Siliceous spicules in a vauxiid sponge (Demospongia) from the Kaili Biota (Cambrian Stage 5), Guizhou, South China

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Fossils of the sponge Angulosuspongia sinensis from calcareous mudstones of the middle and upper part of the Kaili Formation (Cambrian Stage 5) in the Jianhe area of Guizhou province, South China, exhibit an apparently reticulate pattern, characteristic of the Vauxiidae. Energy Dispersive X-Ray Spectrometry (EDS) and Raman spectroscopy analysis indicate the presence of silica in the skeletal elements of these fossils, suggesting that this taxon possessed a skeleton comprised of spicules. This is the first confirmation of siliceous skeletal elements in fossils of the family Vauxiidae, and it lends support to the hypothesis that some early demosponges possessed biomineralized siliceous skeletons, which were subsequently lost and replaced by spongin later in the evolutionary history of this lineage. The new materials provide critical insight into the phylogeny and evolution of biomineralization in the Demospongiae.

The earliest stages of animal evolution remain highly unclear1. Recent studies of molecular genetics suggest that sponges lay near the base of the animal phylogenetic tree2–4. Whether the sponges are monophyletic or polyphyletic is subject to interpretation5. Based on aspects of cellular structure and chemistry, some workers have favored the possibility that sponges have multiple origins from two or three different single-celled ancestors6,5. Sponges certainly have a deep evolutionary history7. Articulated body fossils and isolated spicules have been reported from the Ediacaran8–12 and the earliest Cambrian13–16. Biomarker evidence suggests that sponges evolved even earlier, perhaps as early as the Cryogenian Period17. The majority of Proterozoic fossils interpreted as sponges, however, have been questioned18, owing to the presumed non-spicular nature of some19, which would have resulted in a poor fossil record of the group20. Thus, while fossils of early aspiculate sponges are unusual, they are essential in unraveling sponge origins and in understanding demosponge phylogeny and the history of spicule evolution.

To date, the best-known early aspiculate poriferans are those assigned to the family Vauxiidae Walcott, 1920, which is known primarily from the Cambrian. The Vauxiidae are characterized an apparently reticulate, aspiculate fibrous skeleton21. The skeletal composition of the Vauxiidae has been reinterpreted a number of times7,21–26, but recently, Ehrlich et al.27 confirmed chitin in the skeleton of Vauxia gracilenta, and concluded that it was a “keratose” demosponge rather than mineralized spicules, and proposed that the Vauxiidae were likely to be the most basal definitive demosponge group known. This contrasts with a recent hypothesis that some aspiculate sponge skeletons were derived evolutionarily through demineralization of siliceous spicules and loss of spicules may have happened at least twice in the Demospongiae20. Botting et al.28 identified spicules within skeletal strands of Vauxia bellula from the Cambrian Burgess Shale. On the basis of this evidence, Botting et al.29 suggested that diactines were primitive for some Keratosa, and that spicules predate the appearance of the demosponge crown group and were subsequently lost in Myxospongiae and Keratosa.

This study seeks to test the loss of spicules hypothesis in order to shed light on the major pathways in the skeletal evolution of Demospongiae and the skeletal composition of the Vauxiidae. For this reason we have studied a new genus and species, Angulosuspongia sinensis30, a vauxiid sponge from the middle and upper part of the Kaili Formation (Cambrian Series 3, Stage 5) of Jianhe, Guizhou, South China (Fig. 1). The skeletal elements of the
taxon consistently show a hexagonal to slightly irregular, quadrangular-hexagonal architecture (Figs 1e,f, 2a,b and 3a,b), which similar to forms from the Burgess Shale of British Columbia, Canada21, and the Spence Shale and Wheeler formations of Utah, USA25. The Jianhe material illustrates apparent spicular structures, composed of silica and preserved in relief, which were previously unknown in the Vauxiidae. Although spicules were previously described in Vauxia bellula28 the original mineralogy of its spicules is unknown. The Jianhe specimens add an interesting dimension to the evolutionary history of the Demospongea, as they clearly indicate that some early Paleozoic ancestors of nonbiomineralizing ‘Keratosa’ possessed siliceous spicules. The new material lends support to the hypothesis that some early demosponges possessed biomineralized siliceous skeletons, and later in their evolutionary history, silica was replaced by spongin.

