispenses with the major questions that might arise in the minds of both specialist and general readers.

The scope of the background required for this paper means that a wide range of supporting publications must be cited. The authors seem to have done justice to the main publications in sponge paleobiology and present a competent review of Ediacaran and other material required for comparison.

The abstract is succinct, efficiently covering the fossil characteristics, their interpretation, and their implications.

With the subject matter consisting of simple, large macrofossils having a very distinctive, familiar morphology and a well-constrained depositional age, there is little to quibble over in this material, its description and interpretation, or its importance to the evolutionary history of animals.

Finding readily identifiable crown-group sponges in late Ediacaran strata is stupendous, but at the same time almost predictable. The existence of crown-group (probably) non-biomineralised sponges at that time implies a substantial evolutionary pre-history, which the authors (like others) suggest probably took place in aspiculate, non-biomineralised sponges. This adds another very valuable waypoint in the early fossil record of sponges, regarded by many as the most basal animal type. This is a fraught topic with an abundance of controversy and opinions, but virtually no physical evidence, so every scrap of possibly relevant fossil material is exquisitely precious. The broad importance of this material and this paper merit publication in Nature.

We welcome and appreciate this positive and detailed review of our work.

Referee #2

The authors describe a new genus and species, *Helicolocellus cantori*, from upper Ediacaran strata of China, based on seven specimens. Part of the body of *Helicolocellus cantori* in some specimens shows a gridded structure interpreted as an organic skeleton composed of spicule-like elements. They interpret the specimen as a crown-group sponge, related to the extant class Hexactinellida, on the basis of this inferred spicule-like framework.

The mystery of why there are no Ediacaran sponges, despite the long Precambrian range for phylum Porifera indicated by molecular-clock analyses, is an important issue in present-day palaeontology. Even a single Ediacaran crown-group sponge would be enormously informative. Unfortunately, there are several major flaws in the interpretation of *H. cantori* as a hexactinellid, or even as a sponge. Firstly, the supposed sponge-like features of the organism are not convincing, mostly for taphonomic reasons. Secondly, the organism exhibits features that argue against a sponge affinity. Thirdly, there are problems with the cladistic analysis on which the conclusion of a hexactinellid affinity is based.

1). Supposed sponge-like features are unconvincing

The interpretation of the fossil as a crown-group sponge is not tenable on taphonomic grounds. All specimens are preserved in relief, i.e. not flattened. This relief preservation means that the organism would have been either very robust or very thick-bodied, or both. The organism (if a sponge) must have had a fused skeleton (as in the late Palaeozoic *Hydnoceras* and *Clathrosporgia*) to have been preserved in this way. Cambrian (and early Palaeozoic in general) spiculate sponges, which did not have fused skeletons, are preserved flattened (see for example the Burgess Shale and Hetang sponges).

Organisms with soft bodies can also be preserved with reliefs. It is true that protospongiid sponges, which did not have fused skeletons, are preserved flattened in shales (e.g., Burgess Shale and Hetang Formation), but they are also preserved with three-dimensional reliefs in carbonates (see Fig. 1 below). Whether an organism can be preserved in three dimensions is dependent on its mechanical strength as well as when and how it is taphonomically mineralised, e.g., Doushantuo fossils from Weng'an, have no skeletons but are preserved three-dimensionally because early diagenetic phosphatisation was able to preserve them before they were flattened by compaction. The Shibantan biota and other Ediacara-type biotas are known for the three-dimensional preservation of soft-bodied organisms. Other soft-bodied fossils from the Shibantan Member (e.g., *Wutubus*) are preserved in relief, not as flattened compressions.

As such, we would argue that non-flattened preservation is not sufficient to either rule out an unfused skeleton, or a crown-group sponge affinity (see Fig. 1).

However, the regular grid pattern is only present in parts of some specimens: it cannot be seen in the basal part of any specimen, or at all in NIGP176536. NIGP176532 and 176533 show only small portions of regular mesh. This lack of regularity is attributed to partial disarticulation of the grid during degradation or compaction; in this case, the presumed spicules must have been unfused.

Thus, there is a contradiction: the skeletal grid must have been fused (to be preserved in relief) and unfused (to have disarticulated). The interpretation of the grid as a sponge-like skeleton therefore does not work.

The reviewer is correct in interpreting our suggestion that the skeleton has partially disarticulated in some regions of some specimens, and that we therefore suggest it was unfused. But as above, we do not consider the proposed taphonomic argument to conversely suggest it was also fused, so there is no contradiction. The three-dimensional preservation of *Helicolocellus* is entirely consistent with the lack of biomineralised skeletons and unfused skeletal elements. Therefore, we believe the reviewer's taphonomic argument is insufficient to rule that *Helicolocellus* is not a sponge.

[REDACTED]

In addition, the authors interpret the gridded structure as “an organic skeleton comprising orthogonally arranged cruciform elements” (abstract; they say elsewhere in the manuscript that the grid may have been lightly mineralised). However, no traces of the presumed spicules are preserved. This is surprising, given that the elements forming the grid structure must have been present when the rock was lithified (or the grid structure would not have been preserved at all). In the holotype, the first-order rectangles are separated by “grooves that are 1.1 mm wide and approximately 0.4 mm deep”. The first-order rectangles are 12 mm long and ca. 9 mm wide. Assuming that each ray of the largest “cruciform elements” extended over half a grid square (as in the Palaeozoic reticulosan *Cyathophycus*; see Botting 2003), the largest spicules should have had long rays 6 mm long, shorter rays 4.5 mm long, and been 0.4 to 0.5 mm wide. This would be quite large for Palaeozoic sponge spicules (which is not in itself a problem), but then one would expect to see imprints of the spicules in the sediment, because the largest spicules would be markedly larger than the grain size of the rock (see Fig. 2). No spicule imprints are visible in Fig. 2, suggesting that spicule-like elements were not present.

We think there may be a slight misunderstanding here. Our interpretation of the specimen is that the negative relief grooves between the rectangles (e.g. in the holotype in Fig. 2) are the *imprints* of a skeletal grid consisting of cruciform elements. We argue that the *Helicolocellus* has an organic skeleton rather than biomineralized spicules. Its preservation is consistent with an organic skeleton. We have added a paragraph in the revised manuscript to further clarify the description and interpretation.

Added text: “The preservation style of *Helicolocellus* is identical to that of other non-biomineralised macrofossils in the Shibantan Member, such including as *Arborea*²¹, *Flabelllophyton*²², and *Wutubus*²³. Specifically, the distinction between the fossils and the sedimentary matrix is defined by lithological contrast, with proportionally more carbonate cement in the former (compare Fig. 1c with fig. 7.2 of Wang et al.²¹, fig. 9E of Wan et al.²², fig. 6g–h of Chen et al.²³). The fossils are preserved as casts and moulds, but the body walls and their constituent elements themselves are not preserved. This taphonomic style is sharply different from that of biomineralised tubular fossils such as *Cloudina*²⁴ and *Sinotubulites*²⁵ from the Shibantan Member, which preserve well-defined although secondarily replaced tests. This difference is likely due to the fact that, relative to biomineralized tests, organic walls are more easily degraded and subsequently compacted (whereas a biomineralised test would be expected to retain the original 3D morphology).”

It is also relevant to mention that sponge taxa that are not hexactinellids can possess an approximately grid-like structure. See for example the Burgess Shale demosponge *Vauxia gracilentia* (<https://burgess-shale.rom.on.ca/fossils/vauxia-gracilentia/>, see ROM 53564 and USNM 66515) and the Cambrian ascosponge *Leptomitus* and relatives (previously identified as protomonaxonid demosponges; see illustrations in the Treatise on Invertebrate Paleontology, part E (revised), vol. 3).

Good suggestion. We have added comparisons with *Vauxia* and *Leptomit*us.

Added text: “Some other Cambrian sponges, e.g., the demosponge *Vauxia* and the ascosponge *Leptomit*us, also exhibit a grid pattern⁴⁵. However, these patterns differ significantly from the regular and hierarchical grid pattern found in *Helicolocell*us and other Palaeozoic hexactinellid sponges in having less fractality in their organisation, and less regularity in the spicule arrangements.”

2). *Helicolocell*us possesses features that are not sponge-like

The stem plus basal disc of *Helicolocell*us are not sponge-like features; they are, however, very like those of other Ediacara Biota taxa, specifically fronds such as *Charnia*. The authors claim that the holdfast/basal disc represent a convergent adaptation to a soft substrate in several (unrelated) taxa living in the same environment. Convergence is certainly something that has occurred commonly in evolution, and should always be considered as a possibility. In this case, however, some sort of rigid framework could also have evolved convergently, meaning that *H. cantori* is not a sponge.

We would like to point out that there are multiple extant sponges that have a stem and a discoidal holdfast, and in at least some of those, the spicule arrangement and morphology in the stem are very different to those in the rest of the body (e.g. *Advhena magnifica* Cristiana Castello-Branco et al., 2020).

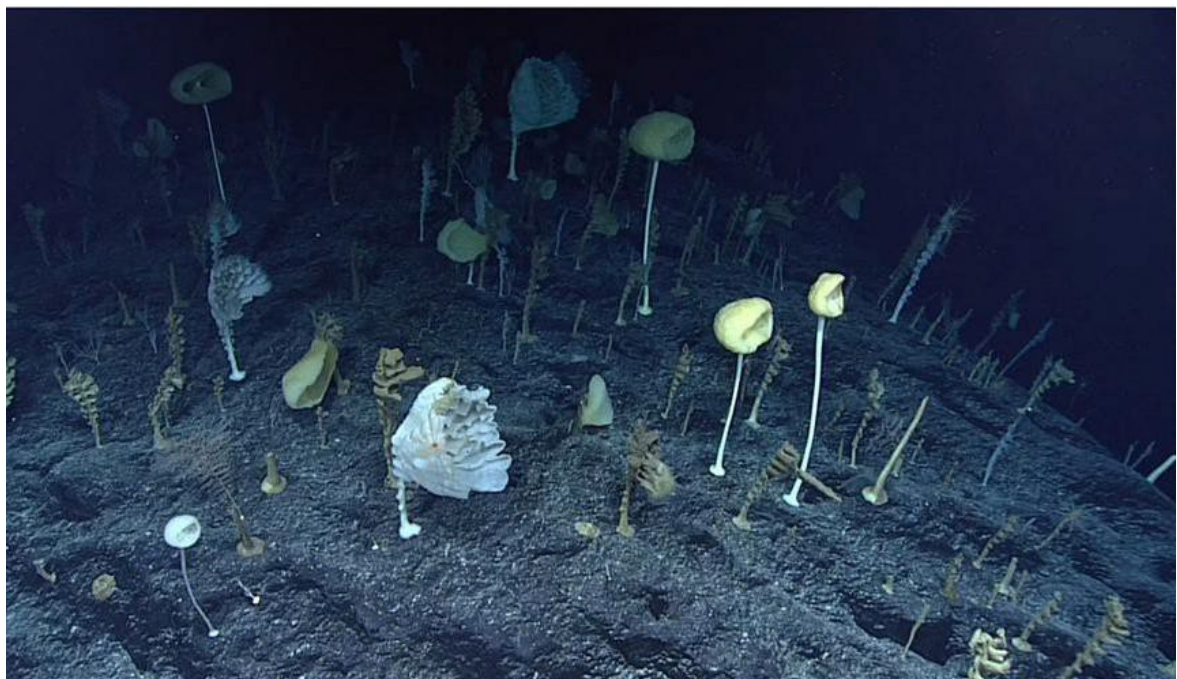


Figure 2. An extant deep sea hexactinellid sponge forest in Ridge Seamount, Johnston Atoll, Pacific Ocean (image in Mitchell & Harris, 2020, original image was recorded on Dive 11 of Okeanos Explorer expedition EX1706 of the Johnston Atoll Unit in

PRIMNM). Many of these extant organisms show a stem with a disc-like holdfast.

The second part of this argument (proposing that a rigid framework could have evolved convergently) appeals to hypothetical reasoning, and is not testable. We can confidently state that the stem and holdfast are convergent because they have been found across different kingdoms (e.g., algae and sponges, Wang et al., 2021 & Mitchell & Harris, 2020). In contrast, a regular, hierarchical meshwork is only known in sponges and *Helicolocellus*.

The presence of a marginal fringe implies that *H. cantori* is bilaterally symmetrical, rather than radially, as would be expected for a sponge. (There is evidence that early sponges were tetradially symmetric [Botting et al. 2014], but there is no sign of tetradial symmetry here.) If the organism is bilaterally symmetrical, then character 91 of the cladistic analysis is incorrectly coded (coded as radially symmetric in the analysis). This incorrect coding may have affected the results, because extant sponges are radially symmetric (or asymmetric); thus, incorrectly coding *H. cantori* as radially symmetric may have erroneously caused the organism to cluster with sponges.

We interpreted the fringe as a marginal expression of a partially flattened conical body, where the skeletal structures extend out from the margin of the body wall (n.b. the revised manuscript now more strongly supports this interpretation, following removal of the alternative contraction option on the recommendation of reviewer 4). This interpretation is compatible with a cone/radial symmetry body plan of *Helicolocellus*. If we accept for a moment that the reviewer is correct though and that *Helicolocellus* was a ‘fan’-shaped organism, then radial symmetry would be ruled out for *Helicolocellus*; however, this wouldn’t rule out a sponge interpretation, because extant sponges can in fact be fan-shaped (e.g., *Clathria rugosa*).

The presence of a second fringe in the holotype is also not a sponge-like feature: the authors suggest that the second fringe may have resulted from contraction of the body. If the body has contracted, then the grid-like pattern cannot represent a spicule net, because the spicular framework of sponges supports the soft tissue and stops it moving. Allowing the soft tissue to contract would close the inhalant canals, meaning that the organism could not have filter-fed. The presence of the fringes is thus evidence against a sponge affinity.

