Unearthing Giants: Novel 3D Reconstruction of Carboniferous Lycophyte Roots from Coal Ball Material

CHICAGO BOTANIC GARDEN

2021 Research Experiences for Undergraduates Program

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Introduction

During the Carboniferous period (~350 million years ago), giant arborescent lycophytes towered in swamp-like ecosystems and were dominant among the macroflora. How these massive plants achieved their height, up to 50 meters, has been attributed to their highly branched, root and root-like structures. Studying fossil lycophytes gives insight into the evolution of root and root-like structures and allows for further understanding of how these plants grew and reveals important steps in their evolutionary journey to the surviving lycophytes we see today (e.g., clubmosses, spikemosses, and quillworts). Much of the history of giant aroberescent lycophytes is preserved in Carboniferous coal balls. Coal balls are permineralized peat deposits composed of plant parts preserved in calcium carbonate. One of the most common ways to document the composition of coal balls is through the acetate peel technique. A major limiting factor that accompanies acetate peels of fossil plants is their two-dimensional nature. This limited dimensionality of the fossils restricts what can be gathered and learned about these ancient plants. Furthermore, because coal balls form a sphere around the preserved plant matter, studying plants in this material naturally presents two obstacles:
1) the fossil plants are inaccessible without the use of destructive methods, and even when those methods are used or the plant's anatomy is exposed,
2) the flat, two-dimensional, surface view limits the study of these fossils.