Results
Skeletal composition. Taphonomic characteristics of the vauxiid sponge Angulospongia sinensis from the Kaili Biota suggest a rather rigid skeletal network, in contrast to flexible fibers comprised of spongin or chitin. Skeletons of Jianhe materials, although compacted in mudstone, are consistently preserved three-dimensionally, with robust rays extending beyond the margin of the sponge body (Figs 1 and 3a). This manner of preservation indicates that the skeletal elements were capable of resisting flattening during sediment compaction.
Energy-dispersive X-ray spectroscopy (EDS) and elemental mapping were used to determine the composition of skeletal elements. The analyses reveal O and Si to be major components in both the sponge body and rock matrix, but the sponge spicules have far less Al, K and Fe than rock matrix, and the sponge body is enriched in C relative to the matrix (Fig. 4; Table 1). It is worth noting that skeletons were more silicon-rich and with less carbon than the inner of polygonal openings produced by fused spicules (Fig. 4a,b,d,e). Raman spectroscopy analysis (Fig. 5) indicates that the vauxiid sponge skeletons of the Kaili Biota are composed of kerogen and silica and demonstrates that the kerogen is composed of geochemically moderately altered amorphous carbonaceous matter (interlinked polycyclic aromatic hydrocarbons) like that of other Burgess Shale-type fossils of Cambrian age. Confirmation of the presence of SiO2 in the spicular skeleton provides strong support for the interpretation that this species possessed siliceous spicules. It is unlikely that silica is a secondary diagenetic product in specimens of vauxiid sponges of the Kaili Biota, as co-occurring biomineralized brachiopods do not show evidence of mineral replacement (Fig. 4j–o). As is typical of the Burgess Shale-type preservation, high-fidelity preservation of labile soft tissues in fossils of the Kaili biota resulted primarily from conservation of primary organic remains,
as two-dimensional carbonaceous (kerogen) films. These films are sometimes augmented by early diageneric mineralization by pyrite or apatite rather than silicification or aluminosilicification.

Discussion

Prior to the description of the Jianhe materials, the family Vauxiidae was monogenic, embracing only Vauxia. In Vauxia and putatively related sponges, three interpretations of skeletal composition have emerged.

(1) Siliceous spicule hypothesis. Walcott and later de Laubenfels classified Vauxia as a hexactinellid sponge. Finks regarded Vauxia as a specialized offshoot of the hexactinellid Protospongidae. Implicit in this classification is a skeletal composition of opaline silica. Walcott reported that in all specimens he examined from the Burgess Shale of British Columbia, Canada, the original siliceous matter of the spicules was removed and replaced by pyrite or a black carbonaceous material, or a combination of the two.

(2) Keratose spongin fiber hypothesis. Rigby reassigned the family Vauxiidae to the lithistid Demospongiae on the basis of skeletal symmetry. He stated that the symmetry of Vauxia is more similar to the symmetry of the Demospongea than the Hexactinellida. Later, Rigby concluded that the vauxiid skeleton was probably comprised of spongin fibers rather than biomineralized spicules, and Rigby and Collins characterized the skeleton as having a double-layer construction of apparently fused keratose fibers. According to Ehrlich et al., spongin in poriferans results from a hierarchical, multilevel organization of collagen microfibrils. The
collagen microfibrils are densely packed, and arranged in a preferential orientation, usually in concentric layers.