The contraction hypothesis was only one of the suggestions we offered, and has now been removed (see comment of reviewer 4). The manuscript now considers the second fringe to more likely result from compaction and be taphonomic in origin (effectively viewing the two fringes as the two sides of a ‘break’ in a cone-shaped specimen). As a result, this comment is no longer an argument against *Helicolocellus* being a sponge, and indeed all of the features considered by the referee not to be sponge-like can be either explained by the preservational mode of the fossils, or are in fact features of extant sponges. The morphology of *Helicolocellus* is most parsimoniously rationalized

as it being a poriferan.

3). Cladistic analysis

There are several issues with the cladistic analysis.

- Only extant taxa are included, meaning that the fossil must cluster with one of these taxa.

This is a valid point. In response to this and Reviewer 3 below, more fossil sponge taxa have been included in the cladistic analysis (see our response to reviewer 3 for details).

- As above, *Helicolocellus* appears to be bilaterally symmetrical; thus, character 91 is coded incorrectly.

See above for response to comment #2 of this reviewer.

- Character 176 in the cladistic analysis (highly regular hierarchical reticulate skeleton [quadrate mesh]) is stated to be present. However, the regular grid pattern is only present in parts of some specimens: it cannot be seen in the basal part of any specimen, or at all in NIGP176536. NIGP176532 and 176533 show only small portions of regular mesh.

We argue the partial irregularity is attributed to partial disarticulation of the grid during degradation or compaction of the unfused organic skeleton, as the reviewer proposed above, and is thus taphonomic in origin. The regular grid pattern observed in several specimens, including the distal end of the holotype, is inferred to be the original morphology of the organism, and is unquestionably present in multiple specimens of *Helicolocellus*.

- Character 182 in the cladistic analysis (skeletal organisation): *Helicolocellus* is coded as dictyonine-like, i.e., a rigid framework; however, a rigid framework would not show partial disarticulation, as is claimed for *Helicolocellus*. Lyssacine-like skeletal organisation is known in sponge taxa with regular grid patterns, such as *Protospongia* and *Cyathophycus*; thus, the presence of a regular grid is not sufficient to interpret the skeleton as being dictyonine.

Thank you for this important clarification. We agree, and have modified the character matrix. This character (Skeletal organisation) is now numbered as 183. In order to focus on the morphology of the skeletal organization and to reduce phylogenetic implications, the terms dictyonine-like and lyssacine-like have been changed to “an unfused regular grid pattern that is held together by organic tissue” and “a fused rigid three-dimensional scaffold”.

Revised text: “183. Skeletal organisation. This character is coded as 0 for *Helicolocellus*, because of its regular grid pattern. 0 – an unfused regular grid pattern that is held together by organic tissue. 1 – a fused rigid three-dimensional scaffold.”

- If *Helicolocellus* did have a spicule network, then the coding of all spicule types as being absent (characters 191 to 218), rather than unknown, would be problematic. The “spicules” (if they existed) would have had some morphology, which the authors say is cruciform.

We make a distinction between spicules (biomineralised skeletal elements) and skeletons (somewhat rigid structures that can be organic or biomineralized). Since we interpreted *Helicolocellus* as a non-biomineralising sponge with organic skeletons, we coded absent for characters related to biomineralised spicules. Nonetheless, we have re-run the analysis with those characters coded as unknown (as suggested by the reviewer), the new topology remains essentially the same (please see Figure 4b).

- As far as I can make out, the stalk/basal disc and the fringe are not included in the cladistic analysis; thus, the analysis considers only some of the morphology of the organism.

As mentioned above, the stem and basal disc are apparent homoplastic characters, since they have been found in distinctively different, unrelated lineages (e.g. rangeomorphs, sponges and algae, which also include members without a stem or basal disc). Homoplastic characters are not suitable for cladistic analysis. We also argue in the manuscript that the fringe is a preservational artefact, and it would therefore not be appropriate to include these characters in the cladistic analysis.

- *Charnia* was included in the original dataset of Dunn, on which the cladistic analysis is based; however, the authors have chosen to exclude *Charnia*. I strongly suspect that if extinct taxa such as *Charnia* had been included, *H. cantori* would (on the basis of the stalk/basal disc) have been included in that group.

We have included *Charnia* in the new cladistic analysis (Figure 4b), and it grouped with eumetazoans as shown by Dunn et al. (2021). As mentioned above, inclusion of other poriferan fossils has strengthened this analysis, while the presence of a stem and disc appears to have been an evolutionarily convergent feature of several late Ediacaran lineages (certainly worthy of investigation in a separate paper! See also the extant sponges with such a stem and disc in the figure included in our response to point 2 above).

To summarise, given the lack of plausible sponge features, the presence of decided non-sponge morphology, and the issues with the cladistic analysis, the conclusions of the paper are not supported, and the paper is not suitable for publication.

We appreciate the referee’s comments, and feel that they have undoubtedly helped us to refine and improve our paper. We consider our response here to demonstrate that we have resolved the issues raised with our original cladistic analysis (with *Helicolocellus* still being resolved as a stem group hexactinellid in our revised analysis); demonstrated that the highlighted ‘non-sponge’ features are either present in some living sponges, or

can be explained taphonomically; and emphasized that there are in fact characters within the specimens that are plausibly poriferan, and that the taphonomic mode of the fossils is consistent with an unfused skeleton in a sponge. We hope that our response goes some way to addressing the referee concerns both in this response document, and in the revised manuscript.

Specific comments

Line 66: change “late Ediacaran Shibantan Member” to “upper Ediacaran Shibantan Member”.

We have revised the text.

Line 119: “In addition to the holotype, five other specimens are known”, but six additional specimens are listed (“Referred material” in line 65; NIGP176532, 33, 34, 35, 36, 37). Please clarify.

Sorry for the confusion. It should be “six other specimens are known”.

Line 120: NIGP176532 is referred to as a paratype, but in line 65 is part of “Referred material”, which would normally imply that it is not a paratype. Please specify whether specimens NIG176532–176537 are paratypes or not.

We have revised this to clarify that NIGP176532 is the paratype, and NIGP176533–176537 are just other specimens.

Line 218: *Pontagrossia* was not interpreted as a sponge by ref. 40.

“The shape and organization of the fossil resembles that in some sponges

We have rephrased this part more carefully to “...the Devonian fossil *Pontagrossia* (Extended Data Fig. 3f)⁴⁶, which has been compared to a sponge and is inferred to have possessed a largely organic skeleton characterised by a reticulate pattern.”

Reference

Castello-Branco, C., et al. "A collection of hexactinellids (Porifera) from the deep South Atlantic and North Pacific: new genus, new species and new records." *PeerJ* 8 (2020): e9431.

Dunn, F., et al. “The developmental biology of *Charnia* and the eumetazoan affinity of the Ediacaran rangeomorphs.” *Science advances* 7 (2021) eabe0291.

Mitchell, E., and Harris, S. "Mortality, population and community dynamics of the glass sponge dominated community “The Forest of the Weird” from the RIDGE seamount, Johnston Atoll, Pacific Ocean." *Frontiers in Marine Science* 7 (2020): 565171.

Wang, X., et al. "Evolution of holdfast diversity and attachment strategies of Ediacaran benthic macroalgae." *Frontiers in Earth Science* 9 (2021): 783427.

Referee #3

Wang and colleagues describe a new fossil - *Helicolocellus* – from the Ediacaran of China. They describe *Helicolocellus* as a sponge and, after phylogenetic analysis conclude that it represents a stem-group hexactinellid and therefore a crown-group sponge. They interpret *Helicolocellus* as having been either weakly or non-biomineralised and use this as a springboard to suggest that the living Classes of sponge may have biomineralised independently of each other. I found the description of the fossil interesting and compelling, but the manuscript falls down in the execution of the phylogenetic analysis (and subsequent interpretations), the inferences about the evolution of biomineralisation and the discussion of other, relevant taxa. I hope my comments and suggestions are useful.

1). 1. Other taxa: The discussion of other, relevant fossil sponges is limited to very specific comparisons with reference to obvious Ediacaran or Cambrian taxa cursory. Two obvious examples from the Ediacaran Period are *Gibbavasis* which shares many clear similarities to *Helicolocellus* and the potential sponge material described by Turner in Nature last year which – if interpreted correctly – would likely represent a much more ancient crown-group sponge and it also interpreted as non-mineralised. If this interpretation is correct it undermines the unique selling point of the current manuscript and therefore needs to be dealt with appropriately and in the main text.

Discussion of *Gibbavasis* was already included in the manuscript. Brief discussion of the Turner material has now been included.

Text added: "...see for example Tonian candidate keratose sponge material from Canada⁷..."

The regular cross-hatch pattern is reminiscent of the Palaeozoic reticulosans which are mentioned only (from my reading) through *Protospongia* and *Diagonella*, which is described confidently as a hexactinellid, when my reading of the literature is that affinities of *Protospongia* (and other reticulosans) are far less certain. In fact, the reticulosans share several characters with other non-reticulosan Cambrian sponges, including taxa like *Eifellia* which is commonly interpreted as a stem-group calcarerous sponge potentially demonstrating that these characters were much more widespread, plesiomorphically for sponges.

We re-ran the phylogenetic analyses, this time incorporating more reticulosans. Specifically, we considered the characters that are inconsistent with hexactinellids, such as biomineralic spicules and the shape of the cross-section of axial filaments. Current evidence supports reticulosans having rounded cross-sections of axial filaments, instead

of hexagonal (please refer to character 233 in the supplementary material for more discussion). Additionally, rounded cross-sections of axial filaments have also been observed in modern hexactinellids, consistent with the interpretation that reticulosans can be classified as hexactinellids. The results of our phylogenetic analyses further substantiate this interpretation.

We would also like to point out that previous phylogenetic interpretations of reticulosans were not based on cladistic analysis.

The manuscript currently does not give enough time to considering the Ediacaran and early Palaeozoic sponge fauna. The other thing to note here is that when these taxa are discussed it is almost exclusively with reference to the differences between *X* and *Helicolocellus* and does not really reference shared derived similarities, of which there are plenty, and which underpins phylogenetic thinking today. I suggest the authors change the tone of the discussion to focus more on this.

We do consider several Ediacaran taxa, but we acknowledge the referee's point that we did not really focus on the similarities, we have changed the tone accordingly. When comparing with Palaeozoic sponges, our focus has been on similarities such as overall morphology and the unfused skeleton. Furthermore, we have now included a number of these Paleozoic sponge fossils in the cladistic analysis.

Revised text: "The putative sponge *Thectardis*³¹⁻³³ from Newfoundland has a conical body that is similar to *Helicolocellus*, but *Thectardis* typically exhibits a featureless external surface, and lacks a stem or basal disc. Another cylindrical fossil, the putative sponge *Ausia* from the Nama Group of Namibia³⁴, displays mm-scale pore-like structures on its surface, albeit notably smaller than the first-order rectangles observed in *H. cantori* and lacking second-order divisions. *Palaeophragmodictya*, a discoidal fossil found in South Australia and the White Sea region of Russia, was initially interpreted as a sponge³⁵ and shares reticulate surface patterns but smaller in size than those of *H. cantori*. However, it differs markedly in shape, being discoidal rather than conical, and more recent research suggests it may represent an attachment disc of frondose organisms or a microbially-produced texture⁵. The vase-shaped fossil *Gibbavasis*^{36,37} from Iran and Moldova shares a lattice-like appearance with *Helicolocellus*, but only possesses a single order of orthogonally arranged "boxes". Furthermore, *Gibbavasis* is much smaller (4 to 14 mm long, 2 to 7 mm wide) than *Helicolocellus*. Perpendicular cross-hatching structures have also been described in an unnamed fossil from Nevada (fig. 3a-b in Smith et al.³⁸). However, its surface ornamentation can be readily distinguished from the reticulation observed in *H. cantori* by the absence of secondary latticework."

I think the authors spend too much time arguing against a cnidarian affinity – certainly I would not think a cnidarian affinity likely from looking at the fossil, but the authors might consider devoting some text to *Paramackenzia*, recently described as sharing

similarities to modular Ediacarans and which shows some similarity to *Helicolocellus*.

More discussion regarding *Paramackenzia* has been added. We have chosen to keep our discussion of cnidarian affinity however, as the points we raise are also relevant in ruling out broader eumetazoan comparisons.

Text added: “The enigmatic Cambrian fossil, *Paramackenzia*, which has previously been compared to modular Ediacaran organisms and exhibits potential 3D box-like compartmental structures⁴¹, bears passing similarity, but its compartmental structures neither subdivide nor display a helical arrangement.”

2) Phylogenetic analysis: The analysis presented is not appropriate for what the authors want to say or use it for. There are methodological problems: not enough information about parameters etc is presented in the methods and so far as I can see there are no sensitivity tests – the authors do not explain how they have dealt with ascertainment bias and why and do not run additional sensitivity tests, and despite their tree not resembling molecular trees of living sponges in e.g. the reciprocal monophyly of calcareans + homoscleromorphs and demosponges + hexactinellids, the authors have not presented a molecular backbone tree. These and other relevant tests need to be presented as so much relies on the phylogenetic position of *Helicolocellus* having been interpreted correctly. In my view these are absolutely essential before this paper can be accepted in Nature, or any other journal.

To address this concern, we have performed additional sensitivity tests by applying constraints, removing fossil sponge taxa and characters (Extended Data Fig. 5). To test whether coding polymorphisms as missing data has a great impact on the topology of the phylogenetic tree, we removed all the 46 characters with polymorphism states (characters 13, 16, 39, 42, 43, 44, 46, 59, 75, 90, 91, 110, 111, 113, 117, 120, 124, 134, 135, 136, 137, 139, 140, 141, 145, 146, 147, 148, 153, 154, 155, 160, 177, 179, 185, 193, 197, 198, 199, 200, 201, 203, 204, 230, 231, 233), and re-ran the analysis. The results show that the position of *Helicolocellus* did not change significantly (Extended Data Fig. 5d).

