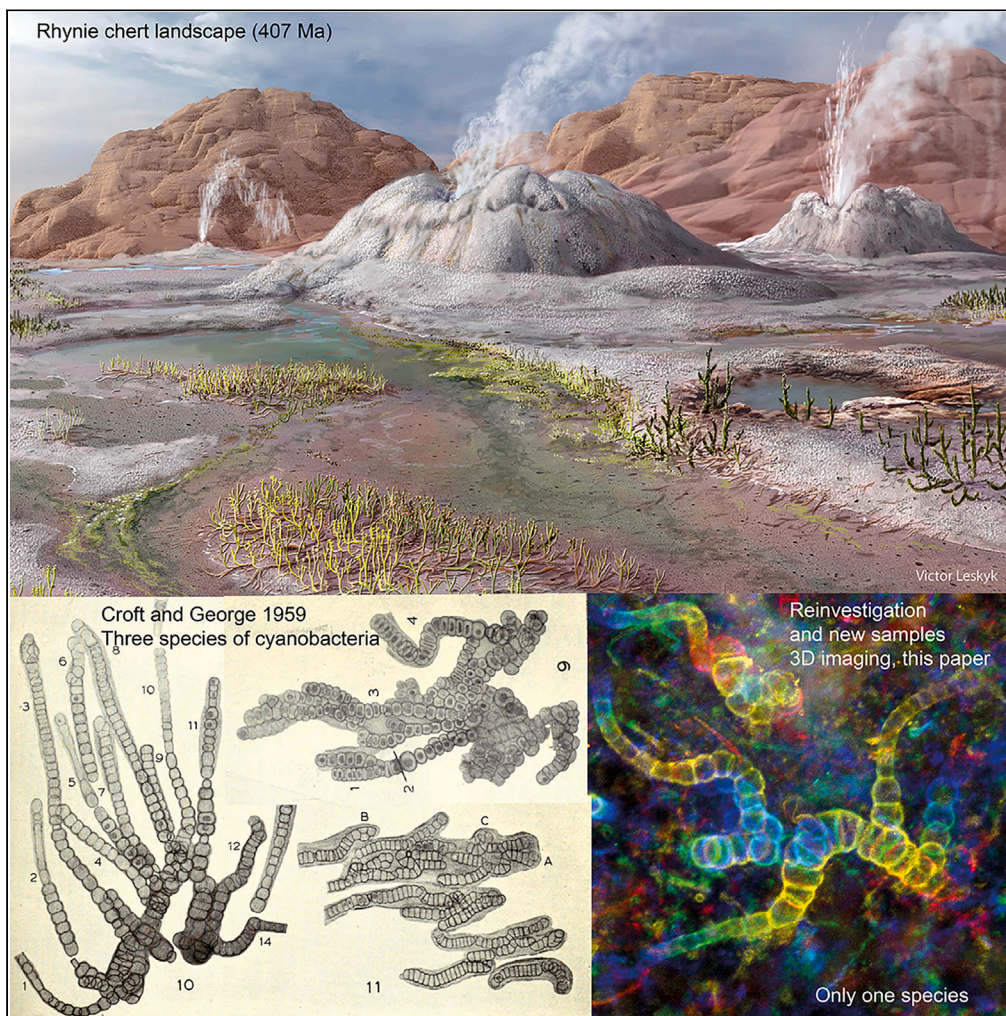Article

Hapalosiphonacean cyanobacteria (Nostocales) thrived amid emerging embryophytes in an early Devonian (407-million-year-old) landscape

Christine Strullu-Derrien, Frédéric Fercoq, Marc Gèze, ..., Marc-André Selosse, Karim Benzerara, Andrew H. Knoll

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Highlights

New and previously described Devonian cyanobacteria are part of a single species

Confocal microscopy permits detailed 3D reconstruction of complex cyanobacteria

Nostocales thrived among early land plants much as their extant relatives do today

Article

Hapalosiphonacean cyanobacteria (Nostocales) thrived amid emerging embryophytes in an early Devonian (407-million-year-old) landscape

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SUMMARY

Cyanobacteria have a long evolutionary history, well documented in marine rocks. They are also abundant and diverse in terrestrial environments; however, although phylogenies suggest that the group colonized land early in its history, paleontological documentation of this remains limited. The Rhynie chert (407 Ma), our best preserved record of early terrestrial ecosystems, provides an opportunity to illuminate aspects of cyanobacterial diversity and ecology as plants began to radiate across the land surface. We used light microscopy and super-resolution confocal laser scanning microscopy to study a new population of Rhynie cyanobacteria; we also reinvestigated previously described specimens that resemble the new fossils. Our study demonstrates that all are part of a single fossil species belonging to the Hapalosiphonaceae (Nostocales). Along with other Rhynie microfossils, these remains show that the accommodation of morphologically complex cyanobacteria to terrestrial ecosystems transformed by embryophytes was well underway more than 400 million years ago.