(3) Spongin-chitin hypothesis. Ehrlich et al. demonstrated the presence of \(\alpha\)-chitin in spongin fibers of two extant demosponges of the order Verongida (\textit{Aplysina} sp. and \textit{Verongula gigantea}). Chitin, or poly \([\beta(1\rightarrow 4)]-2\text{-acetamido}-2\text{-deoxy-D-glucopyranose}\), is a polymer of the aminosugar \(N\)-acetylglucosamine, and is often associated with proteinaceous skeletons in invertebrates. Ehrlich et al. also identified calcium carbonate in the form of aragonite in the two verongiids, and stated that it is responsible for the stability of the sponge skeleton. Later, Ehrlich et al. identified the presence of chitin in \textit{Vauxia gracilenta} from the Burgess Shale.

Maldonado hypothesized that spongin skeletons evolved at least twice in the Demospongiae. Spicules, which he considered to have been siliceous, were, in his view, lost and replaced by spongin fibers at least once in the Chondrosida-Verongida lineage, and also in the Haplosclerida - Dictyoceratida - Dendroceratida lineage. The skeletal composition of the Jianhe specimens are intriguingly consistent with Walcott's interpretation that \textit{Vauxia} from the Burgess Shale originally possessed siliceous spicules that were later replaced, and offer support for Maldonado's hypothesis that spongin fibers replaced siliceous spicules in some demosponge lineages. These findings support the view that spicules were present among skeletal strands of \textit{Vauxia bellula} and confirm their

| C   | O   | Mg  | Al  | Si  | K   | Ca  | Fe  | S   |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Fig. 2a-1 | 47.65 | 27.88 | 0.18 | 1.57 | 19.97 | 0.60 | 0.59 | 0.70 | 0.87 |
| Fig. 2a-2 | 44.98 | 30.84 | 0.38 | 0.61 | 22.83 | 0.12 | 0.04 | 0.20 |
| Fig. 2a-3 | 40.49 | 40.96 | 0.46 | 1.08 | 46.32 | 0.36 | 0.12 | 0.21 |
| Fig. 2a-4 | 38.74 | 28.49 | 1.52 | 3.75 | 23.75 | 1.34 | 0.22 | 2.18 |
| Fig. 2a-5 | 47.30 | 26.96 | 1.71 | 4.27 | 10.71 | 1.53 | 0.26 | 6.67 | 0.58 |
| Fig. 2a-6 | 71.70 | 6.59 | 1.18 | 4.15 | 8.31 | 1.83 | 0.32 | 5.35 | 0.56 |
| Fig. 2a-7 | 5.71 | 45.40 | 2.20 | 10.99 | 20.04 | 2.64 | 0.43 | 12.78 |
| Fig. 2a-8 | 10.68 | 39.98 | 1.19 | 4.15 | 8.31 | 1.83 | 0.32 | 5.35 | 0.56 |
| Fig. 2a-9 | 13.97 | 41.13 | 2.71 | 10.75 | 21.06 | 3.26 | 1.21 | 5.92 |
| Fig. 2b-1 | 21.95 | 26.64 | 0.71 | 1.36 | 47.12 | 0.45 | 0.36 | 1.39 |
| Fig. 2b-2 | 26.86 | 28.70 | 0.70 | 1.99 | 39.31 | 0.50 | 0.47 | 1.48 |
| Fig. 2b-3 | 34.25 | 30.29 | 0.85 | 1.83 | 30.60 | 0.54 | 0.60 | 1.04 |
| Fig. 2b-4 | 38.08 | 23.16 | 0.78 | 3.01 | 30.36 | 0.99 | 1.05 | 2.56 |
| Fig. 2b-5 | 56.25 | 19.17 | 1.00 | 4.25 | 10.95 | 1.44 | 1.28 | 5.66 |
| Fig. 2b-6 | 12.71 | 30.76 | 2.74 | 14.22 | 27.00 | 6.39 | 0.31 | 5.87 |
| Fig. 2b-7 | 12.08 | 30.79 | 1.50 | 13.71 | 28.04 | 7.41 | 0.55 | 5.92 |
| Fig. 2b-8 | 17.84 | 23.98 | 2.21 | 11.32 | 29.85 | 4.81 | 0.51 | 9.47 |

Table 1. Energy dispersive X-ray spectroscopy (EDS) point analyses of \textit{Angulospongia sinensis}. EDS points are marked on Fig. 2. Elemental compositions are shown in estimated weight percentages.