We also calculated the Effective Sample Size (ESS) to examine the robustness of the phylogenetic analysis. The ESS=2749, which is significantly greater than 200, indicating our result is robust. To summarise, the results of the sensitivity tests and the parameters indicate that the result of phylogenetic analysis is reliable. The constraints applied also reflect the molecular backbone tree: 1. relationships of Porifera classes constrained (Aguilar-Camacho et al., 2019; Extended Data Fig. 5a); 2. ctenophores constrained as sister-group to all other animals (Dunn et al., 2015; Extended Data Fig. 5c).

Revised text: “A total of 233 morphological characters were coded for 75 taxa. We scored *Helicolocellus cantori* for 85 of these morphological characters (see Extended

Data File for details). Bayesian phylogenetic analysis was run in MrBayes 3.2.6 (ref.⁶⁶), We used the Mk model with a gamma distribution of modelled rate variation. Analyses were run for 5,000,000 generations, sampled with a frequency of every 1000 generations, discarded the first 25% samples as burn-in. The Effective Sample Size (ESS) was calculated to be 2749 using Tracer⁶⁷,

The dataset used is also not appropriate because it only really samples hexactinellids and includes no other sponge fossils. The Dunn et al. matrix only scored sponges at Class level, and only considered the living members of the group; polymorphisms are essentially treated as missing data in these analyses and therefore you are reducing the signal massively in groups which are clearly and obviously relevant for this study. The authors need to improve taxon sampling of living sponges and include additional relevant fossil sponges (including reticulosans and *Eiffelia*, for example). This expanded dataset would then be appropriate for downstream ancestral state reconstructions, directly testing hypotheses about the plesiomorphic nature of sponges.

We thank the reviewer for these useful suggestions. The dataset has been expanded by including 6 more fossil taxa (*Protospongia*, *Eiffelia*, *Cyathophycus*, *Minitaspongia*, *Vauxia*, *Charnia*) and 8 more modern sponge taxa (*Sycon*, *Leucosolenia*, *Uteopsis*, *Agelas*, *Cymbastela*, *Metania*, *Oscarella*, *Plakortis*). Additionally, 15 more characters (characters 177 and 220–233) are also included to accommodate the enlarged taxon sampling. The position of *Helicolocellus* remains stable in these tests.

The suggestion to run ancestral state reconstructions is welcome, but something we would prefer to reserve for a future manuscript with even more fossil data from early sponges.

3). Evolution of biomineralization: The authors do not have enough information to draw conclusions about the evolution of biomineralization. They are, after all, working with one data point which they concede may also be lightly biomineralised and so their argument is not compelling even in the absence of other relevant fossils which will have significant bearing on this. I suggest this is either removed or greatly reduced in the manuscript and the focus be shifted to encompass a more thorough discussion of contemporaneous sponges and discussion of the phylogenetic implications of this fossil.

We respect the reviewer's opinion here, but feel that our discussion on biomineralisation is a natural implication of the discovery of this new soft-bodied fossil. Our biomineralization discussion is mainly restricted to the evolution of biomineralization in sponges, and also considers available earliest Cambrian data, and we think it is written cautiously enough to acknowledge the uncertainty surrounding this topic given the available data. We would refer also to the comments of reviewer 1, who appears to view this discussion as an important part of our manuscript: "*The existence of crown-group (probably) non-biomineralised sponges at that time implies a substantial evolutionary pre-history, which the authors (like others) suggest probably*

took place in aspiculate, non-biomineralised sponges. This adds another very valuable waypoint in the early fossil record of sponges, regarded by many as the most basal animal type. This is a fraught topic with an abundance of controversy and opinions, but virtually no physical evidence, so every scrap of possibly relevant fossil material is exquisitely precious.”

References mentioned in our response above:

Aguilar-Camacho, J. M., Doonan, L. & McCormack, G. P. “Evolution of the main skeleton-forming genes in sponges (phylum Porifera) with special focus on the marine Haplosclerida (class Demospongiae)”. *Molecular Phylogenetics and Evolution* 131 (2019): 245 – 253.

Dohrmann, M. *et al.* "Phylogeny and evolution of glass sponges (Porifera, Hexactinellida)." *Systematic Biology* 57 (2008): 388–405.

Dunn, C.W., Leys, S.P. & Haddock, S.H. The hidden biology of sponges and ctenophores. *Trends in ecology & evolution* 30 (2015): 282 – 291

Referee #4

This manuscript describes and names a new species of crown group sponge from the late Ediacaran Dengying Formation of southern China. Important Ediacaran fossils are not new from the Dengying, however *Helicolocellus cantori* might prove to be the most influential one. Understanding the Ediacaran-Cambrian transition has been extremely controversial; whether it be due to preservational biases associated with the fossilization of soft-tissues, or bonafide evolutionary innovations in response to large scale climatic or biological competition, fossil communities on either side of the boundary are extremely disparate. Although there have been some strong cases for Eumetazoa in the Ediacaran (many of which were interpreted by this authorship team), the search for sponges remains difficult in the absence of silicious or calcareous spicules that, according to molecular clock studies, should be present. With this said, *Helicolocellus cantori* represents an exceedingly rare instance where conclusive morphological evidence is present in a presumed Ediacaran sponge. In my opinion, this is the strongest case for Ediacaran sponges published to date. The diagnostic rectangular fractal pattern is so similar to *Diagoniella/Hydnoceras* that I would be hard pressed to think of an alternative to explain the morphological similarities. Furthermore, the apparent absence of a mineralized lattice strengthens the hypothesis that these structures were derived from organic precursors and evolved independently in Hexactinellida and Demospongiae. Honestly, *Helicolocellus* could represent a Rosetta Stone for our understanding of the evolution of Metazoa as a whole.

We thank the reviewer for their positive comments, and for recognizing the evolutionary importance of our specimens, and the morphological evidence in support

of our interpretation of *H. cantori* as a poriferan.

I do have a few comments that I hope will help the authorship team. The first thing that struck me was the apparent presence of a featureless smooth stem at the base of the presumably filter-filtering cup. I'm not a sponge expert but I find it peculiar that a sponge built from a modular fractal pattern to filter feed would forgo feeding from the lower tiers, and importantly construct a non-fractal morphological structure with little use other than elevating the feeding cup off the seafloor. We see a similar problem with many Ediacaran taxa (esp. the Rangeomorphs), which has been tentatively explained as a means of reducing competition with lower-tier feeders or alternatively as a means of elevating reproductive units to improve dispersal. I did try to investigate this problem with Ediacaran fronds back in 2008/2009, but never really got a good answer. Do the authors have a hypothesis for this? Why do modern sponges forgo a stem/stalk? I'm wondering if it would prove useful to run some Computational Fluid Dynamics (CFD) tests on the benefits of elevating the cup above the sediment/water interface? I'd recommend reaching out to Brandt Gibson (he's a postdoc here at UofT with me) to see if this is something that can be investigated. It might prove to be too much additional work for this manuscript specifically, but if not, I'd love to see some data to help interpret this weird phenomenon.

Thank you for proposing the idea! We will certainly reach out to Brandt to discuss the possibility of CFD in the future, but we do not think that it would benefit this particular manuscript. Importantly, some modern sponges *do* have a stem and basal disc that are not involved in filter feeding (please see the figures below), so this isn't as peculiar a problem for sponges as it might appear. Moreover, some modern sponges have choanoderm in their stem (Dohrmann et al., 2008), demonstrating that not only have some modern taxa not forgone the stem/stalk entirely, but it is possible for sponges to possess different structural architectures for these different aspects of their anatomy.

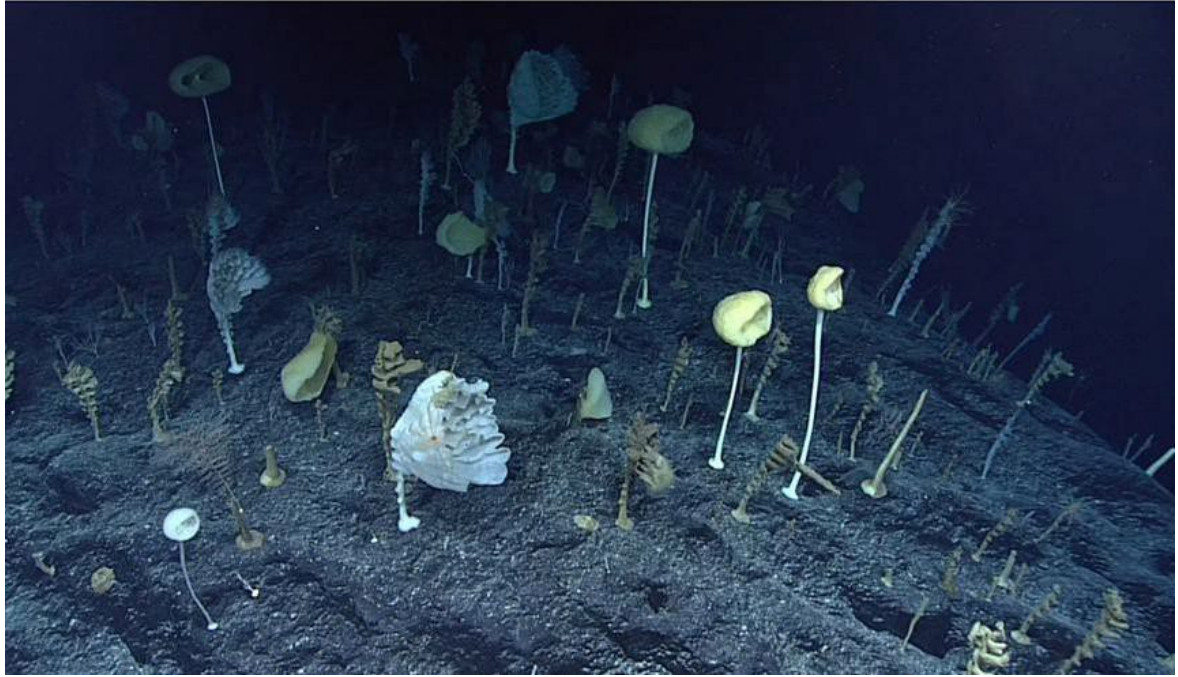


Figure 1. An extant deep sea hexactinellid sponge forest in Ridge Seamount, Johnston Atoll, Pacific Ocean (image in Mitchell & Harris, 2020, original image was recorded on Dive 11 of Okeanos Explorer expedition EX1706 of the Johnston Atoll Unit in PRIMNM). Many of them show a stem with a disc-like holdfast.

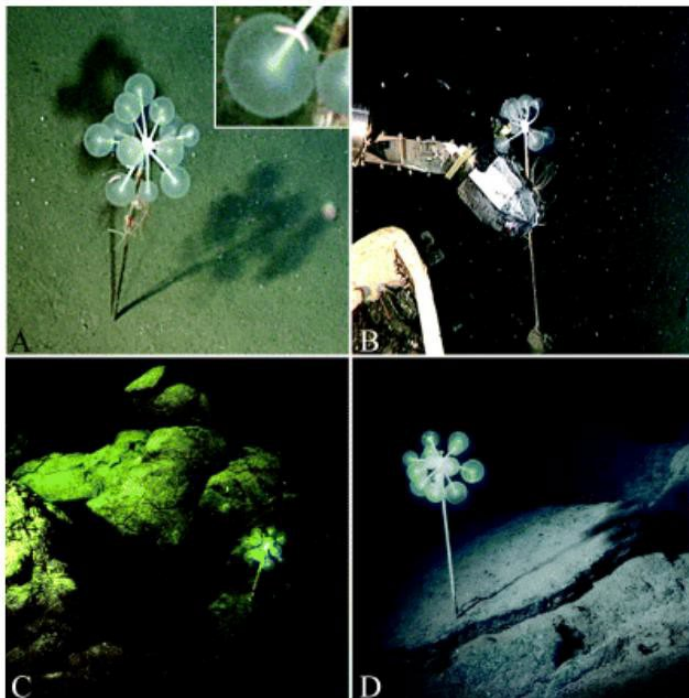


Figure 2. Carnivorous Chondrocladia lampadiglobus with a stem found on the East Pacific Rise during Ifremer /Naudur/Geocyarise expeditions (Vacelet, 2006).

Another minor point would be to clarify a few comments from the text. For example, L52-54 *H. cantori* also suggests that biomineralised skeletons may have evolved

independently among sponge classes and emphasizes biomineralization as the major innovation driving the Cambrian explosion. – Can the authors comment on why mineralization is viewed as a “driver” of the Cambrian explosion, rather than a “response” to other factors driving the diversification? The cause/effect implication here might be worth discussing.

We are sorry for the confusion. The driver of the Cambrian explosion largely remains unresolved. The investigation of this driver is beyond the scope of this paper; therefore, we have rephrased this part.

Revised text: “*H. cantori* also suggests that biomineralised skeletons may have evolved independently among sponge classes, and emphasises biomineralisation as a major innovation during the Cambrian explosion.”

Ediacaran fossils are notoriously difficult to photograph and given the similarity of *H. cantori* to these fossils, I wanted to make sure the images were described correctly. In Figure 2, 2b–d is described as “negative relief preserved on a bed sole (Fig. 2b–d)”, however they appear to be in positive relief in the photo. Is it just a case of lighting from a different angle, or are 2b–d from the same positive relief specimen as 2a (and not the counterpart)?

Yes, it was difficult to photograph the specimens. We are positive that in Figure 2, 2b–d is the negative relief with a different lighting angle. We tried to photograph the positive relief but it looked even more like the negative relief, and it is more difficult to find the best lighting condition for the positive relief due to its large size. We have added some explanations about the lighting direction in the caption to avoid confusion.

I would also suggest adding an image of the casting and molding of *Pontagrossia* from the cited Chahud & Fairchild paper (maybe within the Extended Data Fig. 3?) The similarities are striking and would help with their argument.

Thank you for the suggestion. A picture of *Pontagrossia* has been added (Extended Data Fig. 3f), and credited to the original researchers.