INTRODUCTION

Cyanobacteria, the only group to have evolved oxygenic photosynthesis, originated early in our planet's history and still accounts for an estimated 25 percent of global primary production.[1] Because cyanobacteria were the principal source of dioxygen gas on the early Earth, the Great Oxygenation Event, beginning ca. 2.4 Ga, provides a minimum date for cyanobacterial origins.[2] Geochemical signatures of regional, transient oxygenation in older sedimentary rocks suggest a still earlier origin of the clade,[3,4] consistent with molecular clocks that point to an Archean emergence of crown group cyanobacteria (e.g.,[5–7]). Although most early cyanobacterial fossils occur in rocks deposited in marine environments, phylogenies indicate that the group colonized non-marine habitats early on.[5–7] Indeed, fossils of cyanobacteria have been reported from late Mesoproterozoic shales interpreted as non-marine,[8,9] although a marine influence has been suggested for at least some of these deposits.[10,11] In any event, *Gloeobacter*, the sister of all other extant cyanobacteria, lives on rock surfaces,[12] and a close relative of *Gloeobacter*, recently identified from metagenomic data, was also discovered in a non-marine environment.[13] Moreover, the cyanobacterial endosymbiont that gave rise to photosynthesis in eukaryotes is also inferred to have lived in a low salinity habitat.[6,14,15]

The preceding paragraph makes it clear that the Paleozoic radiation of embryophytes was not life's initial colonization of the land surface by phototrophs but rather a much younger event that transformed terrestrial and freshwater ecoystems established billions of years earlier.[16] With this in mind, we can view the 407.1 $\pm$ 2.2 Ma (^{40}Ar/^{39}Ar age) Rhynie chert[17]—biostratigraphic age of Pragian-?earliest Emsian[18]—as one of the Scotland's geological jewels (e.g.,[19,20]), not only as a chronicle of early land plant evolution (e.g.,[21–26]), but also as a record of cyanobacterial accommodation to the changing face of terrestrial habitats.

Cyanobacteria are relatively common and diverse elements of the Rhynie biota (e.g.,[27–29]) frequently encountered in ever wet or episodically flooded parts of the local environment.[30] Several colony-forming coccoidal taxa have been reported,[28,31–35] but filamentous cyanobacteria are more common. Simple filaments, preserved as trichomes or cylindrical sheaths, were first recognized by Kidston and Lang,[23] who

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described *Archaeothrix contexta* and *A. oscillatoriformis,* which closely resemble living oscillatorian cyanobacteria. The latter species is either occasionally[35] or more commonly[27] observed within necrotic lesions in axes of Rhynie plants. Several other described cyanobacteria have also been referred to the Oscillatoriales,[34–37] including one suggested to have developed a symbiotic association with the embryophyte *Aglaophyton majus*[38] and interpreted as an on-again-off-again association linked to episodic flooding. However, whether or not the plant axes were alive when colonized remains an open question.

The Nostocales, distinguished among cyanobacteria by both their morphological complexity and high diversity, have also been observed in the Rhynie cherts. Croft and George[39] discovered true-branching cyanobacteria in a single minute chip of chert measuring 1mm in thickness and nearly 5 mm across. They erected two species, *Langiella scourfieldii* and *Kidstoniella fritschii,* which they placed within the Stigonematales, as well as a third, *Rhyniella vermiformis,* which they excluded from this clade because evidence of branching was, at best, incomplete. Traditionally, cyanobacteria with true branching were divided into two classes, the Nostocales and Stigonematales, based on whether filaments were uni- or multiseriate. Molecular phylogenies, however, show that the stigonematalean clade nestles within the Nostocales.[40] Recently Krings[29] described *Rhystigonema obscurum* from the Rhynie chert, a multiseriate, true-branching form, placed with confidence within the Stigonemataceae.

Here we describe another population of nostocalean cyanobacteria, discovered in Rhynie chert thin sections from the collections of Sorbonne University, Paris. In order to address the difficulties of reconstructing thallus morphology of these minute organisms, we use light microscopy and confocal laser scanning microscopy (CLSM). Similarities between these new fossils and those described by Croft and George[39] prompted a reinvestigation of their original material. Our study shows that the newly discovered fossils and the three species described by Croft and George are part of a single fossil species, belonging to the Hapalosiphonaceae (Nostocales) that thrived in soils, freshwater, and hot springs, much as their extant relatives do today.[41]

RESULTS

We studied fossils from thin sections of samples collected from the famous Rhynie chert in Scotland. The cyanobacteria reported here occur as part of a ground cover comprising amorphous material (mucilage), small tubular filaments of possible cyanobacterial affinity (possibly an oscillatorian mat) and plant microdebris (Figures 1A, 2B, S1A, and S2B). The newly discovered fossils (Figures 1, 2, 3, 4, S1, and S2) resemble Rhynie cyanobacteria described decades ago by Croft and George[39] (Figure 5). Comparison with the three species described by these authors resulted in a revised view of cyanobacterial diversity and taxonomy in the Rhynie chert. The cyanobacteria are represented by uniseriate (Figures 1B–1I, 2, 3, 4, and S2B–S2E) or lightly multiseriate trichomes (asterisks in Figures 1B, 1H, 2F, 2I, and S2F). Associated short uniseriate trichomes resemble hormogonia (i.e., structures specialized for dissemination) produced by some nostocalean cyanobacteria (Figures 1C, 2B, 2D, and S2D). Trichomes show true branching with a differentiation of prostrate and erect portions (Figures 1F, 1G; 2A, 2E, 2F, 3, and 4B). 3D movies obtained from the stack of CSLM images unequivocally show that what is observed in light microscopy corresponds to true branching and not to an overlap of filaments (Figure 4B, Videos S1 and S2). As detailed below, the combination of characters in our new fossils leads us to consider them as part of the same fossil species divided into three by Croft and George and to attribute all of them to *Langiella scourfieldii* emend.