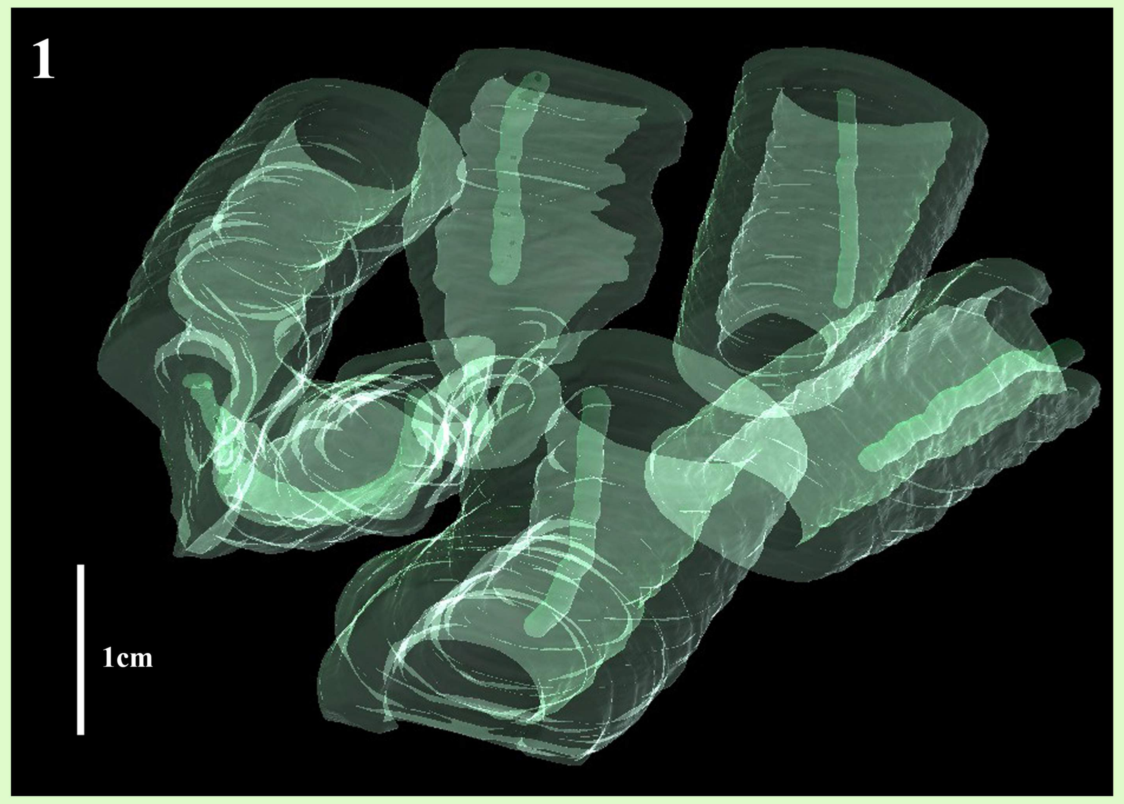
Hypothesis

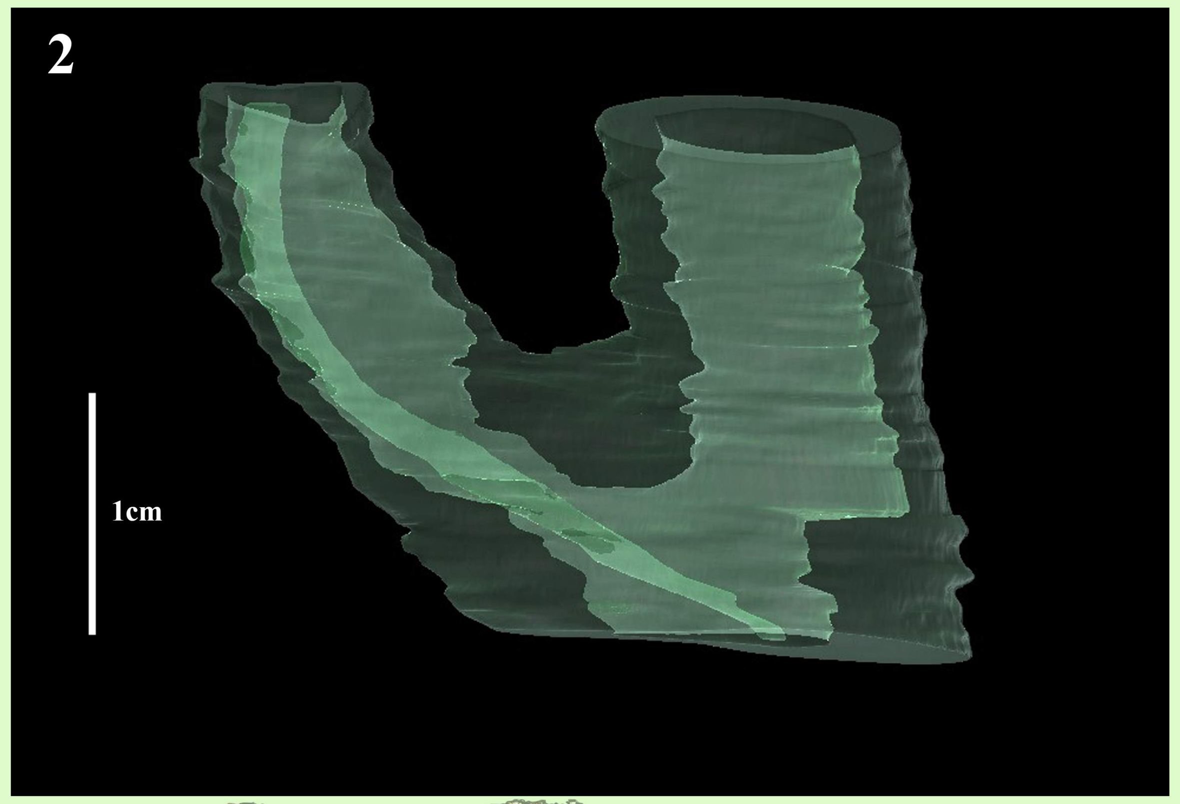
Coal balls preserve plant material to a degree that allows for the study of fossilized plant material and preservation is fine enough for micro-computed x-ray tomography (μCT) reconstruction of that plant material.