The “fringe” structures are interesting. The authors provide two alternatives to explain them, but I found the “contraction” argument to be less likely. I’d argue that the potential “contractions” of *Dickinsonia* and *Aspidella* is difficult to explain without musculature. I’ve also found it very hard to explain how you would capture two “phases” (pre and post contraction, one of which clearly being very short lived) via deathmask casting and molding (I have the same issues with true Ediacarans mind you). Again, this doesn’t take away from the study, but it might not be necessary to include it at all.

We agree with the reviewer, and the contraction hypothesis has been removed.

References referred to in our response above:

Dohrmann, M, *et al.* "Phylogeny and evolution of glass sponges (Porifera, Hexactinellida)." *Systematic Biology* 57.3 (2008): 388-405.

Mitchell, E., and Harris, S. "Mortality, population and community dynamics of the glass sponge dominated community “The Forest of the Weird” from the RIDGE seamount, Johnston Atoll, Pacific Ocean." *Frontiers in Marine Science* 7 (2020): 565171.

Vacelet, J. "New carnivorous sponges (Porifera, Poecilosclerida) collected from manned submersibles in the deep Pacific." *Zoological Journal of the Linnean Society* 148 (2006): 553-584.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The summary below addresses the collective commentary of four reviewers, and does not repeat or revise the content of my own original review.

Collectively, the negative aspects of the four reviews centre on the following:

- 1) Preservation style and relief
- 2) Identity of the grid-like skeletal components
- 3) Whether basal disc and stem are problematic for a sponge interpretation
- 4) Implications of the marginal fringe for a poriferan versus bilaterian identity
- 5) Content of the cladistic analysis
- 6) Whether, how, and what other material (other sponge types and ages) is explicitly compared in the text.

The rebuttal or explanation of each of these items seems to be satisfactory, as summarised below.

The first three items would be serious problems if true, but are in fact based on misunderstandings of the descriptions and interpretations in the original text, and of sponge biology.

- 1) The existence of relief was taken by a reviewer as an indication that the skeleton had some degree of rigidity, perhaps conferred by a fused skeleton, which would contradict the disarticulation interpreted in part of the fossils by the authors. The authors note that relief is possible through other means, especially in carbonate rocks, and in the rebuttal provide, as an example, an image of a garden-variety 3-d sponge body fossil without fused spicules, in a carbonate rock.
- 2) The grid-like skeletal components were misconstrued by a reviewer as spicules, rather than impressions of non-mineralised skeletal elements as intended by the authors. The revision contains new text to help prevent this misunderstanding in other readers.
- 3) A reviewer stated that basal discs and stems are not characteristics of sponges, and so the fossils cannot be interpreted as sponges. The authors' rebuttal includes an image of these exact characteristics in modern sponges.

Item #4 (marginal fringe). According to one reviewer, the marginal fringe suggests a bilaterian affinity, rather than poriferan. However, the authors' interpretation does not require that the fossil have bilateral symmetry.

Item #5 - Two reviewers offer a number of important commentaries on the cladistic analysis, and the authors respond with a variety of modifications and additions (e.g., inclusion of fossil sponge taxa, sensitivity tests, non-hexactinellid taxa). I am not an expert in this type of analysis, but the responses seem to be appropriate. At any rate, the changes do not substantially alter the outcome.

Item #6 – A reviewer suggested that more attention be given to comparison with other possible Ediacaran sponges (rather than only contrasting). The authors provide a revised paragraph that both

compares and contrasts the main examples of putative Ediacaran sponges with *Helicolocellus*.

There are a few other comments that are not substantial enough to influence the viability of the paper, but which have been accommodated by the authors.

I am satisfied that all concerns of the reviewers have been appropriately dealt with, and reiterate my support for the publication of this paper.

Referee #2 (Remarks to the Author):

Re-review of Wang et al. "A late Ediacaran crown-group sponge animal"

The authors have answered some of my points, but they appear to have misunderstood (and thus not adequately replied to) other points. There are also problems with the revised cladistic analysis. The authors have still not adequately demonstrated that the organism in question is a sponge, and there are still major issues with the sponge interpretation of *Helicolocellus*. I regret that I therefore cannot support publication of this paper.

Taphonomy

I thank the authors for clarifying that they consider the grid to have been made up of separate elements (i.e., was unfused). However, the authors appear to have not entirely understood my point about the taphonomy of the grid/soft tissue. Although it is true (but unusual) that a sponge with an unfused spicule skeleton can be preserved with some degree of three-dimensionality, this can only happen if the soft tissue was intact at the time of burial. The authors are also correct that soft-bodied organisms can be preserved three-dimensionally (e.g., as they say, in Ediacara-type assemblages), but this could only have happened if the soft tissue was intact at the time of burial. If a skeletal grid had been present, its disarticulation would mean that the soft tissue had decayed in that part of the organism. If the soft tissue had decayed, then the soft tissue would not have been preserved three-dimensionally. Thus, the presence in *Helicolocellus* of (what the authors interpret as) an area in which the grid has disarticulated, but in which the soft tissue has been preserved in three dimensions, does not make sense taphonomically. Several specimens of *Helicolocellus* are available: all show both three-dimensional preservation of the organism and absence of the grid-like structure over at least part of the body. This absence of grid is best interpreted as a feature of the organism's morphology, rather than loss of an originally present grid-like structure.

The authors' argument that the grid structure was made of separate units that could disarticulate would be much stronger if they illustrated some of these separate units. In a partially disarticulated fossil sponge, some loose spicules are usually visible around the area with an intact skeletal grid. Given that several specimens of *Helicolocellus* that (if the authors are correct) are partially disarticulated are available, it would seem likely that at least one of them would show at least one element that had separated from the grid. Given the large size of the largest elements, this should be easy to illustrate by means of a photomicrograph. (Disarticulated elements might well be more visible on a latex cast of the specimen than on the specimen itself.)

Imprints of skeletal elements

The authors also appear to have not understood my point that imprints of the separate elements making up the grid should be visible. I do understand that (if a grid composed of cruciform elements of an organic composition had been present), those elements have vanished at some point between burial of the organism and discovery of the fossil, and that thus the preserved grooves represent the imprint of the original grid. My point was, however, that if the imprint of a grid is preserved, imprints of at least some of the individual elements making up the grid should be visible. Some of the larger elements would have been much larger than the grain-size of the rock. (In the holotype, the first-order rectangles are separated by “grooves that are 1.1 mm wide and approximately 0.4 mm deep”. The first-order rectangles are 12 mm long and ca. 9 mm wide. Assuming that each ray of the largest “cruciform elements” extended over half a grid square (as in the Palaeozoic reticulosan *Cyathophycus*; see Botting 2003), the largest spicules should have had long rays 6 mm long, shorter rays 4.5 mm long, and been 0.4 to 0.5 mm wide.)

Again, a latex cast would be helpful in showing up this feature, if present. See Botting & Muir (2011) for an example of using a latex cast on a fossil sponge to clarify spicule details.

Stem and holdfast of *Helicolocellus*

The authors are correct that some modern hexactinellids possess root tufts that can look rather different from the body of the sponge; however, the tufts/stalks of extant hexactinellids are made of spicules, and are thus completely different in construction from the *Helicolocellus* holdfast, in which there is no sign of spicules. Comparing the holdfast of *Helicolocellus* with that of extant hexactinellids is misleading. The authors may be correct that a fleshy holdfast has evolved convergently in several taxa of Ediacaran age; however, that a feature has evolved convergently several times does not mean that every example of that feature is an independent derivation. If *Helicolocellus* were a close relative of *Charnia*, then the holdfast of *Helicolocellus* would be homologous with that of *Charnia*.

The authors make the point that they consider *Helicolocellus* to be a sponge because of the presence of a “regular, hierarchical meshwork”, which they consider to be a feature otherwise possessed only by sponges. This is not in fact correct: some specimens of *Palaeophragmodictya* show a rectangular grid structure with two size orders of quadrules (Gehling & Rigby 1996). As *Palaeophragmodictya* is now generally accepted to not be a sponge, it is therefore not the case that any organism showing a grid structure with multiple size order is a sponge.

Cladistic analysis

I appreciate that the authors have included additional fossil and extant sponge taxa to their cladistic data matrix. Unfortunately, there are serious problems with the revised cladistic analysis (listed in detail below). The cladistic data matrix appears to have been constructed (with some characters included and some excluded, and codings manipulated) so as to yield the desired result. Given the problems with the coding of characters, omission of important characters, and incorrect phylogenetic positions for taxa for which the relationships are well understood, the results of the analysis cannot be used to support the conclusion that *Helicolocellus* is a sponge.

Firstly, the results obtained for fossil sponges for which the phylogenetic position is known are inconsistent with current knowledge. The phylogenetic position of *Vauxia* is (unusually for a

Cambrian sponge) agreed on by all workers. *Vauxia* is agreed to be a demosponge (e.g. Botting & Muir 2018), and some workers classify it as a verongioid demosponge (i.e., part of an extant group: Yang et al. 2017; Luo et al. 2019); thus, *Vauxia* should fall out within the Demospongiae in the cladistic analysis. It does not; instead, it appears in the stem group of (Silicea plus Homoscleromorpha). In addition, *Eiffelia*, which is well understood to be an early member of the Heteractinida (early Calcarea) and part of the lineage leading to taxa such as *Astraeospongia* (Mehl & Reitner 1996; Botting & Muir 2018) should cluster with the Calcarea, and yet it appears in the Hexactinellida. I assume that this is the result of including siliceous spicules and biomineralic spicules in the data matrix, but not calcareous spicules. As calcareous spicules are a diagnostic feature of modern Calcarea, this omission is puzzling.

Secondly, the recovered relationships of modern sponges are problematic. The Euplectellidae (*Euplectella*, *Malacosaccus*, etc.) are recovered as sister group to the rest of extant Hexactinellida. This is peculiar, to say the least, because most analyses recover the Euplectellidae as sister-group to the Rossellidae (e.g. Dohrmann et al. 2017): note that the purely morphological analysis of Dohrmann et al. (2008), i.e., the same data as used in the present study, yielded hexactinellid families but the relationships between those families was an unresolved polytomy (fig. 3 of Dohrmann et al. 2008). The recovery of Homoscleromorpha in an unresolved polytomy with Demospongiae and Hexactinellida, rather than as sister-group to Calcarea, is also problematic.

Thirdly, although the authors have included *Charnia* in the revised analysis, they are excluding the feature that is shared by both *Helicolocellus* and *Charnia*—a fleshy holdfast—from the data matrix, on the basis that they believe the feature to be homoplastic. This type of conclusion ought to come out of the analysis, rather than be a basic assumption of it.

Fourthly, the authors do not sufficiently explain how they achieved their character codings for fossil sponges. Most of the sponge-specific characters (and their codings, for extant taxa) are from the phylogenetic analysis of Hexactinellida by Dohrmann et al. (2008) and of Calcarea by Manuel et al. (2003). Neither Dohrmann et al. (2008) nor Manuel et al. (2003) included fossil taxa or extant demosponge genera in their morphological datasets. I assume that the authors coded Protospongia, *Cyathophycus*, *Eiffelia* and the other fossil taxa, and extant demosponges, from either their own observations of actual physical specimens or from the literature. If the former, they should state this (and provide the museum numbers of studied material); if the latter, they should provide citations to the relevant literature. There are some obvious problems with the current character coding; for example, *Cyathophycus* is coded as having fused spicules (character 182), as being basiphytous (i.e., attached to hard substrates; character 185), and as possessing hexasters (a particular type of microsclere; character 190), but I am not aware of any study that has documented spicule fusion, attachment to a hard substrate, or any type of microsclere in *Cyathophycus*. (Note for the editor: extant hexactinellids possess both megascleres [large, obvious spicules] and microscleres [very small, generally <150 microns in size, spicules that are very important in taxonomy.] Microscleres are extremely rare in the fossil record, and there are no records of microscleres for the overwhelmingly vast majority of early Palaeozoic sponges.) Furthermore, *Cyathophycus* is coded as lacking hexactins as major dermal megascleres (i.e., the outer layer of spicules in a hexactinellid sponge according to Dohrmann et al. 2008; character 209), but is coded as ? for stauractins as major dermal megascleres (character 210). This is puzzling, because *Cyathophycus loydeli* has an outer layer of hexactins

(Botting 2004). Similarly, *Cyathophycus* is coded as lacking diactins as major choanosomal megascleres (inner layer of the hexactinellid skeleton; character 212) and as major atrial megascleres (the inner surface of a hexactinellid sponge; character 217), and as ? for stauractins as major choanosomal megascleres (character 213)—yet *Cyathophycus loydelli* has an inner layer of monaxonid (not stauractine) spicules (Botting 2004).

I am also puzzled by the coding of *Eiffelia* as having a basiphytous attachment (character 185) and hexaster microscleres (character 190). To the best of my knowledge, a root tuft and microscleres are not known for *Eiffelia*. Incidentally, the authors refer to *Eiffelia* as a reticulosan in the text, although it is generally classified as a heteractinid. Presumably the authors are regarding *Eiffelia* as a reticulosan based on the presence of hexactine spicules, despite it also bearing heteractine spicules (which do not seem to be included as a character in the phylogenetic analysis, and might have altered the results if included).

The authors' coding of the shapes of axial filaments (character 233) requires further explanation. I am not aware of any reference giving the shape of the axial filament of *Protospongia* (coded as round in the analysis). I also strongly disagree with the coding of *Cyathophycus* as having a circular axial filament: the axial filament, being made of protein, may become altered during diagenesis and thus may not retain its original shape, but a hexagonal axial canal (as illustrated by Botting & Muir 2013 for *Cyathophycus loydelli*) cannot be the result of partial spicule dissolution and thus is a reliable guide to the original shape of the axial filament.

These apparent inaccuracies in the coding of the morphology of taxa means that the results of the analysis cannot be relied on.