Systematics

Description: Filaments up to 175 μm long, densely packed (Figures 1A, 1B, and S1B), and commonly interlaced with one another (Figures 1B, 2B, 4A, and S2C) or occasionally more isolated (Figure S1C). They are mainly uniseriate filaments with true branching (Figures 1F, 1G; 2A, 2E, 2F, 3, and 4B). Main axes can be lightly multiseriate (Figures 1B, 1E, 2D, 2F, and S1E), with rounded cells wider than high, 10 to 12.5 μm in maximum dimension. Branches are uniseriate; constituent cells higher than wide tend to be smaller and barrel-shaped (5 μm in their maximum dimension) (Figures 1E, 3A, 3B, and 4B). No obvious evidence of heterocysts or akinetes, although it is hard to rule out little differentiated heterocysts (yet, no cell wall thickening that often occurs to protect heterocysts from atmospheric oxygen was observed). No sheath has been observed around the filaments. Rarely, filaments seem to show a multiseriate end (Figures 2A and 2D), which could reflect incipient branching. Small rounded cells, 5 μm in their larger dimension, occur along some filaments (Figures 1C, 1D, 2F, 4B, and S2D).
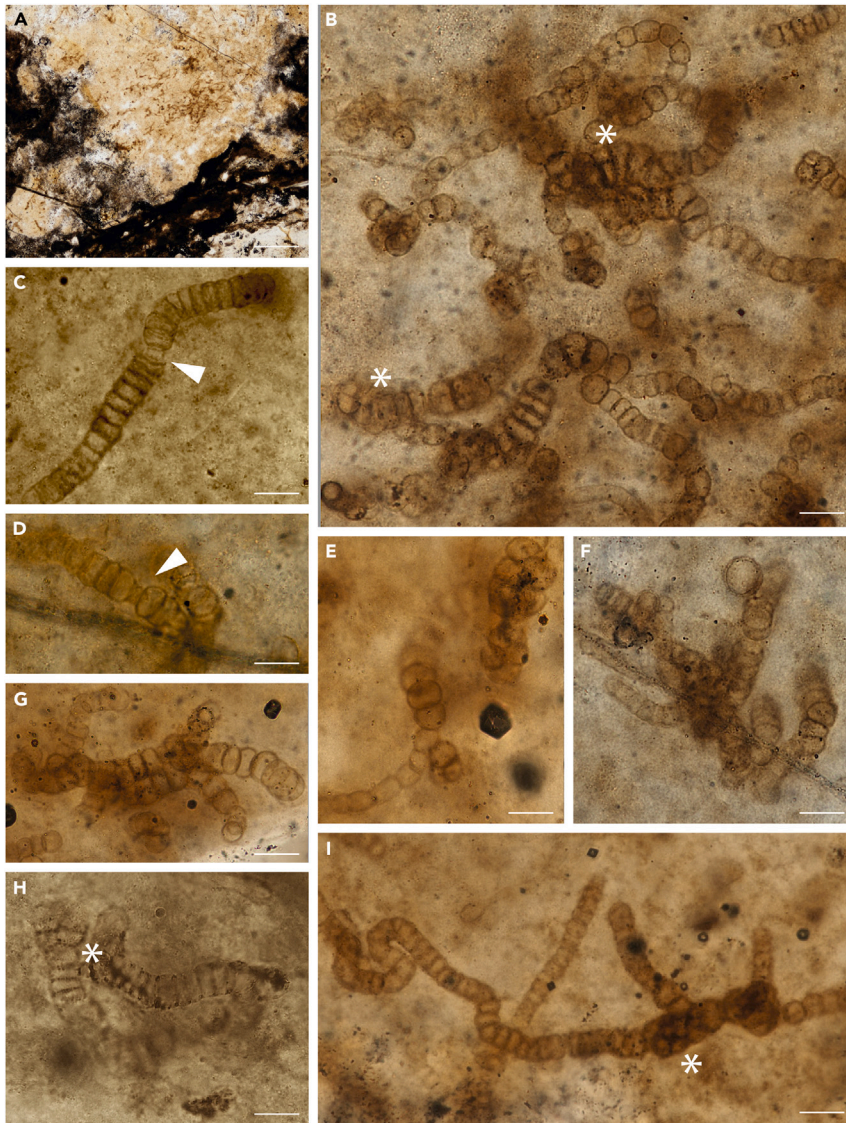
Figure 1. Light microscopy images of _Langiella scourfieldii_ (Croft and George 1959, emend. Strullu-Derrien and Knoll)
(A) Cyanobacteria as part of a ground cover; dark areas represent plant remains. (B–I) Filaments are mainly uniseriate, sometimes slightly multiseriate (asterisks in B and H). Filaments densely packed and interlaced with one another (B). Short uniseriate trichome resembling hormogonia (C). Necridia visible on some of the filaments (arrow in C, D). A–H: from thin section SU.PB. 2023.0.1.2.8; I: from thin section SU.PB. 2023.0.1.2.9. Scale bars: 0.31mm (A), 20 μm (F, I), 14 μm (C, D, E, H), 18 μm (B, G).

Taxonomy

Division: Cyanobacteria.

Order: Nostocales Borzi[42]

Family: Hapalosiphonaceae Elenkin[43]

Genus: _Langiella_ Croft and George,[39] emend. Strullu-Derrien and Knoll.