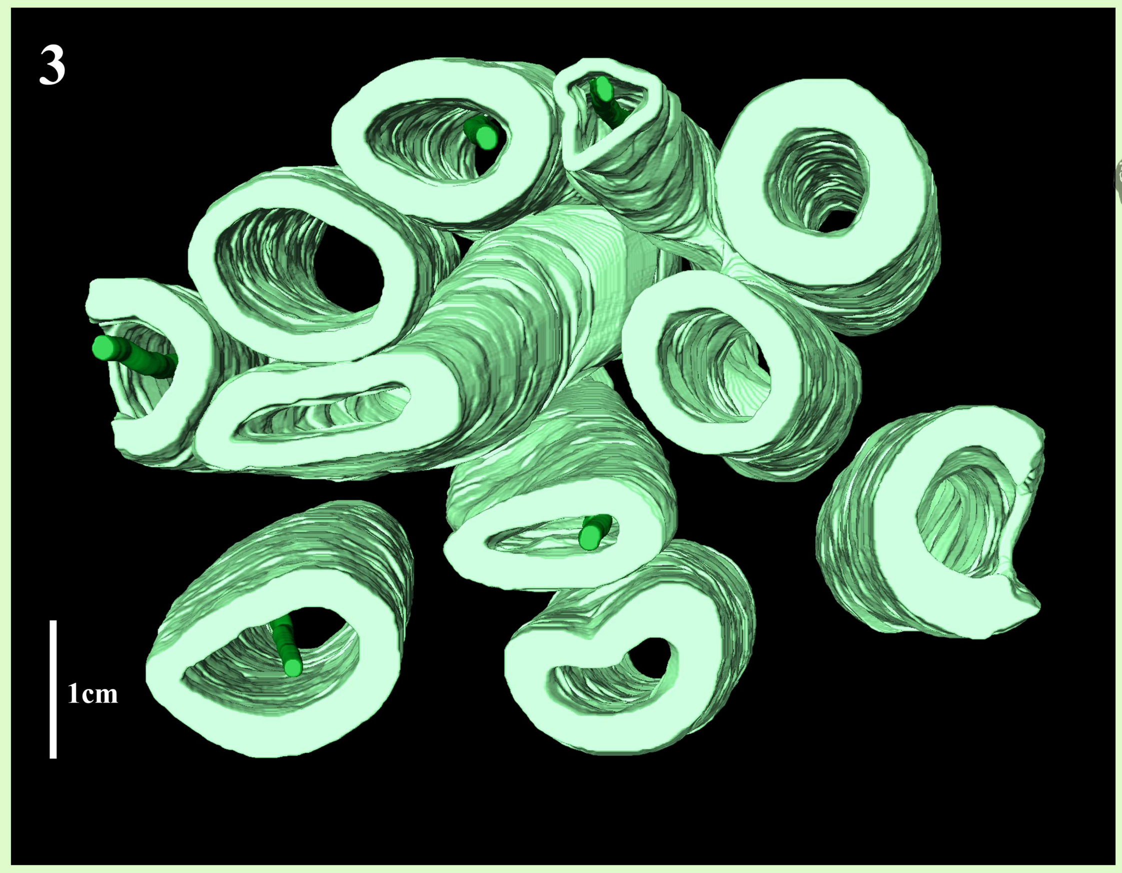
Here, we present the first three-dimensional reconstruction of lycophyte roots (i.e., stigmarian axes) from coal ball material. The preservation potential of coal balls allows for distinguishing different layers of root tissue and even individual cells within these rooting structures. Furthermore, three-dimensional rendering of individual roots is possible to an expectional degree, even at low-resolution scans.