References

- Botting, J.P., 2004. An exceptional Caradoc sponge fauna from the Llanfawr Quarries, central Wales, and phylogenetic implications. *Journal of Systematic Palaeontology* 2, 31–63.
- Botting, J.P., Muir, L.A., 2011. A new Middle Ordovician (late Dapingian) hexactinellid sponge from Cumbria, UK. *Geological Journal* 46, 50–506.
- Botting, J.P., Muir, L.A., 2013. Spicule structure and affinities of the Late Ordovician hexactinellid-like sponge *Cyathophycus loydelli* (Llanfawr Mudstones Lagerstätte, Wales). *Lethaia* 46, 454–469.
- Botting JP, Muir LA. 2018. Early sponge evolution: A review and phylogenetic framework. *Palaeoworld*, 27, 1–29.
- Dohrmann et al. 2017 An integrative systematic framework helps to reconstruct skeletal evolution of glass sponges (Porifera, Hexactinellida). *Frontiers in Zoology* (2017) 14:18.
- Gehling, J.G., Rigby, J.K., 1996. Long expected sponges from the Neoproterozoic Ediacara Fauna of South Australia. *Journal of Paleontology* 70, 185–195.
- Mehl, D. & Reitner, J. (1996). Observations on *Astraeospongium meniscum* (Roemer, 1948) from the Silurian of western Tennessee; constructional morphology and palaeobiology of the *Astraeospongiidae* (Calcarea, Heteractinellidae). *Berliner geowiss. Abh.* 18 243–255.
- Luo et al. 2019. The first report of a vauxiid sponge from the Cambrian Chengjiang Biota. *Journal of Paleontology*, 94, 28–33.
- Yang, XL., Zhao, YL., Babcock, L. & Peng, J., 2017. A new vauxiid sponge from the Kaili Biota (Cambrian Stage 5), Guizhou, South China. *Geological Magazine*, 154, 1334–1343.

Lucy Muir

Referee #3 (Remarks to the Author):

I would like to thank Wang and colleagues for considering my suggestions. The revised version of the manuscript is, in my opinion, much improved. I have a few more comments regarding the phylogenetic analyses, some of which are based on the revision files, but some I feel haven't properly been addressed from the original files. If the authors are happy to accommodate these, then I am happy to sign-off on this manuscript.

The authors have still not, in my view, provided enough information on the parameters of their analyses. The authors report ESS, but not what the deviation of split frequencies was - similarly important in assessing convergence. Furthermore, I would like to see either justification of use of the gamma distribution to model rate variation, or additional analyses using a lognormal distribution. I would also like justification for employing a symmetric transition rate, as opposed to asymmetric, or additional analyses with some specified asymmetry. Both of these parameters have been shown to be better fitting in some phylogenetic scenarios (and impact resultant topology) and when so much rests on the phylogenetic analysis I think it's really important to be sure of the position. These can be easily implemented in MrBayes. I also think, given the authors have opted to leave in the discussion regarding the evolution of biomineralisation and have chosen not to perform ASR, that it would now be worth conducting some Bayes Factor comparisons to assess the strength of support for *Helicolocellus* as a stem-group hexactinellid, versus e.g. a stem-group sponge. The authors have this phylogenetic data, and it would be good to have some quantitative measure of support for the hypothesis. Again, stepping stone analyses can be very easily implemented in MrBayes and would provide confidence in the conclusions regarding biomineralisation.

Finally, I have a couple of comments about the character list as well: it would be useful if the authors could indicate next to each number where characters have come from, so the list is more readily interpretable to the reader. The authors have provided information as to why *Helicolocellus* has been scored in the way that it has, but it doesn't seem they have provided the same information for any other fossils. As the authors point out, this is the first phylogenetic dataset in which these fossils will be scored in, so it's important that all this information is available for the reader.

These comments aside, it's a fantastic fossil and I look forward to seeing it in the literature.

Referee #4 (Remarks to the Author):

I am pleased with the replies to my comments. I support publication in its current state. Really cool to learn about the stalked sponges!

Author Rebuttals to First Revision:

Comments from editor and reviewers: black fonts, with emphasis in **bold** fonts added by the authors

Response: red fonts

Referees' comments:

Referee #1 (Remarks to the Author):

The summary below addresses the collective commentary of four reviewers, and does not repeat or revise the content of my own original review.

Collectively, the negative aspects of the four reviews centre on the following:

- 1) Preservation style and relief
- 2) Identity of the grid-like skeletal components
- 3) Whether basal disc and stem are problematic for a sponge interpretation
- 4) Implications of the marginal fringe for a poriferan versus bilaterian identity
- 5) Content of the cladistic analysis
- 6) Whether, how, and what other material (other sponge types and ages) is explicitly compared in the text.

The rebuttal or explanation of each of these items seems to be satisfactory, as summarised below.

The first three items would be serious problems if true, but are in fact based on misunderstandings of the descriptions and interpretations in the original text, and of sponge biology.

- 1) The existence of relief was taken by a reviewer as an indication that the skeleton had some degree of rigidity, perhaps conferred by a fused skeleton, which would contradict the disarticulation interpreted in part of the fossils by the authors. The authors note that relief is possible through other means, especially in carbonate rocks, and in the rebuttal provide, as an example, an image of a garden-variety 3-d sponge body fossil without fused spicules, in a carbonate rock.
- 2) The grid-like skeletal components were misconstrued by a reviewer as spicules, rather than impressions of non-mineralised skeletal elements as intended by the authors. The revision contains new text to help prevent this misunderstanding in other readers.
- 3) A reviewer stated that basal discs and stems are not characteristics of sponges, and so the fossils cannot be interpreted as sponges. The authors' rebuttal includes an image of these exact

characteristics in modern sponges.

Item #4 (marginal fringe). According to one reviewer, the marginal fringe suggests a bilaterian affinity, rather than poriferan. However, the authors' interpretation does not require that the fossil have bilateral symmetry.

Item #5 - Two reviewers offer a number of important commentaries on the cladistic analysis, and the authors respond with a variety of modifications and additions (e.g., inclusion of fossil sponge taxa, sensitivity tests, non-hexactinellid taxa). I am not an expert in this type of analysis, but the responses seem to be appropriate. At any rate, the changes do not substantially alter the outcome.

Item #6 – A reviewer suggested that more attention be given to comparison with other possible Ediacaran sponges (rather than only contrasting). The authors provide a revised paragraph that both compares and contrasts the main examples of putative Ediacaran sponges with *Helicolocellus*.

There are a few other comments that are not substantial enough to influence the viability of the paper, but which have been accommodated by the authors.

I am satisfied that all concerns of the reviewers have been appropriately dealt with, and reiterate my support for the publication of this paper.

Response: We thank this reviewer for their positive assessment of the manuscript, and for their concise summary of our previous revisions.

Referee #2 (Remarks to the Author):

Re-review of Wang et al. "A late Ediacaran crown-group sponge animal"

The authors have answered some of my points, but they appear to have misunderstood (and thus not adequately replied to) other points. There are also problems with the revised cladistic analysis. The authors have still not adequately demonstrated that the organism in question is a sponge, and there are still major issues with the sponge interpretation of *Helicolocellus*. I regret that I therefore cannot support publication of this paper.

Taphonomy

I thank the authors for clarifying that they consider the grid to have been made up of separate elements (i.e., was unfused). However, the authors appear to have not entirely understood my point about the taphonomy of the grid/soft tissue. Although it is true (but unusual) that a sponge with an unfused spicule skeleton can be preserved with some degree of three-dimensionality, this can only happen if the soft tissue was intact at the time of burial. The authors are also correct that soft-bodied organisms can be preserved three-dimensionally (e.g., as they say, in Ediacara-type assemblages), but this could only have happened if the soft tissue was intact at the time of burial. If a skeletal grid had been present, its disarticulation would mean that the soft tissue had decayed in that part of the organism. If the soft tissue had decayed, then the soft tissue would not have been preserved three-dimensionally. Thus, the presence in *Helicolocellus* of (what the authors interpret as) an area in which the grid has disarticulated, but in which the soft tissue has been preserved in three dimensions, does not make sense taphonomically.

Several specimens of *Helicolocellus* are available: all show both three-dimensional preservation of the organism and absence of the grid-like structure over at least part of the body. This absence of grid is best interpreted as a feature of the organism's morphology, rather than loss of an originally present grid-like structure.

Response: We thank the reviewer for the clarification, and note to the editor that some of the authors have met with this reviewer since receiving this review to attempt to resolve any points of confusion. As a result, we believe that our responses here reflect a correct understanding of the reviewer's concerns.

In response to this first point, we would argue that degradation does not happen in a binary manner that results in a carcass being either entirely degraded or perfectly intact. In reality, degradation, compaction, and disarticulation can occur to varying extents even on individual carcasses. We argue that there is undoubtedly some level of degradation, but that the skeletal structures are preserved despite degradation. Partial compaction has occurred too, evidenced by the fossils' elliptical rather than circular cross-section, which could have resulted in disarticulation (dislocation or dislodgement) of skeletal elements post-burial, leading to irregular arrangements of skeletal elements in some parts of individual specimens.

The reviewer interprets the absence of the grid pattern in the lower part of the body as a feature of the organism's morphology, commenting that "This absence of grid is best interpreted as a feature of the organism's morphology, rather than loss of an originally present grid-like structure". We have added a specimen (Extended Data Fig. 2d in the revised manuscript; see also Fig. R1 below)

to show the grid-like structure at the lower part of its conical body. This specimen supports our interpretation that the grid-like structure originally covered the entire conical portion of the body of *Helicolocellus*, and that the loss of the grid-like structure in the lower part of the conical body is related to preservation due to dislodgement of skeletal elements, rather than being “a feature of the organism’s morphology”.

The reviewer also appears to have conflated disarticulation and degradation, suggesting that “disarticulation would mean that the soft tissue had decayed” and that “if the soft tissue had decayed, then the soft tissue would not have been preserved three-dimensionally.” It is essential to clarify that disarticulation and degradation are not the same process; that disarticulation does not invariably signify complete decay; and that soft-tissue degradation does not necessarily rule out three-dimensional preservation. To illustrate, consider leaves, which can become disarticulated from trees. Notably, fallen or fragmented leaves can also be preserved as moulds (as are the disarticulated skeletal elements of *Helicolocellus cantori*), sometimes maintaining their original three-dimensionality, even if the leaves themselves are completely degraded after burial. As another example, some Ediacaran macrofossils (*Pteridinium*, for example) were torn apart, completely degraded (no organic preservation at all), yet three-dimensionally preserved as casts and moulds.

In the revision, we have replaced “disarticulation” with “dislocation” for a more precise depiction of the skeletal elements’ preservation condition: semi-loose and dislodged but not distanced from the sponge body. However, we maintain our opinion that the distortion of the grid-like pattern in the lower part of the conical body is a preservational artefact, not an original feature of the organism.

Figure R1: A specimen of *Helicolocellus cantori* with regular grid-like structures in the lower part of the conical body – now added to the manuscript as Extended Data Figure 2d.



Revised text

Lines 106–109: “The basal part of the conical body is often poorly preserved, consisting of vaguely defined but poorly aligned rectangles without fine structure, possibly resulting from post-mortem distortion, as evidenced by a separate specimen showing regularly and diagonally arranged rectangles at the basal part (Extended Data Fig. 2d).”

Lines 135–137: “In contrast, specimen NIGP-176538 exhibits well-preserved, diagonally arranged rectangles in the lower part of the conical body (Extended Data Fig. 2d).”

The authors’ argument that the grid structure was made of separate units that could disarticulate would be much stronger if they illustrated some of these separate units. In a partially disarticulated fossil sponge, some loose spicules are usually visible around the area with an intact skeletal grid. Given that several specimens of *Helicolocellus* that (if the authors are correct) are partially disarticulated are available, it would seem likely that at least one of them would show at least one element that had separated from the grid. Given the large size of the largest elements, this should be easy to illustrate by means of a photomicrograph. (Disarticulated elements might well be more visible on a latex cast of the specimen than on the specimen itself.)

Response: See our response to the point immediately below.

Imprints of skeletal elements

The authors also appear to have not understood my point that imprints of the separate elements making up the grid should be visible. I do understand that (if a grid composed of cruciform elements of an organic composition had been present), those elements have vanished at some point between burial of the organism and discovery of the fossil, and that thus the preserved grooves represent the imprint of the original grid. My point was, however, that if the imprint of a grid is preserved, imprints of at least some of the individual elements making up the grid should be visible. Some of the larger elements would have been much larger than the grain-size of the rock. (In the holotype, the first-order rectangles are separated by “grooves that are 1.1 mm wide and approximately 0.4 mm deep”. The first-order rectangles are 12 mm long and ca. 9 mm wide. Assuming that each ray of the largest “cruciform elements” extended over half a grid square (as in the Palaeozoic reticulosan *Cyathophycus*; see Botting 2003), the largest spicules should have had long rays 6 mm long, shorter rays 4.5 mm long, and been 0.4 to 0.5 mm wide.)

Again, a latex cast would be helpful in showing up this feature, if present. See Botting & Muir (2011) for an example of using a latex cast on a fossil sponge to clarify spicule details.

Response: We argue that the grid is composed of skeletal elements that are preserved as moulds. Due to the nature of preservation (mouldic preservation of skeletal elements in limestone) and the propensity for calcite minerals to dissolve, recrystallize, and overgrow, it is difficult to delineate individual skeletal elements when articulated, because the boundaries between neighbouring skeletal elements are much smaller than sediment grain size. In other words, it is the size of the gap

separating neighbouring skeletal elements, not the size of the skeletal elements per se, that makes it difficult to delineate the skeletal elements when articulated. Notably, the skeletal elements appear to be thin, non-biomineralised, cruciform straps. Their non-biomineralized nature and style of preservation makes it inherently difficult for them to leave discernible imprints when disarticulated.

Following the reviewer's advice, we used silicone rubber and resin to cast the fossils, and we also made additional thin sections, but unfortunately were unable to recover discrete skeletal elements. As mentioned above, the absence of discrete cruciform spicule-like elements, commonly found in biomineralizing sponges in the Phanerozoic, does not necessarily negate the interpretation of *Helicolocellus cantori* as a non-biomineralizing sponge.