Emended diagnosis: Thallus heterotrichous, with true branching; main axis uniseriate or lightly multiseriate, showing rounded cells; branches generally uniseriate, with smaller, commonly barrel-shaped cells.
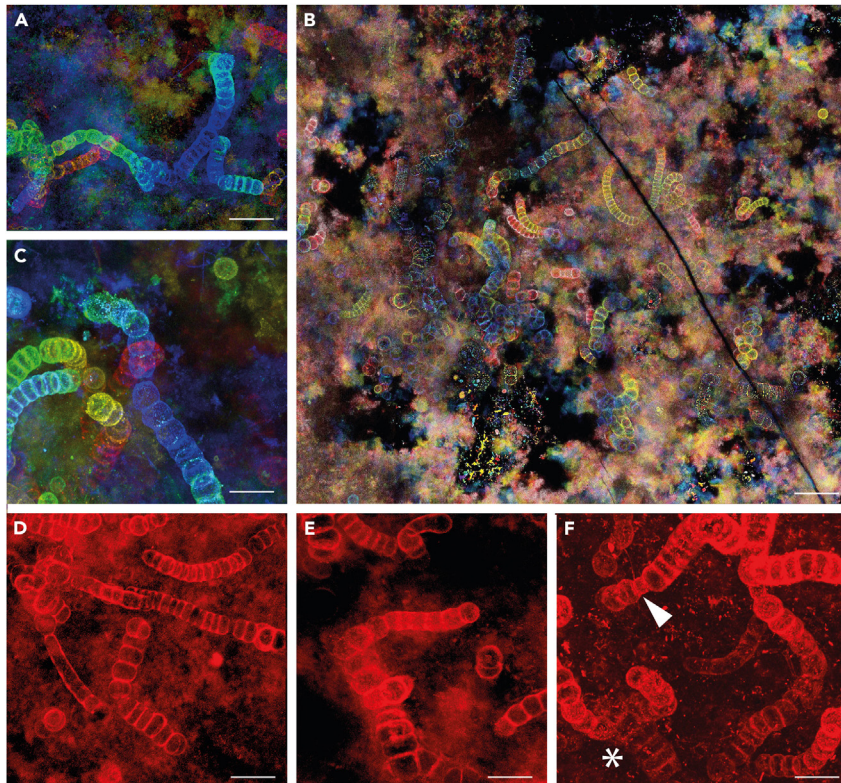
Figure 2. Confocal Laser Scanning Images of *Langiella scourfieldii* (Croft and George 1959, emend. Strullu-Derrien and Knoll)

False-colored for z stack depth (A–C). Cyanobacteria as part of the ground cover (B) showing branching (A, C–F). Necridia visible on some of the filaments (F). From thin section SU.PB. 2023.0.1.2.8. Scale bars: 25 μm (A), 50 μm (B),16 μm (C, F), 20 μm (D), 14 μm (E).

Smaller rounded cells (necridia) present on some filaments. Short uniseriate trichomes resemble hormogonia.

Type species: *Langiella scourfieldii* Croft and George,[39] emend. Strullu-Derrien and Knoll (Figures 1, 2, 3, and 4).

Species: *Langiella scourfieldii* Croft and George,[39] emend. Strullu-Derrien and Knoll.

Emended diagnosis: As for the genus and rounded cells in main axis 10–12.5 μm in maximum dimension and cells in branches ca. 5 μm in maximum dimension.

Epitype: *hic designatus*, Pôle Collections scientifiques et patrimoniales, Bibliothèque de Sorbonne Université: assemblage from thin section SU.PB. 2023.0.1.2.8. Figures 1A–1H, 2, 3, and 4.

Holotype: *Langiella scourfieldii* Croft and George[39] (Bulletin of the British Museum (Natural History) Geology 3: 342–343) – Type illustrated in Plate 41, Figure 10. Specimen *pro parte* NHMUK V32409!

= *Kidstoniella fritschii* Croft and George[39] – Type illustrated in Plate 41, Figure 9. Specimen *pro parte* NHMUK V32409!

= *Rhyniella vermiformis* Croft and George[39] – Type illustrated in Plate 41, Figure 11. Specimen *pro parte* NHMUK V32409!

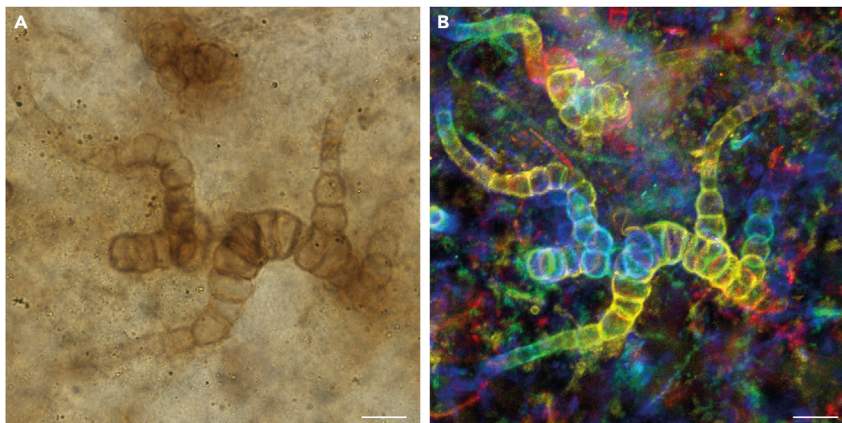Locality: Rhynie, northwest of Aberdeen (Scotland, UK)

Figure 3. Light microscopy (A) and confocal laser scanning, false-colored for z stack depth (B) images of heterotrichous thallus of *Langiella scourfieldii* (Croft and George 1959, emend. Strullu-Derrien and Knoll)
Branches with cells tending to be smaller and barrel-shaped. From thin section SU.PB. 2023.0.1.2.8. Scale bars: 12 μm (A), 14 μm (B).