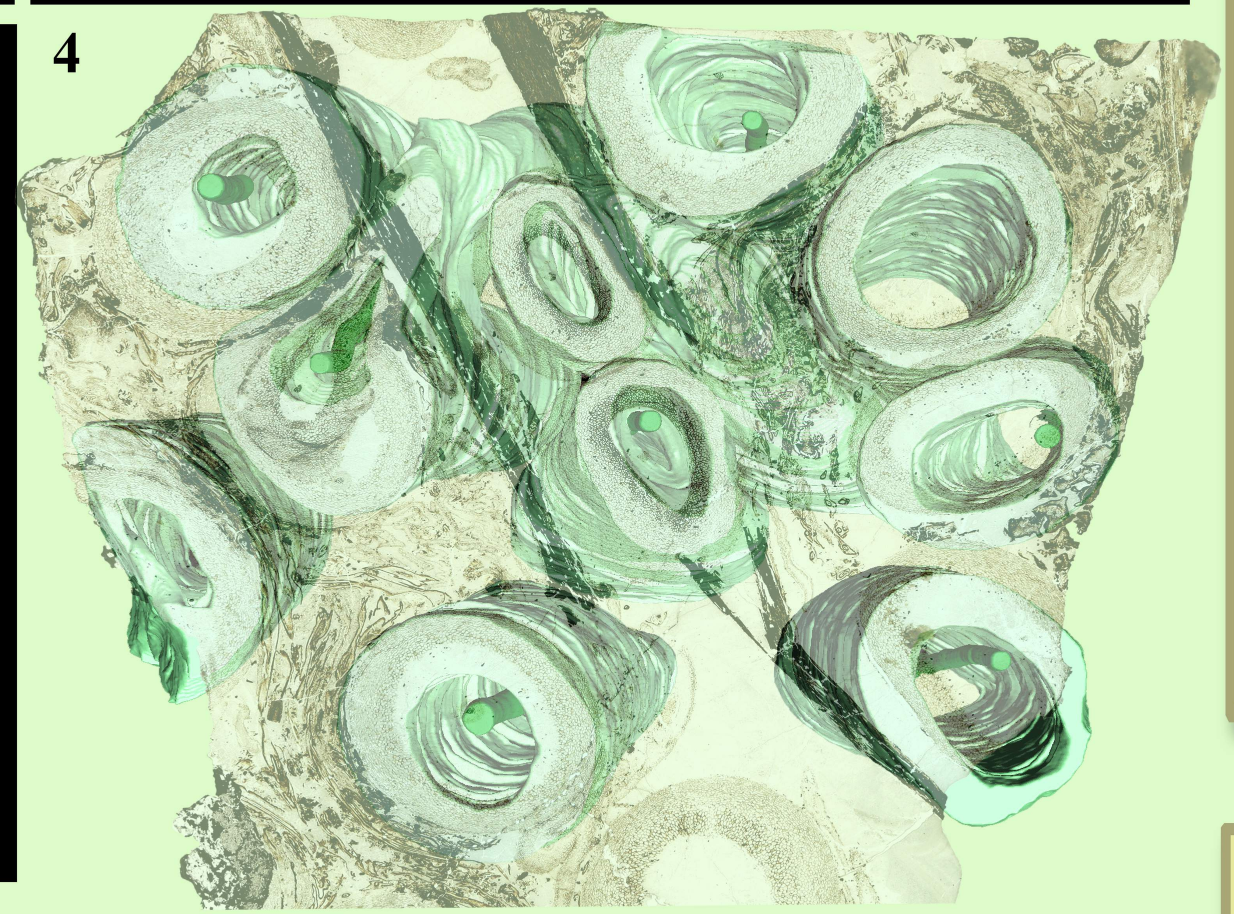
Methods

The fossil material studied comes from the Phillips Coal Ball Collection at the PRI Center for Paleontology, Prairie Research Institue, University of Illinois. Cellulose acetate peels were prepared using standard techniques (Joy et al. 1956), using dilute hydrochloric acid. Reconstruction and cellulose acetate peel image analysis were achieved through ImageJ. Photographs of cellulose acetate peels were collected by using a StackShot, light-stand system and Leica MZ12 light microscope, integrated camera system using LAS software. Three-dimensional images, resolution of 66.63 voxels (μm), were collected using a micro-computed tomogrpahy x-ray scanner (μCT) in the Department of Organismal Biology and Anatomy, University of Chicago. Scans were performed with 1,300 projections at a voltage of 164 (kV) for a total scan time of 29 minutes. The three-dimensional reconstruction was created using Avizo Lite. Resizing the three-dimensional reconstruction used MeshLab and Vectary.com, and 3D print of construction was provided by 3D Print Chicago.