Stem and holdfast of *Helicolocellus*

The authors are correct that some modern hexactinellids possess root tufts that can look rather different from the body of the sponge; however, the tufts/stalks of extant hexactinellids are made of spicules, and are thus completely different in construction from the *Helicolocellus* holdfast, in which there is no sign of spicules. Comparing the holdfast of *Helicolocellus* with that of extant hexactinellids is misleading. The authors may be correct that a fleshy holdfast has evolved convergently in several taxa of Ediacaran age; however, that a feature has evolved convergently several times does not mean that every example of that feature is an independent derivation. If *Helicolocellus* were a close relative of *Charnia*, then the holdfast of *Helicolocellus* would be homologous with that of *Charnia*.

Response: While the stalks of extant hexactinellids are composed of spicules, these spicules may be too small to be discerned by the naked eye. For example, Gong et al. (2023) noted that, for *Caulophacus*, "the stalks of the sponge are hollow and there are no visible spicules on the surface." Reiswig et al. (2021, their fig. 12) also provided additional information and pictures of the stalk of *Caulophacus*, substantiating Gong et al.'s (2023) observations.

We argue that the stems and holdfasts are not phylogenetically informative, as pointed out in the manuscript. It would be problematic to assume that stems and holdfasts are homologous to any group, as many distantly related groups had, and still have, them (e.g., sponges, cnidarians, mushrooms, and various algae; e.g. Fig. R2 below). Such examples are cited in the manuscript.

With regard to a possible relationship with *Charnia*, we would like to point out that *Charnia* and *Helicolocellus* are fundamentally different in terms of body plan (flattened frond with "fractal" construction versus conical body with hierarchical grids). As pointed out in the manuscript, these

two groups are distantly related to each other (see also Dunn et al., 2021 for a detail description and a phylogenetic analysis of *Charnia*), and the stems and holdfasts are almost certainly homoplastic.

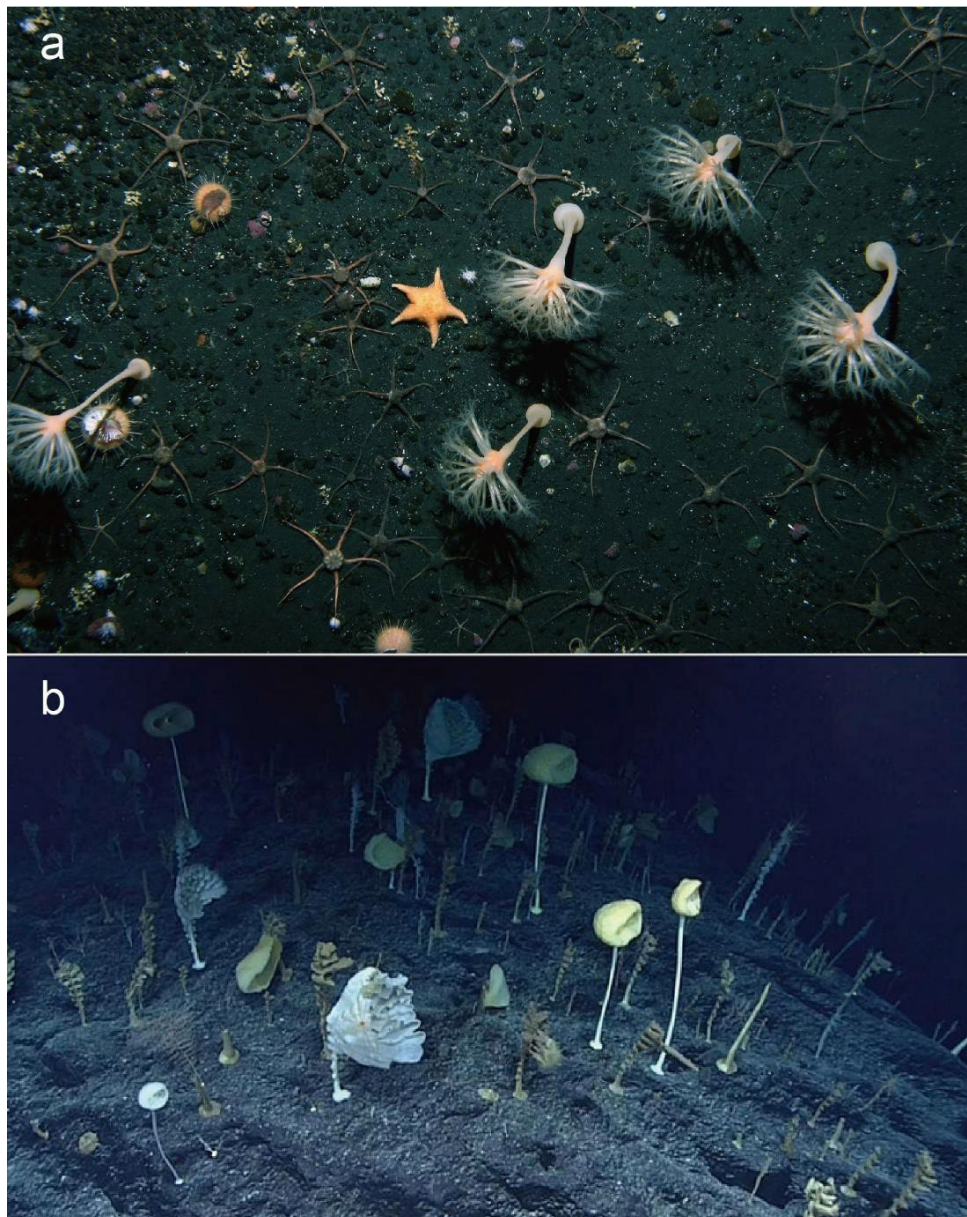


Figure R2. **a.** The seapen *Umbellula pallida*, with a stem and a disc-like holdfast from Scott Island Seamount in the Ross Sea, Antarctica (image by the National Institute of Water and Atmospheric Research, <https://www.facebook.com/NIWAINvertebrateCollection/photos/a.1603775579690322/1603780043023209/?type=3>, accessed on 27/12/2023). **b.** An extant deep sea hexactinellid sponge forest in Ridge Seamount, Johnston Atoll, Pacific Ocean (image from Mitchell & Harris, 2020, original image was recorded on Dive 11 of Okeanos Explorer expedition EX1706 of the Johnston Atoll Unit in PRIMNM). Many of the sponges show a stem with a disc-like holdfast.

We have also included a comparison with another fossil sponge that exhibits disc-like holdfasts.

Revised text:

Lines 261-267: “The attachment strategy of *Helicolocellus* can also find analogues among younger sponges. The Jurassic protospongiid-like sponge *Ammonella*, for example, displays a regular and hierarchical meshwork of stauractines⁴⁸, resembling the pattern observed in *Helicolocellus*, and was also anchored to a potentially microbially-stabilised substrate by a discoid root plate⁵¹. This attachment strategy bears similarity to the way *Helicolocellus* adheres to the (presumably microbially-stabilised⁵²) Ediacaran substrate.”

The authors make the point that they consider *Helicolocellus* to be a sponge because of the presence of a “regular, hierarchical meshwork”, which they consider to be a feature otherwise possessed only by sponges. This is not in fact correct: some specimens of *Palaeophragmodictya* show a rectangular grid structure with two size orders of quadrules (Gehling & Rigby 1996). As *Palaeophragmodictya* is now generally accepted to not be a sponge, it is therefore not the case that any organism showing a grid structure with multiple size order is a sponge.

Response: *Palaeophragmodictya* is an Ediacaran genus characterised by a disc-like morphology and originally interpreted as a hexactinellid sponge (Gehling and Rigby, 1996). Only one illustrated specimen exhibits semi-regular grids, and it is not clear at all that this specimen displays two size-orders of quadrules (see Fig. R3 below); in light of our study on *Helicolocellus*, the anatomy and phylogenetic affinity of *Palaeophragmodictya* certainly warrants further investigation. Regardless, *Palaeophragmodictya* surface impressions differ significantly from the highly regular, hierarchical grid observed in *Helicolocellus*. Additional specimens and further investigation of *Palaeophragmodictya* are required to verify the existence of the claimed two size-orders of quadrules and to contribute to the ongoing controversy surrounding its biological affinity.

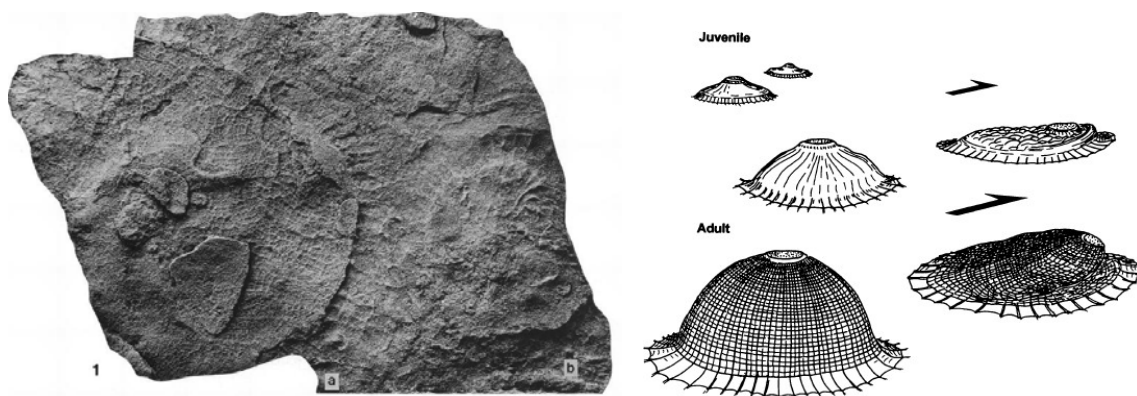


Figure R3. *Palaeophragmodictya reticulata*. Left: holotype. Right: ontogeny and morphological reconstruction. There do not seem to be two size-orders of quadrules in *Palaeophragmodictya reticulata*. From Gehling and Rigby (1996).

Cladistic analysis

I appreciate that the authors have included additional fossil and extant sponge taxa to their cladistic data matrix. Unfortunately, there are serious problems with the revised cladistic analysis (listed in detail below). The cladistic data matrix appears to have been constructed (with some characters included and some excluded, and codings manipulated) so as to yield the desired result. Given the problems with the coding of characters, omission of important characters, and incorrect phylogenetic positions for taxa for which the relationships are well understood, the results of the analysis cannot be used to support the conclusion that *Helicolocellus* is a sponge.

Response: We thank the reviewer for providing thorough comments and suggestions for improving the phylogenetic analysis. The reviewer's specific concerns regarding character coding have been addressed in the revised data matrix. Following the reviewer's suggestions (in her review comments and in our discussion with her, we have carefully revised the coding, added several characters (e.g., character 233: Skeletal elements contain calcium carbonate; character 235: Chitin in sponge skeletal elements), added the fossil taxon *Diagoniella* in our phylogenetic analysis, and carried out various sensitivity tests. Details are provided below. The updated analysis shows that *Helicolocellus* and other fossil taxa (i.e., *Diagoniella*, *Protospongia*, *Eiffelia*, *Cyathophycus*, *Minitaspongia*, and *Eiffelia*) are likely stem-group hexactinellids (Fig. 4b). Thus, the main conclusion of the manuscript that *Helicolocellus* is a crown-group sponge is substantiated in the updated analysis.

Revised text

Lines 336–339: "Our phylogenetic analysis indicates that at least some extinct reticulosan taxa, i.e., *Diagoniella*, *Protospongia*, *Eiffelia*, *Cyathophycus*, and *Minitaspongia*, along with the heteractinid *Eiffelia*, are grouped with extant hexactinellids (Fig. 4b)."

Lines 642–644: "*Helicolocellus cantori* is resolved as a stem-group hexactinellid along with *Eiffelia*, *Diagoniella*, *Protospongia*, *Cyathophycus*, *Minitaspongia*."

Firstly, the results obtained for fossil sponges for which the phylogenetic position is known are inconsistent with current knowledge. The phylogenetic position of *Vauxia* is (unusually for a Cambrian sponge) agreed on by all workers. *Vauxia* is agreed to be a demosponge (e.g. Botting &

Muir 2018), and some workers classify it as a verongioid demosponge (i.e., part of an extant group: Yang et al. 2017; Luo et al. 2019); thus, *Vauxia* should fall out within the Demospongiae in the cladistic analysis. It does not; instead, it appears in the stem group of (Silicea plus Homoscleromorpha). In addition, *Eiffelia*, which is well understood to be an early member of the Heteractinida (early Calcarea) and part of the lineage leading to taxa such as *Astraeospongia* (Mehl & Reitner 1996; Botting & Muir 2018) should cluster with the Calcarea, and yet it appears in the Hexactinellida. I assume that this is the result of including siliceous spicules and biomineralic spicules in the data matrix, but not calcareous spicules. As calcareous spicules are a diagnostic feature of modern Calcarea, this omission is puzzling.

Response: First, we would like to point out that previous phylogenetic interpretations of early sponges, including *Vauxia* and *Eiffelia*, were based on narratives and remained uncertain. These narratives must be subjected to rigorous phylogenetic analysis, as is done in our manuscript.

Second, we refer the reviewer to the statistics reported along with the cladogram, which show that the posterior probability of Silicea + Homoscleromorpha to the exclusion of *Vauxia* is relatively low (only 67%). Considering the topology of the cladogram and the posterior probability values, it is more appropriate to regard *Vauxia* + Demospongiae + Hexactinellida + Homoscleromorpha as a polytomy. The same is true in the updated analysis, with a posterior probability of 61% for Demospongiae + Hexactinellida + Homoscleromorpha to the exclusion of *Vauxia* (see Extended Data Fig. 4 in the manuscript). These results show that the phylogenetic position of *Vauxia* is not unambiguously resolved in our analysis.