Age: Early Devonian (407.1 ± 2.2 Ma)[17]

Other materials examined that are attributable to *Langiella scourfieldii* Croft and George, emend. Strullu-Derrien & Knoll are the specimens considered and illustrated here (Figures 1I and S2) from assemblages in thin section SU.PB. 2023.0.1.2.9, also housed in the same repository as the epitype.

Epitypification is necessary because the holotype is ambiguous: there are no evident sheaths, heterocysts, or akinetes as stated by Croft and George, the size of the filaments is not correct (likely Croft and George included in the measurement what they interpreted as a sheath). The epitype shows the form of the filaments and the connection of filament types more clearly. 3D movies also equivocally show true branching and not overlapping of filaments. Necridia were not reported (these were interpreted as heterocysts by Croft and George).

Our nomenclature follows the Shenzhen Code.[44] Since the three species names were simultaneously published, they compete for priority. In accordance with Article 11.5 we, therefore, choose one of these to establish priority. We choose *Langiella scourfieldii* because it is the first specimen named in the original publication by Croft and George.[39] Our higher level classification follows Cavalier-Smith[45] and Komárek et al.[40]

DISCUSSION

Comparison with the three species described by Croft & George[39]

The newly discovered fossils resemble *Langiella scourfieldii* Croft and George in most of their characters (Figures 5C and 5D). Croft and George[39] reported that the compacted cells of the basal system of *Langiella scourfieldii* are generally broader and thicker-walled than those composing the erect filaments. Reinvestigation of the original specimens shows that it is impossible to confirm that statement. Croft and George[39] (p. 341) themselves wrote that "Examination and illustration of the plants are rendered difficult by the thickness of the chip and the amount of debris contained in it … Moreover, the cell walls often contrast little with the matrix and the outlines and visibility of the cells and of the sheaths vary considerably with the setting of the mirror and the width of the illuminating cone". Our specimens show variations of size and shape in some of the erect branches (Figures 3 and S2F), similar to a pattern observed by Croft and George in filaments 2 and 5 of their figure 10 of plate 41. We remeasured the specimens illustrated plate 41 figure 10 in Croft and George's publication (Figures 5C and 5D). The diameter of the prostrate axes is up to 26 μm and not 40 μm; the diameter of the prostrate cells is up to 12.5 μm and not 20 μm; the maximum width of the trichome is 12.5 μm not 16 μm as reported in the publication. These new measurements fit well with measurements of the newly discovered specimens.

Croft and George[39] reported the occurrence of a sheath around the trichomes. Our reinvestigation based on light microscopy shows that no sheath is present, although filaments may have a variably thin coat of
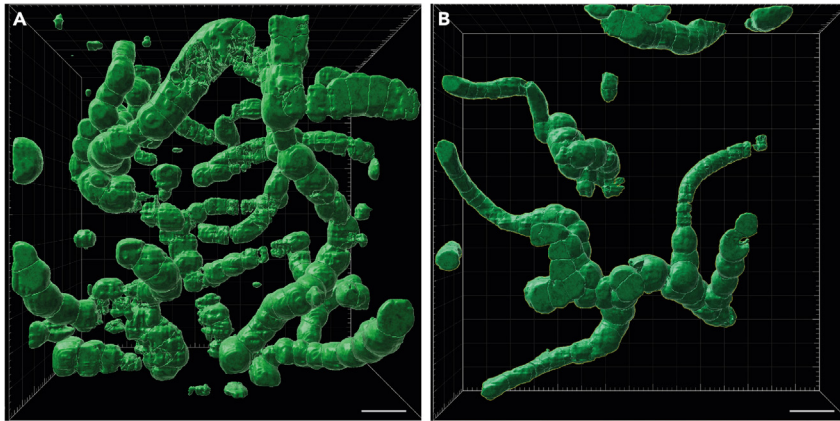
Figure 4. Confocal laser scanning images of *Langiella scourfieldii* (Croft and George 1959, emend. Strullu-Derrien and Knoll) in one plane from movies
(A from Video S1; B from Video S2). From thin section SU.PB. 2023.0.1.2.8. Scale bars: 15 μm (A), 18 μm (B).

amorphous, mucilage-like organic matter (Figure 5D). Croft and George[39] also identified a limited number of distinctly small cells in their population and interpreted them as heterocysts. Nostocalean cyanobacteria are well known for their capacity to differentiate distinct cell types along filaments. Akinetes, specialized reproductive structures, are commonly larger than surrounding vegetative cells and can be spheroidal or elongated along the axis of the filament or branch. Akinetes also commonly have a distinct granular internal structure that distinguishes them under the light microscope. Heterocysts, cells specialized for nitrogen fixation, may also have distinctive morphology, although generally less pronounced than that of akinetes. Heterocysts can be more rounded, more elongate, slightly larger, or slightly smaller than associated vegetative cells. And, like akinetes, they appear as distinctive structures under the light microscope due to their yellowish color and distinctive polar plugs. Of the distinguishing features of akinetes and heterocysts, only distinctive morphology is likely to be recognizable in the fossil record. To this end, we find diminutive cells like those noted by Croft and George[39] in our population (Figures 1C, 1D, and 2F), but suggest that they are necridia (cells whose death, self-organized or by accident, allows division of trichomes[46]) rather than heterocysts. Indeed, while Komárek et al.[47] (Figure 16) noted relatively small heterocysts in extant populations of *Hapalosiphon arboreus*, we know of no heterocysts among living cyanobacteria with a size as small as that of the Rhynie necridic cells.