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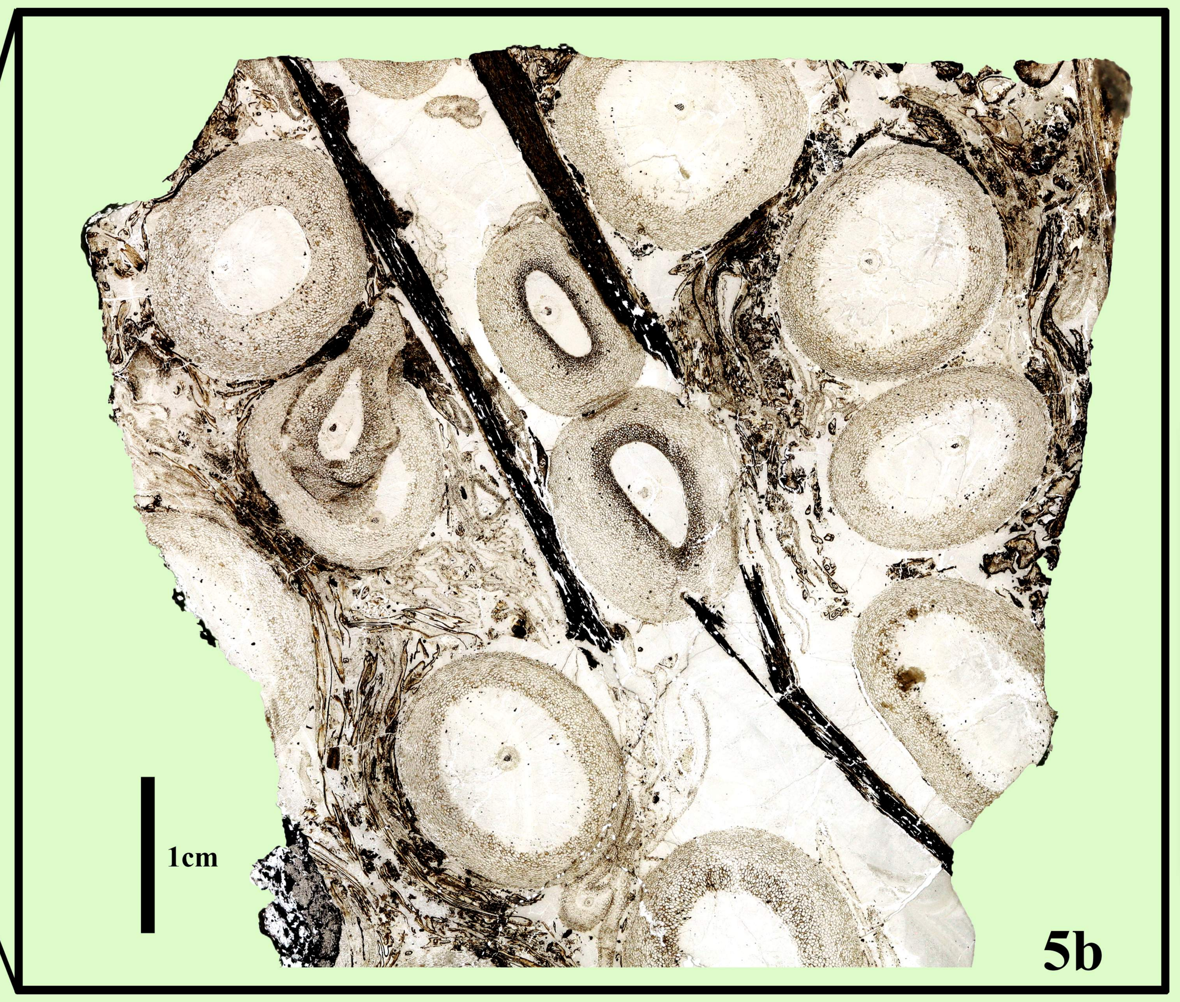

5b

Table 1

Root	Volume (mm^3)
1	2558.26
2	2669.10
3	2736.96
4	3112.44
5	2898.31
6	3435.14
7	2898.31
8	5972.29
9	2969.18
10	5151.60

Results

Fig. 1: Transparent model of 3D reconstruction
Fig. 2: Transparent detail of Root 10, vascular bundle visible
Fig. 3: Complete 3D model of coal ball
Fig. 4: Acetate peel detail superimposed on 3D model
Fig. 5a: Acetate peel of coal ball
Fig. 5b: Detail of acetate peel, filpped
Table 1: Approximate volumes of each root from 3D model

Discussion

Three-dimensioanl visualization of fossilized rooting systems can reveal new information about the morpholoy of rooting systems, as seen in the illustrated branching in reconstructions. The structures rendered from this coal ball are isotomous, dichotomous branching Stigmarian "roots," the expected branching pattern of lycophytes. Stigmarian "roots" are early rooting structures found in Carboniferous lycophytes that are similar to shoots and covered in "rootlets." "Rootlets" are believed to be modified leaves since these structures are shoot-like in that they arise exogenously, whereas true roots arise endogenously. The exact classification of rooting systems in lycophytes is still debated amoing reserachers. Roots and root-like structures evolved twice in vascular plants, once in lycophytes and later in euphyllophytes (ferns and seed plants). This fossil supports the hypothetical model of the stepwise evolution of rooting systems in lycophytes and adds to the broader debate about the evolutionof rooting systems and how we might classify these morphotypes.

In addition to being able to calculate the volume of a structure, other physical characteristics can now be analyzed utilizing three-dimensional reconstructions, like the movment of vascular cylinders along the Stigmarian axes, surface details, and the three-dimensional relationship of roots in the peat. Tracking this movement along the axes and calculations of other physical aspects of these systems has implications to better comprehend how rooting systems might have sequestered and used carbon dioxide. Our understanding of how extant lycophytes interact with carbon dioxide coupled with this new ability to calculate physical traits from fossils can help inform our hypotheses about how extinct lycophytes interacted with carbon dioxide, whether that be cellular respiration or carbon sequestration. This new technique is exciting and informative, but only by employing both new and old techniques can the full picture of extinct lycophytes be revealed.

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