Third, in our updated analysis, we have now coded for calcareous spicules and chitin in sponge skeletal elements, and have added the fossil sponge *Diagoniella* into the data matrix. In the updated analysis, *Vauxia* remained as a sister-group to Silicea + Homoscleromorpha, and *Eiffelia* as a basal branch in the total-group Hexactinellida, although the posterior probability values are relatively low. The results mean that the phylogenetic affinities of both *Vauxia* and *Eiffelia* remain a matter of debate, although both are clearly within the phylum Porifera (with a posterior probability of 100%). Although we do not state this in the revised manuscript because we consider it beyond the scope of this specific study, our analysis does raise questions about the “accepted” interpretation of early sponge evolution, and it would be timely to reassess several traditional paradigms within this field in light of this and other recent fossil discoveries.

The added characters: “Character 233. Skeletal elements contain calcium carbonate. Character 235. Chitin in skeletal elements.”

Secondly, the recovered relationships of modern sponges are problematic. The Euplectellidae (*Euplectella*, *Malacosaccus*, etc.) are recovered as sister group to the rest of extant Hexactinellida. This is peculiar, to say the least, because most analyses recover the Euplectellidae as sister-group to the Rossellidae (e.g. Dohrmann et al. 2017): note that the purely morphological analysis of Dohrmann et al. (2008), i.e., the same data as used in the present study, yielded hexactinellid families but the relationships between those families was an unresolved polytomy (fig. 3 of Dohrmann et al. 2008). The recovery of Homoscleromorpha in an unresolved polytomy with Demospongiae and Hexactinellida, rather than as sister-group to Calcarea, is also problematic.

Response: Phylogenetic analyses of sponges using different datasets (molecular vs. morphological) and different methods (maximum parsimony vs. Bayesian inference) give incongruent results. For example, a Bayesian consensus tree from molecular data (fig. 2 of Dohrmann et al. 2008), maximum parsimony trees from morphological data (fig. 3 of Dohrmann et al. 2008), a TNT tree from morphological data (fig. 6 of Dohrmann et al. 2017), and a maximum likelihood tree from molecular data (fig. 7 of Dohrmann et al. 2017) show different topologies for the Hexactinellida. Thus, the phylogenetic relationship among hexactinellid families remains a matter of debate and, in principle, we refrain from using previously published phylogenetic results to justify or refute our phylogenetic results.

That said, we would like to point out that our phylogenetic results are broadly consistent with the morphological trees of Dohrmann et al. (2008; their fig. 3). In both our analyses (Extended Data Fig. 4 in the original submission, first revision, and second revision) and Dohrmann et al.'s (2008, their fig. 3b), the Amphidiscophora, Pheronematidae, Aphrocallistidae, Scopularia, Scepтрulophora, Hexactinosida, and Euplectellidae are well defined, whereas the Rossellidae is paraphyletic (which means that the Euplectellidae cannot be a sister group to the Rossellidae).

The broadly similar results between our and Dohrmann et al.'s (2008) phylogenetic analyses are remarkable given that different data matrices and methods were used. In terms of taxon sampling, our analyses include all hexactinellid taxa of Dohrmann et al. (2008), plus several additional living hexactinellids (e.g., *Leucopsacus*, *Caulophacus*, and *Oopsacas*), several fossil taxa, and many non-hexactinellid animals that were not included in Dohrmann et al. (2008). In terms of methods, we used Bayesian inference whereas Dohrmann et al. (2008) used maximum parsimony. These differences likely contributed to the differences between our and Dohrmann et al.'s (2008) hexactinellid phylogenetic trees (e.g., the polytomy at the base of the Hexactinellida in Dohrmann et al., 2008 vs. better resolution in our phylogenetic tree).

With regard to the reviewer's comment that "The recovery of Homoscleromorpha in an unresolved polytomy with Demospongiae and Hexactinellida, rather than as sister-group to Calcarea, is also problematic", we note that morphological data tend to favour a Homoscleromorpha-Demospongiae grouping (as in traditional sponge classification and in our updated phylogenetic analysis, though with a relatively low posterior probability) because of the siliceous spicules in the Homoscleromorpha, whereas molecular data tend to favour a Homoscleromorpha-Calcarea or Homoscleromorpha-Eumetazoa grouping (e.g., Gazave et al., 2012). Thus, our results are not surprising given that we used only morphological data in our analysis. Nonetheless, we have carried out multiple sensitivity tests where we constrained the relationships between Porifera classes and Hexactinellida genera based on molecular phylogenies, and the phylogenetic position of *Helicolocellus* as a crown-group sponge remained unchanged. See lines 276-280 in the revised text: "Our results remain stable in multiple sensitivity tests: when ctenophores are constrained as the sister-group to all other animals⁵⁴ (Extended Data Fig. 5a), and when the relationships of the classes within Porifera and the internal relationships within Hexactinellida are constrained to follow recent molecular phylogenies^{53,55} (Extended Data Fig. 5b)."

We would like to emphasize that our repeated phylogenetic analyses of the refined data matrix with multiple sensitivity tests consistently recovered *Helicolocellus* as a crown-group sponge and within the total-group Hexactinellida. Thus, the main conclusion of the manuscript (i.e., *Helicolocellus* is a crown-group sponge) is robust.

A detailed description of the dataset, methods, and sensitivity tests are provided in the methods section of the main text and the supplementary material.

Thirdly, although the authors have included *Charnia* in the revised analysis, they are excluding the feature that is shared by both *Helicolocellus* and *Charnia*—a fleshy holdfast—from the data matrix, on the basis that they believe the feature to be homoplastic. This type of conclusion ought to come out of the analysis, rather than be a basic assumption of it.

Response: See our response to the reviewer's comment on "Stem and holdfast of *Helicolocellus*." Since we already know that a fleshy holdfast is homoplastic (present in sponges, cnidarians, mushrooms, and various algae, for example), the inclusion of this feature in the phylogenetic analysis would simply introduce noise because it does not reflect shared ancestry, and would confound phylogenetic signals.

Fourthly, the authors do not sufficiently explain how they achieved their character codings for fossil

sponges. Most of the sponge-specific characters (and their codings, for extant taxa) are from the phylogenetic analysis of Hexactinellida by Dohrmann et al. (2008) and of Calcarea by Manuel et al. (2003). Neither Dohrmann et al. (2008) nor Manuel et al. (2003) included fossil taxa or extant demosponge genera in their morphological datasets. I assume that the authors coded *Protospongia*, *Cyathophycus*, *Eiffelia* and the other fossil taxa, and extant demosponges, from either their own observations of actual physical specimens or from the literature. If the former, they should state this (and provide the museum numbers of studied material); if the latter, they should provide citations to the relevant literature.

Response: We have added citations and explanation for the coding of fossil taxa next to relevant characters in the Supplementary Information file (Section 2: Character List). We also added a statement in Supplementary Material to indicate that the taxon coding (except for *Helicolocellus*) in this study relies exclusively on literature research.

Lines 34–36 in Supplementary material: “The taxon coding (except for *Helicolocellus*) in this study relies exclusively on literature cited in this document (ref.¹⁻⁴ and others cited in the character list).”

There are some obvious problems with the current character coding; for example, *Cyathophycus* is coded as having fused spicules (character 182), as being basiphytous (i.e., attached to hard substrates; character 185), and as possessing hexasters (a particular type of microscle; character 190), but I am not aware of any study that has documented spicule fusion, attachment to a hard substrate, or any type of microscle in *Cyathophycus*. (Note for the editor: extant hexactinellids possess both megascleres [large, obvious spicules] and microscleres [very small, generally <150 microns in size, spicules that are very important in taxonomy.] Microscleres are extremely rare in the fossil record, and there are no records of microscleres for the overwhelmingly vast majority of early Palaeozoic sponges.) Furthermore, *Cyathophycus* is coded as lacking hexactins as major dermal megascleres (i.e., the outer layer of spicules in a hexactinellid sponge according to Dohrmann et al. 2008; character 209), but is coded as ? for stauractins as major dermal megascleres (character 210). This is puzzling, because *Cyathophycus loydelli* has an outer layer of hexactins (Botting 2004). Similarly, *Cyathophycus* is coded as lacking diactins as major choanosomal megascleres (inner layer of the hexactinellid skeleton; character 212) and as major atrial megascleres (the inner surface of a hexactinellid sponge; character 217), and as ? for stauractins as major choanosomal megascleres (character 213)—yet *Cyathophycus loydelli* has an inner layer of monaxonid (not stauractine) spicules (Botting 2004).

Response: We thank the reviewer for pointing out the coding errors in *Cyathophycus*. Some of these were mistakes, and others came from *Cyathophycus* species other than *Cyathophycus loydelli*. Following the reviewer's leads, we have revised the coding of *Cyathophycus* as follows: unfused spicules (character 182), lophophytous (i.e., the sponge is anchored by tufts of protruding spicules; character 185), lacking hexasters (character 190), having hexactins as major dermal megascleres (character 209), lacking stauractins as major dermal megascleres (character 210), unknown for diactins as major choanosomal megascleres (character 212) and as major atrial megascleres (character 217), and lacking stauractins as major choanosomal megascleres (character 213). We have explained that the coding is mostly based on *Cyathophycus loydelli*, one of the better known and better described species of *Cyathophycus*.

I am also puzzled by the coding of *Eiffelia* as having a basiphytous attachment (character 185) and hexaster microscleres (character 190). To the best of my knowledge, a root tuft and microscleres are not known for *Eiffelia*. Incidentally, the authors refer to *Eiffelia* as a reticulosan in the text, although it is generally classified as a heteractinid. Presumably the authors are regarding *Eiffelia* as a reticulosan based on the presence of hexactine spicules, despite it also bearing heteractine spicules (which do not seem to be included as a character in the phylogenetic analysis, and might have altered the results if included).

Response: We have revised the coding for *Eiffelia*: being lophophytous (character 185), lacking hexasters (character 190). We have also revised the text to refer *Eiffelia* as a heteractinid, and added the character of hexaradiate spicules in the data matrix. *Eiffelia* was still resolved as a stem-group hexactinellid, but in a basal polytomy of Hexactinellida. *Helicolocellus* remained as a crown-group sponge and within the total-group Hexactinellida. Thus, the main conclusion of the manuscript (i.e., *Helicolocellus* is a crown-group sponge) remains the same.

The authors' coding of the shapes of axial filaments (character 233) requires further explanation. I am not aware of any reference giving the shape of the axial filament of *Protospongia* (coded as round in the analysis). I also strongly disagree with the coding of *Cyathophycus* as having a circular axial filament: the axial filament, being made of protein, may become altered during diagenesis and thus may not retain its original shape, but a hexagonal axial canal (as illustrated by Botting & Muir 2013 for *Cyathophycus loydelli*) cannot be the result of partial spicule dissolution and thus is a reliable guide to the original shape of the axial filament.

Response: Church et al. (1999) reported rod-like axial canals in *Protospongia* spicules. However, we agree with the reviewer that a cylindrical axial canal may be a result of dissolution and thus is not a reliable indicator of the original shape of the axial filament. As a result, we have revised this character to “unknown” for *Protospongia*. In the case of *Cyathophycus*, Botting and Muir (2013) illustrated multiple cylindrical canals (their fig. 3B, G, H) and a “sub-circular axial filament within rounded canal” (their fig. 3I), although they also illustrated “hexagonal canals” (e.g., their fig. 3F). Nonetheless, we agree with the reviewer that a hexagonal axial canal is a reliable guide to the original shape of the axial filament. Therefore, we have revised the cross-sectional shape of the axial filament as round or hexagonal for *Cyathophycus*. Again, the phylogenetic position of *Helicolocellus* and hence the main conclusion of the manuscript did not change.

These apparent inaccuracies in the coding of the morphology of taxa means that the results of the analysis cannot be relied on.

Response: We accept the reviewer’s point here that the original errors in our character scoring may have undermined confidence in the results, and we thank them for patiently and clearly pointing out these inconsistencies. We have addressed all of the points raised, and re-run the analyses (and multiple sensitivity tests) with this corrected and expanded character matrix. We find that the original main conclusion of our manuscript (that *Helicolocellus* is a crown-group sponge) remains robust.

References

- Botting, J.P., 2004. An exceptional Caradoc sponge fauna from the Llanfawr Quarries, central Wales, and phylogenetic implications. *Journal of Systematic Palaeontology* 2, 31–63.
- Botting, J.P., Muir, L.A., 2011. A new Middle Ordovician (late Dapingian) hexactinellid sponge from Cumbria, UK. *Geological Journal* 46, 50–506.
- Botting, J.P., Muir, L.A., 2013. Spicule structure and affinities of the Late Ordovician hexactinellid-like sponge *Cyathophycus loydelli* (Llanfawr Mudstones Lagerstätte, Wales). *Lethaia* 46, 454–469.
- Botting JP, Muir LA. 2018. Early sponge evolution: A review and phylogenetic framework. *Palaeoworld*, 27, 1–29.
- Dohrmann et al. 2017 An integrative systematic framework helps to reconstruct skeletal evolution of glass sponges (Porifera, Hexactinellida). *Frontiers in Zoology* (2017) 14:18.
- Gehling, J.G., Rigby, J.K., 1996. Long expected sponges from the Neoproterozoic Ediacara Fauna of

South Australia. *Journal of Paleontology* 70,185–195.

Mehl, D. & Reitner, J. (1996). Observations on *Astraeospongium meniscum* (Roemer, 1948) from the Silurian of western Tennessee; constructional morphology and palaeobiology of the Astraeospongiidae (Calcarea, Heteractinellidae). *Berliner geowiss. Abh.* 18 243–255.

Luo et al. 2019. The first report of a vauxiid sponge from the Cambrian Chengjiang Biota. *Journal of Paleontology*, 94, 28–33.

Yang, XL., Zhao, YL., Babcock, L. & Peng, J., 2017. A new vauxiid sponge from the Kaili Biota (Cambrian Stage 5), Guizhou, South China. *Geological Magazine*, 154, 1334–1343.