Our newly described fossils also bear close similarities to *Kidstoniella fritschii* (Figures 5E and 5F), differing largely in the absence of internal contents within cells. The postmortem collapse of cell contents within cyanobacteria is well established from both paleontological and experimental studies (e.g.,[48]), with the complete loss of internal contents a predictable endmember. Therefore, we interpret the differences between cells with and without contents as a taphonomic feature. *Kidstoniella* was also considered distinct from *Langiella* on the basis of occasional branching of putatively erect branches, a character also seen in our newly observed specimens (Figures 1B and 2F). Given that the orientation of branches was strongly affected by burial, it is difficult to ascertain whether or not this feature might reflect branching of the main axis. Croft and George[39] described *Kidstoniella fritschii* as epiphytic; however, the cyanobacteria are not in physical contact with the adjacent plant axis, thus providing little evidence for an epiphytic lifestyle.

The newly observed specimens also show features similar to *Rhyniella vermiformis* (Figures 1B, 1G, 1F, and 1I). Croft and George[39] described this single specimen as a distinct species, but it is more parsimoniously interpreted as a fragmented specimen of *Langiella scourfieldii*. Alternatively, modern hapalosiphacean cyanobacteria commonly reproduce by releasing Oscillatoria-like hormogonia,[49] providing another possibility for interpretation. Although Croft and George[39] reported a multicellular tip of the *Rhyniella* trichome, we have not been able to confirm this.

In combination, these observations lead us to consider the newly discovered fossils as part of the same fossil species divided into three by Croft and George[39] and to attribute all to *Langiella scourfieldii* emend. This conclusion is consistent with the close physical proximity of all three taxa, originally described.
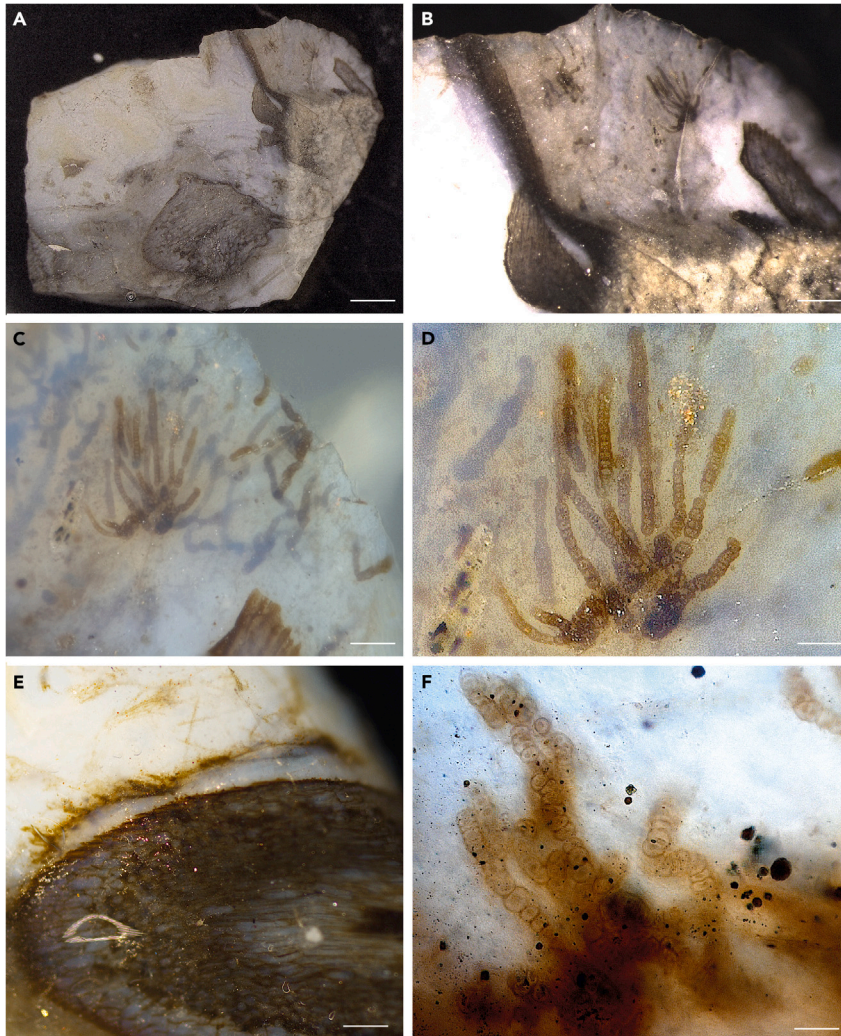
Figure 5. Light microscopy images of cyanobacteria from the chip studied by Croft and George (1959)
(A) Overview of one face of the chip.
(B) Higher magnification of the upper right part of image A.
(C) *Langiella scourfieldii* (holotype) on the left and *Rhyniella vermiformis* (holotype) on the right.
(D) Higher magnification of *Langiella scourfieldii* (holotype).
(E) From the other side of the chip: *Kidstoniella fritschii* (holotype) close to the plant axis but without physical contact.
From specimen NHMUK V32409. Scale bars: 0.55 mm (A); 210 μm (B, E); 87 μm (C); 44 μm (D); 22 μm (F).