Figure 5. Overlapping Raman spectra showing the major bands of silica and kerogen that comprise the spicule of \textit{Angulospongia sinensis}. (a) Raman spectroscopy image of spicule of GTBM17-1761b; (b) Raman spectrum taken from the cross in Fig. 5a. Thanks to Yuning Yang from Northwest University for taking these photos.
originally siliceous composition. This new approach does not entirely refute Rigby's interpretation of the Vauxiidae as non-biominerized, as siliceous spicules appear to have been present in some species, but not others. The simultaneous occurrence of spicules and spongin fibers in vauxiid sponges may also support Botting's hypothesis of a single origin of spicules prior to the appearance of crown-group Silicea, and the subsequent loss of spicules in early ‘keratosan’ sponges rather than the traditional and molecular-based views of demosponge phylogeny.

Methods
Sixty-two specimens of vauxiid sponge remains were examined in this study. They are housed at the Guizhou Research Center for Paleontology, Guizhou University, Guiyang, China (GRCP, GU). Some specimens (Figs 1 and 2) were imaged using a Canon EOS Rebel T3i Digital SLR camera with MP-E 65 mm macro lens. Others (Figs 3 and 4) were imaged at finer scale using a LEO1530VP Scanning Electron Microscope (SEM) equipped with an Energy-dispersive X-ray Spectrometer (EDS), located in the State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences. One sample, GM-16-1192 was gold-coated, whereas GTBM9-4598a imaged under the SEM were left uncoated. Raman spectroscopy analyses for skeletal composition of vauxiid sponges from the Kaili Biota were performed on an Invia Raman spectograph of the Renishaw company. In this instrument, two laser devices with wavelengths of 785 nm and 325 nm were used. The simultaneous occurrence of spicules and spongin fibers in vauxiid sponges may also support Botting's model of Raman spectroscopy analyses for skeletal composition of vauxiid sponges from the Kaili Biota were performed on an Invia Raman spectograph of the Renishaw company.

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**Acknowledgements**

This research was supported by the National Natural Science Foundation of China (grant numbers 41362002, 41330101), the National Basic Research Program of China (2013CB835002), the State Key Laboratory of Palaeobiology and Stratigraphy (Nanjing Institute of Geology and Palaeontology, CAS) (No.153113), the Guizhou science and technology plan and the Study Abroad Scholarship of China. Special thanks are due to Feng Liu and Zhefu Liu, Balang Village, Jianhe County, for assistance in fieldwork, and to Yan Fang and Yang Tang, Nanjing Institute of Geology and Palaeontology (NIGP) and Guiyang Institute of Geochemistry of the Chinese Academy of Sciences (GIGCAS) respectively, for assistance with SEM microphotography and EDS analyses, and to Yuning Yang, Northwest University, for assistance with Raman spectroscopy analysis, and to Maoyan Zhu of NIGP and Robert R. Gaines of Pomona College, for providing helpful comments, and to Robert R. Gaines and Steve LoDuca of Eastern Michigan University, for improving the English writing of the manuscript.

**Author Contributions**

Collection of material was made by Chinese members (Y.Z., X.Y. and J.P.). X.Y. designed the study of these fossils. X.Y. and L.B. wrote the main manuscript text and X.Y. prepared all Figures. All authors reviewed the manuscript.

**Additional Information**

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Yang, X.-L. *et al.* Siliceous spicules in a vauxiid sponge (Demospongia) from the Kaili Biota (Cambrian Stage 5), Guizhou, South China. *Sci. Rep.* **7**, 42945; doi: 10.1038/srep42945 (2017).

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