Lucy Muir

References cited in response:

Church, S. B., Rigby, J. K., Gunther, L. F., & Gunther, V. G., 1999. A large *Protospongia hicksi* Hinde, 1887, from the middle Cambrian Spence Shale of southeastern Idaho. *Brigham Young University Geology Studies* 44, 17–26.

Botting, J.P., Muir, L.A., 2013. Spicule structure and affinities of the Late Ordovician hexactinellid-like sponge *Cyathophycus loydelli* (Llanfawr Mudstones Lagerstätte, Wales). *Lethaia* 46, 454–469.

Dunn, F.S., Liu, A.G., Grazhdankin, D.V., Vixseboxse, P., Flannery-Sutherland, J., Green, E., Harris, S., Wilby, P.R., & Donoghue, P.C.J., 2021, The developmental biology of *Charnia* and the eumetazoan affinity of the Ediacaran rangeomorphs. *Science Advances* 7, eabe0291, doi: doi:10.1126/sciadv.abe0291.

Gazave, E., Lapébie, P., Ereskovsky, A.V. et al. (2012) No longer Demospongiae: Homoscleromorpha formal nomination as a fourth class of Porifera. *Hydrobiologia* 687, 3–10.

Gehling, J.G., Rigby, J.K., 1996. Long expected sponges from the Neoproterozoic Ediacara fauna of South Australia. *Journal of Paleontology* 70, 185–195.

Gong, L., Yang, M., Janussen, D., Dohrmann, M., & Li, X., 2023. A new species of *Caulophacus* (Hexactinellida: Lyssacinosida: Rossellidae) from the western Pacific Ocean, with new insights into the mitochondrial genome characteristics of hexactinellid sponges. *Frontiers in Marine Science* 10, 979912.

Mitchell, E. G., & Harris, S. (2020). Mortality, population and community dynamics of the glass sponge dominated community “The Forest of the Weird” from the RIDGE seamount, Johnston Atoll, Pacific Ocean. *Frontiers in Marine Science*, 7, 565171.

Reiswig, H. M., Dohrmann, M., Kelly, M., Mills, S., Schupp, P. J., & Wörheide, G., 2021. Rossellid glass sponges (Porifera, Hexactinellida) from New Zealand waters, with description of one new genus and six new species. *ZooKeys* 1060, 33-84.

Referee #3 (Remarks to the Author):

I would like to thank Wang and colleagues for considering my suggestions. The revised version of the manuscript is, in my opinion, much improved. I have a few more comments regarding the phylogenetic analyses, some of which are based on the revision files, but some I feel haven't properly been addressed from the original files. If the authors are happy to accommodate these, then I am happy to sign-off on this manuscript.

Response: We thank the reviewer for providing helpful comments and suggestions to improve the phylogenetic analysis. We have further revised the manuscript to accommodate the suggestions provided here.

The authors have still not, in my view, provided enough information on the parameters of their analyses. The authors report ESS, but not what the deviation of split frequencies was - similarly important in assessing convergence. Furthermore, I would like to see either justification of use of the gamma distribution to model rate variation, or additional analyses using a lognormal distribution. I would also like justification for employing a symmetric transition rate, as opposed to asymmetric, or additional analyses with some specified asymmetry. Both of these parameters have been shown to be better fitting in some phylogenetic scenarios (and impact resultant topology) and when so much rests on the phylogenetic analysis I think it's really important to be sure of the position. These can be easily implemented in MrBayes. I also think, given the authors have opted to leave in the discussion regarding the evolution of biomineralisation and have chosen not to perform ASR, that it would now be worth conducting some Bayes Factor comparisons to assess the strength of support for *Helicolocellus* as a stem-group hexactinellid, versus e.g. a stem-group sponge. The authors have this phylogenetic data, and it would be good to have some quantitative measure of support for the hypothesis. Again, stepping stone analyses can be very easily implemented in MrBayes and would provide confidence in the conclusions regarding biomineralisation.

Response: We have added more information on the parameters of the analyses, including
1) The deviation of split frequencies is 0.0079 (lines 557–559).

- 2) To compare the different rate models, we have carried out stepping-stone analyses. The marginal model likelihoods for different models were (presented in lines 38–42 in supplementary material):
- gamma distribution with symmetrical transition frequencies (-1816.26);
 - gamma distribution with asymmetrical transition frequencies (-1792.39);
 - lognormal distribution with asymmetrical transition frequencies (-1790.86);
 - and lognormal distribution with symmetrical transition frequencies (-1813.49)
- These values show that a model with a lognormal distribution and an asymmetric transition rate best fits the dataset.
- 3) We have also implemented stepping-stone analyses to assess the strength of support for *Helicolocellus* as a stem-group hexactinellid versus a stem-group sponge. The marginal model likelihoods for the two hypotheses were (presented in lines 46–47 in supplementary material):
- Helicolocellus* is a stem-group sponge (-1540.46)
 - Helicolocellus* is a stem-group hexactinellid (-1532.55)
- Therefore, the stem-group hexactinellid interpretation is better supported.

Revised text

Lines 560–570: “We compared the Mk model with gamma and lognormal distributions, considering both symmetric and asymmetric transition frequencies. Additionally, we evaluated the topological hypotheses, specifically whether *Helicolocellus* is a stem-group sponge or a stem-group hexactinellid. To assess the strength of support for different models and hypotheses, we compared marginal likelihoods, which were computed using stepping stone sampling with 50 steps and 20,000,000 generations. Hard constraints were applied to all nodes during the stepping-stone sampling analysis for the two hypotheses. The results revealed that the model with a lognormal distribution and asymmetrical transition frequencies, and the topology of the stem-group hexactinellid hypothesis were better supported (see Supplementary Information for further details).”

Lines 49–60 in supplementary material: “These marginal model likelihoods (the average fit of a model to a data set⁵, with higher values meaning better fit) were computed using stepping stone sampling with 50 steps and 20,000,000 generations. The data best support the model with a lognormal distribution and asymmetrical transition frequencies, and the topology of the stem-group sponge hypothesis.

Sensitivity tests have been conducted in order to assess whether the phylogenetic interpretation of *Helicolocellus* remains robust under different topologies for early metazoans. These include constraint of ctenophores as sister-group to all other animals⁶ (Extended Data Fig. 5a), and constraint of the relationships of sponge classes and the internal relationships within Hexactinellida to following recent molecular phylogenies^{2,7} (Extended Data Fig. 5b)."

Finally, I have a couple of comments about the character list as well: it would be useful if the authors could indicate next to each number where characters have come from, so the list is more readily interpretable to the reader. The author have provided information as to why *Helicolocellus* has been scored in the way that it has, but it doesn't seem they have provided the same information for any other fossils. As the authors point out, this is the first phylogenetic dataset in which these fossils will be scored in, so it's important that all this information is available for the reader.

Response: The character list in the supplementary information file has been comprehensively updated to include information about the characters and their coding for the fossil taxa, with justification and citations where applicable. The source of each character is now listed in the first paragraph of the Supplementary Information document.

These comments aside, it's a fantastic fossil and I look forward to seeing it in the literature.

Referee #4 (Remarks to the Author):

I am pleased with the replies to my comments. I support publication in its current state. Really cool to learn about the stalked sponges!

Reviewer Reports on the Second Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

I had no further items of concern as of R1. Now reviewing the authors' responses to concerns raised by other reviewers as of R2, the items in which I have relevant expertise seem to have been adequately and succinctly addressed. My opinion is that the scientific importance and rigour of this paper has met the bar for publication in Nature.

Referee #2 (Remarks to the Author):

The authors have made appropriate changes in response to my comments and those of the other reviewers. I still think that they are misinterpreting this fossil, but consider that their interpretation is now sufficiently well supported that the debate on its affinities can be conducted in the literature.

Lucy Muir

Referee #3 (Remarks to the Author):

The authors have made all the changes I suggested and so I am happy to sign-off on the manuscript. The only comment I have – and this does need to be addressed before final acceptance - is that not all the new phylogenetic information is presented in the manuscript, and you have to dig into the nexus file in the supplement to find other bits which should be in the methods section. As this is already there or done it should be trivial to address these changes. Below, I have listed the information which needs to be included before publication:

- Did you use Bayes Factor comparison after stepping stone analysis? If so, I couldn't find any mention of Bayes Factor comparison at all in the paper. You should provide information on the likelihood values and then the Bayes Factor scores for the tests between your hypotheses and what this translates to in terms of support from the Kass and Raftery paper. At present, the discussion of these results is not given enough space because the lognormal + asymmetrical analysis is not significantly supported over the gamma + asymmetrical analysis – in the Kass and Raftery terminology the support here is 'not worth more than a bare mention'. This is different to the support for stem-hex over stem-poriferan, where there is significant support – you need to lay this out for the reader.
- Optional, but it seems a shame not to lean on this in your discussion – you find significant statistical support for a crown-group placement of *Helicolocellus* over a stem-group alternative.
- Other information is only present in the nexus file in the extended data, but should be present in the methods section. This would include: confirmation that you used the lognormal + asymmetrical set for downstream analyses, explanation as to why you have included only parsimony-informative characters and addressing the ascertainment bias, etc.

- Was the deviation of split frequencies that in all analyses? Or just the final analysis you took forward? It would be better to just state that it was ~ 0.1 in all runs if that's the case so the reader understands that all your analyses converged.

Referee #4 (Remarks to the Author):

I remain pleased with how the authors addressed my comments.

Author Rebuttals to Second Revision:

Reviews and Response: Round 3

Comments from editor and reviewers: black fonts

Response: red fonts

Referees' comments:

[report from Referee #3]

The authors have made all the changes I suggested and so I am happy to sign-off on the manuscript. The only comment I have - and this does need to be addressed before final acceptance - is that not all the new phylogenetic information is presented in the manuscript, and you have to dig into the nexus file in the supplement to find other bits which should be in the methods section. As this is already there or done it should be trivial to address these changes. Below, I have listed the information which needs to be included before publication:

- Did you use Bayes Factor comparison after stepping stone analysis? If so, I couldn't find any mention of Bayes Factor comparison at all in the paper. You should provide information on the likelihood values and then the Bayes Factor scores for the tests between your hypotheses and what this translates to in terms of support from the Kass and Raftery paper. At present, the discussion of these results is not given enough space because the lognormal + asymmetrical analysis is not significantly supported over the gamma + asymmetrical analysis

- in the Kass and Raftery terminology the support here is 'not worth more than a bare mention'. This is different to the support for stem-hex over stem-poriferan, where there is significant support - you need to lay this out for the reader.

The information about Bayes Factor comparison has been added to the Methods section and details have been provided in Supplementary Information. The Bayes Factor score for the lognormal + asymmetrical analysis is $2\Delta\log_e L = 2*1.53 = 3.06$, falling within the range of positive evidence (2-6) in favor of the lognormal + asymmetrical model (MrBayes version 3.2 Manual).

Revised text

(lines 739-740):

"The marginal likelihoods for each model were used to calculate Bayes factors, to determine the best-fit model⁷²."

(lines 70-83 in Supplementary Information):

"The marginal likelihoods for each model were used to determine the best-fit model through Bayes factors⁵, i.e., values that signify the relative strength of statistical support for one hypothesis/model over another: $2\Delta\log_e L$. Bayes factors calculated in this study indicate that the model with lognormal distribution and asymmetrical transition frequencies is strongly supported over the models with symmetrical transition frequencies, and reasonably supported

over the model with gamma distribution⁵ (Table S1).

	GS	GA	LS	LA
GS	n/a	47.74	5.54	50.8
GA	-47.74	n/a	-42.2	3.06
LS	-5.54	42.2	n/a	45.26
LA	-50.8	-3.06	-45.26	n/a

Table S1. Bayes factor comparison of evolutionary models. GS: gamma distribution with symmetrical transition frequencies; GA: gamma distribution with asymmetrical transition frequencies; LS: lognormal distribution with symmetrical transition frequencies; LA: lognormal distribution with asymmetrical transition frequencies. Interpretations of Bayes factors follow reference⁵.

- Optional, but it seems a shame not to lean on this in your discussion - you find significant statistical support for a crown-group placement of *Helicolocellus* over a stem-group alternative.

Following the reviewer's suggestion, this information has been added to the main text as well as the Supplementary Information.

Revised text
(lines 276-279):

"Moreover, the Bayes factor (15.82) indicates a strong statistical support for a crown-group sponge placement of *Helicolocellus* over a stem-group Porifera alternative (see Supplementary Information)."

(lines 89-91 in Supplementary Information):

"The Bayes factor indicates that the stem-group hexactinellid hypothesis is strongly supported over the stem-group sponge hypothesis (Bayes factor value equals 15.82). In other words, *Helicolocellus* is strongly supported as a crown-group sponge.

- Other information is only present in the nexus file in the extended data, but should be present in the methods section. This would include: confirmation that you used the lognormal + asymmetrical set for downstream analyses, explanation as to why you have included only parsimony-informative characters and addressing the ascertainment bias, etc.

This information has been included in the Methods section. According to the MrBayes version 3.2 Manual: 'Ascertainment bias, in our experience, when there are more than 20-30 taxa, even the most severe bias (only informative characters observed) is associated with an insignificant correction of the likelihood

values.’ As we have included 79 taxa in our analysis, the ascertainment bias is expected to be insignificant.

Revised text

(lines 744–746):

“Therefore, the model with a lognormal distribution and asymmetrical transition frequencies was used in downstream analyses, as presented in Fig. 4b and Extended Data Fig. 4.”

(lines 725–726):

“Only characters that are parsimony-informative were included in the analysis, in order to accentuate shared derived characters (synapomorphies)”

– Was the deviation of split frequencies that in all analyses? Or just the final analysis you took forward? It would be better to just state that it was ~ 0.1 in all runs if that’s the case so the reader understands that all your analyses converged.

It is the deviation of split frequencies for final analysis.

Revised text

(line 730–731):

“The average standard deviation of split frequencies was ~ 0.01 in all runs.”