Phylogenetic relationships of the fossil cyanobacteria

Croft and George[39] assigned two of their three described species to the Stigonemataceae. True branching unambiguously places the augmented microfossil population within the Nostocales, but the uniseriate to modestly multiseriate main axis and multiple uniseriate branches suggest placement with the Hapalosiphona-ceae, another family within the order. As noted above, the single simple trichome ascribed by Croft and George[39] to *Rhyniella*, is readily interpreted as a fragmental specimen or hormogonium from the same population.

Several hapalosiphonacean genera are common in non-marine environments, including *Fischerella, Hapa-losiphon, Westiella,* and *Westiellopsis,* and as Komarek et al.[40] stress—as did Geitler[50] before them—it can be challenging to differentiate among these genera on the basis of morphology alone, especially in the fossil record, where features such as cell ultrastructure are not preserved. For this reason, we have confi-dence in the family relationships of *Langiella scourfieldii* emend but refrain from trying to ally it to any particular extant genus.

Nostocalean cyanobacteria have the capacity to differentiate cells specialized for either nitrogen fixation (heterocysts) or reproduction (akinetes). Our population does not unambiguously exhibit either of these cell types, but it bears mention that such cells occur only rarely in some extant taxa and, if not clearly distinct in terms of morphology, could be hard to distinguish in fossils. This absence does not influence our systematic interpretation, as membership in the *Hapalosiphonaceae* is demonstrated by their heterotrichous habit and uniseriate branches. In fact, cell differentiation in nostocalean cyanobacteria is induced by environmental triggers and so will not be present in all populations. Akinete differentiation has been related to a number of triggers, including phosphorus deficiency,[51,52] and heterocyst development commonly reflects nitrogen starvation.[53] We have no independent insights into nutrient status in the Rhynie environment, but the abundance of decaying vegetation is consistent with the hypothesis that local cyanobacteria were not nutrient stressed.

Importance of cyanobacteria in early terrestrial environments

Our new fossils, along with those described by Croft and George,[39] clearly document distinctive, morphologically complex Nostocales in wet soils, warm springs and/or episodically inundated soils of the time. And, as noted above, further evidence of Rhynie nostocaleans has been provided by Krings,[29] who described clearly multiseriate populations similar to those of extant *Stigonema*. Loron et al.[54] also provide an illustration of a multiseriate cyanobacterial filament from the Rhynie chert. In today's world, true-branching Nostocalean cyanobacteria largely inhabit non-marine to brackish water habitats (e.g.,[50]), and so it is not surprising that the oldest currently recognized Hapalosiphonaceae (this paper) and Stigonemataceae[29] occur in the Rhynie chert, our earliest clear view of emerging terrestrial ecosystems. That said, possible stigonematacean fossils have been reported from older marine shales,[55] perhaps most convincingly multiseriate, true-branching filaments found in ca. one billion-year-old rocks of the Mbuji-Mayi Supergroup, Democratic Republic of the Congo.[56] Thus, complex Nostocalean cyanobacteria may well have a much longer, but seldom recorded evolutionary history, as implied by some molecular clock studies (e.g.,[5,7]). Along with an increasing number of oscillatorian and coccoid taxa (e.g.,[28,33–35,37]), these fossils attest to the importance and diversity of cyanobacteria in early terrestrial ecosystems. Despite the emergence of embryophytes as both competitors for space and nutrients and new structural elements in ecosystems of increasing spatial complexity, cyanobacteria continued to thrive both within Rhynie hot springs and in adjacent wetlands as they do today in comparable terrestrial environments. Able to colonize rapidly, nostocalean cyanobacteria thrived in flooded surfaces with sedimented plant debris.

Limitations of the study

These are induced by the preparation of the original material (chip and thin sections) that cannot be modified because of the risk of damaging or destroying the fossil specimens.

Conclusions

Today, cyanobacteria are important players in extant continental hot springs where they contribute to the development of a favorable environment for further biological establishment. The 407-million-year-old Rhynie chert shows that this was true, as well, 407 million years ago. The Nostocalean cyanobacteria here described allow us to reinterpret three cyanobacterial species previously described and recognize all as part of a single fossil species attributable to the Hapalosiphonaceae. This study adds to our knowledge of photosynthetic prokaryotes in early terrestrial environments and shows something of their diversity. Continuing research should include structural and biogeochemical studies at the nanoscale, as well as investigating the possible role these early cyanobacteria played in Rhynie biomineralization and biogeochemistry of their environment, a role well studied in modern hot springs. For now, Rhynie fossils show that the acclimation of non-marine cyanobacteria to terrestrial ecosystems transformed by embryophytes was well underway more than 400 million years ago.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability

AUTHOR CONTRIBUTIONS

C.S.-D. and A.H.K. conceptualized the study, C.S-D collected the data in light microcopy, C.S.-D., M.G., and F.F. collected the confocal data; F.F. created the 3D reconstructions and generated the movies. C.S.-D. and A.H.K. analyzed the fossils, interpreted the results and wrote the original draft. P.K. and C.S.-D. fixed the taxonomy/nomenclature. All authors contributed to the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Microfossils from the Lower Devonian Rhynie chert, UK	- Palaeobotany Collection in the Pôle Collections scientifiques et patrimoniales. Bibliothèque de Sorbonne Université, Paris (France) - Palaeobotany Collections. Natural History Museum, London (UK)	- Thin sections SU.PB. 2023.0.1.2.8 and SU.PB. 2023.0.1.2.9 - Chert chip in cavity slide NHMUK V32409
Deposited data		
Confocal microscopy data	https://www.zenodo.org/	https://doi.org/10.5281/zenodo.7845413
Software and algorithms		
Three-dimensional reconstruction of the microfossils	- Fiji/ImageJ - Cellpose algorithm-Online platform ZeroCostDL4Mic - Imaris (Bitplane, version 9.9.1)	https://imagej.nih.gov/ij/download.html https://github.com/HenriquesLab/ZeroCostDL4Mic https://imaris.oxinst.com/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Christine Strullu-Derrien (c.strullu-derrien@nhm.ac.uk)

Materials availability

All specimens are curated in publicly accessible collections. The newly discovered cyanobacteria were observed in thin sections SU.PB. 2023.0.1.2.8 and SU.PB. 2023.0.1.2.9 from the Palaeobotany Collection curated in the Pôle Collections scientifiques et patrimoniales, Bibliothèque de Sorbonne Université, Paris (France). Previously described cyanobacteria and reinvestigated here are located within specimen NHMUK V32409 housed in the Palaeobotany Collections of the Natural History Museum, London (UK).

Data and code availability

CSLM data have been deposited at Zenodo and will be publicly available as of the paper's publication date. A DOI is reported in the manuscript and listed in the key resources table.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The Rhynie chert is a geological site located about 50 km NW of Aberdeen, Scotland. Cherts in this locality were precipitated from a hydrothermal spring system that episodically flooded adjacent floodplains, not unlike modern sinter deposits in Yellowstone Park, USA, and the North Island of New Zealand.[57,58] An ^{40}Ar/^{39}Ar date of 407.1 $\pm$ 2.2 Ma constrains the age of the hydrothermal system,[17] corresponding to the early Devonian Period (Pragian-?earliest Emsian[18]). The palaeoenvironment is interpreted as a low-energy alluvial plain in which plants grew on sandy substrates or on sinter surfaces close to a river system with associated ephemeral ponds and small lakes.[58]

METHOD DETAILS

The Rhynie chert block from which thin sections SU.PB. 2023.0.1.2.8 and SU.PB. 2023.0.1.2.9 were cut was collected by palaeobotanist Édouard Boureau (1913–1999) who is known for the landmark synthesis *Traité de Paléobotanique* published in four volumes.[59] The thin sections were prepared by Denise Pons in the 1960-1970s using the petrographic standard method. Sections are ca 30–40 μm in thickness and mounted with glass coverslips.

A Nikon Eclipse Ni-E microscope equipped with a DS-Ri2 camera was used at the Muséum national d'Histoire naturelle (Paris) to examine and photograph the microorganisms under transmitted light. The depth of field of the resultant imagery was enhanced through z-stack montages.

An Axio Zoom V16 (Carl Zeiss) was used to acquire an overview image of the whole slide at the MNHN light microscopy facility (CeMIM, Centre de Microscopie et d'IMagerie numerique, MNHN Paris).

CLSM images were acquired at the MNHN light microscopy facility (CeMIM, Paris) using a Zeiss LSM880 confocal microscope (Carl Zeiss) equipped with Airyscan detectors (Carl Zeiss) and plan Apo objective lenses (20X/0,8 NA - dry or 40X/1.30 NA – oil immersion, Carl Zeiss). The quantum efficiency (QE) of the detector was about 50%. An auto-fluorescence signal was collected with an Airyscan head using a 32 GASP detector array in super resolution mode (0.2 airy unit for each elementary detector of the Airyscan head). Images were recorded with pixel dimensions of 90 nm and 16-bit depth mode. Autofluorescence of the samples was excited with the 561 nm laser line and z-stacks (20-60 μm thick) were acquired with steps of 0.25 μm. Images were then processed (Airyscan processing) and visualized with the Zen software (Carl Zeiss). The fluorescence signal from each z-plane was projected onto a maximum projection image. Color-coded z-projections were used to generate a maximum intensity projection displaying the position in z with a color gradient.

For 3D rendering, brightness attenuation in the z-stacks was compensated using the "bleach correction (histogram matching)" tool in Fiji/ImageJ and a gaussian filter was used to reduce background noise. Then, the Cellpose algorithm[60] was applied using the online platform ZeroCostDL4Mic.[61] Generated label images were then imported into Imaris (Bitplane, version 9.9.1) and a virtual surface object was created for each detected cyanobacterium. Movies were generated using the "animation" tool in Imaris.

The confocal dataset will be publicly available on https://www.zenodo.org/ (https://doi.org/10.5281/zenodo.7845413).

Cyanobacteria described by Croft and George[39] were reinvestigated using sample NHMUK V32409 (a single chip) housed in the collections of the Natural History Museum, London. A Leica 250C stereomicroscope and a Zeiss Axio-Imager M2 were used to observe and photograph the cyanobacteria. The depth of field of the resultant imagery was enhanced through z-stack montages.

QUANTIFICATION AND STATISTICAL ANALYSIS

No quantification or statistical analyses were used in this study.