Three-dimensional reconstructions of the putative metazoan Namapoikia show that it was a microbial construction

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Strata from the Ediacaran Period (635 million to 538 million years ago [Ma]) contain several examples of enigmatic, putative shell-building metazoan fossils. These fossils may provide insight into the evolution and environmental impact of biomineralization on Earth, especially if their biological affinities and modern analogs can be identified. Recently, apparent morphological similarities with extant coraline demosponges have been used to assign a poriferan affinity to Namapoikia rietoiogensis, a modular encrusting construction that is found growing between (and on) microbial buildups in Namibia. Here, we present three-dimensional reconstructions of Namapoikia that we use to assess the organism’s proposed affinity. Our morphological analyses, which comprise quantitative measurements of thickness, spacing, and connectivity, reveal that Namapoikia produced approximately millimeter-thick meandering and branch-merging sheets. We evaluate this reconstructed morphology in the context of poriferan biology and determine that Namapoikia likely is not a sponge-grade organism.

In the Late Ediacaran (~550 Ma), microbe-dominated reefs bore witness to the arrival of putative biomineralizing metazoans. By the Cambrian radiation [beginning 538.6 Ma to 538.8 Ma (1)], a time period during which most modern animal phyla first emerged, skeletal reef dwellers were producing framework constructions and effectively engineering their surroundings (2). Today, biomineralizing organisms are responsible for building some of Earth’s largest organic constructions (e.g., the Great Barrier Reef), which is indicative of the outsized impact that biomineralization has had on the planet’s sedimentological, biological, and geochemical makeup.

To understand when, where, and why animals began to biomineralize, as well as to determine the environmental, ecological, and evolutionary ramifications associated with the first biomineralizers, it is necessary to study the earliest skeletal metazoan fossil record. This record comprises four genera from Ediacaran shallow water settings: Namacalathus, Cloudina, Sinotubulites, and Namapoikia (3). Although morphologically simple, these organisms have proven to be enigmatic, and their growth habits, biological affinities, and environmental impacts are the subject of ongoing debate.

With respect to early biomineralization, modes of shell building appear to have varied among the Ediacaran putative biomineralizers. Exactly how, and to what degree, each organism made hard parts remains unresolved. Workers have suggested that Namacalathus, a flexible, goblet-shaped organism, produced a foliated calcitic ultrastructure (4). Conversely, Namacalathus also has been shown to have been lightly calcified (5, 6). Cloudina, a tubular organism made up of a “cup in cup” morphology, was thought to have precipitated carbonate on an organic matrix (5, 7). More recent work, however, has demonstrated that phosphatized Cloudina share a nanoparticulate fabric with extant biomineralizers, suggesting that the organism formed skeletons in the same way as modern animals (8). That said, reconstructions of Cloudina, made to test the assertion that the organism built wave-resistant frameworks (9, 10), revealed that aggregates comprise transported and deformed individuals (11), furthering the idea that Cloudina produced weakly-to-non-biomineralized tubes. Sinotubulites, another tubular organism, also had plastic walls, but ones that were made up of a predominately organic matrix (12).

Recently, researchers have proposed that Namapoikia, a labyrinthine encrusting construction, produced skeletal material by rapidly calcifying an organic scaffold (13). Studies of polished, two-dimensional (2D) transverse and longitudinal sections of Namapoikia suggest a complex interplay between the construction and surrounding microbial growths, with the two life forms competing and, in some cases, repeatedly encrusting over one another (13). Workers have proposed that Namapoikia shares morphological characteristics with Chaetetid sponges and have inferred a biomineralizing pathway that is like that of the extant demosponges Facelleta and Acanthochaeetes (13, 14). On the basis of these similarities, Namapoikia has been assigned a poriferan affinity.

Molecular clock and phylogenetic estimates (15) suggest that poriferans evolved during the Cryogenian Period (720 Ma to 635 Ma). Indeed, the Precambrian fossil record is replete with examples of purported sponge remains. Spicules, biomarkers, and even full body fossils, all older than the onset of the Ediacaran Period, have been described—and debated—by researchers (for a complete review, see ref. 16; also see refs. 17 and 18 for more recent examples of debate). Additionally, by the early Cambrian, calcifying organisms of definitive poriferan affinity were present.

Significance

Animals that build skeletons have an outsized impact on Earth’s biological, geochemical, and sedimentological cycles. To determine when, where, and why metazoan biomineralization first emerged, it is necessary to study the earliest record of skeletal animals. This record is made up of four genera from the Ediacaran period: Namacalathus, Cloudina, Sinotubulites, and Namapoikia. Here, we measure three-dimensional reconstructions of Namapoikia to test the hypothesis that it is a calcifying sponge. We find that Namapoikia lacks the physical characteristics expected of a sponge, or, for that matter, an animal.

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Data deposition: The computational source code used in this paper is available in Github at https://github.com/giriprinceton/namapoikia.

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on Earth, and some sponges, namely the Archaeocyathids, were even responsible for the world’s first framework reefs (2).

It stands to reason that poriferans, having appeared during the Cryogenian and eventually becoming the dominant engineers of the Early Cambrian, may have first evolved the ability to build calcified skeletons during the Late Ediacaran. To test this idea, we seek to determine whether Namapoikia is, in fact, a sponge-grade organism.

Like many other Ediacaran fossils, specimens of Namapoikia lack soft tissue preservation and exhibit signs of diagenetic alteration (e.g., recrystallization). As a result, it is necessary to analyze the gross morphological characteristics of Namapoikia specimens, such as the size, shape, and distribution of structures, in order to describe growth habit, identify possible analogs, and evaluate biological affinity. Since 2D measurements (e.g., made on polished slabs or on bedding planes in outcrop) are subject to misinterpretation and measurement error (11), three-dimensional (3D) data are required for accurate analysis. Unfortunately, Namapoikia skeletons are preserved as carbonate minerals within carbonate rock, precluding isolation via acid dissolution or imaging with traditional, density-sensitive techniques. To address this problem, we utilize serial grinding and imaging, a method which relies on color and texture to differentiate between features of interest (e.g., fossils) and the surrounding matrix (6, 11, 19). The resulting 3D reconstructions, combined with field observations, enable us to quantitatively assess the affinity and paleoecology of Namapoikia.

Geologic Setting, Field Observations, and Reconstructions

In situ examples of Namapoikia are found in a pinnacle reef outcropping on Driedoornvlakte Farm near Reitoog, Namibia (WGS84 UTM 33K 667792E 7358951N; Fig. 1 A and B). These rocks are part of the Kuibis Subgroup (Omkyk Member) of the Neoproterozoic Nama Group. They formed on a carbonate ramp in the northern Zaris subbasin coincident with convergence along the Damara and Gariep orogens (20, 21). There are no direct radiometric dates from the Driedoornvlakte stratigraphy. However, uranium–lead zircon ages from the Kuibis constrain the maximum depositional age to 548.8 ± 1 Ma (20). Additionally, a uranium–lead zircon date in the overlying Schwarzrand subgroup (22) provides a minimum deposition age of 545.4 ± 1 Ma.

The reef at Driedoornvlakte Farm, which is 500 m thick, 10 km long, and dips 25 to 40° to the southeast, sits on Precambrian quartzite. The carbonate ramp was created over the course of three distinct accommodation cycles; in the final stage, just prior to drowning by shales of the Urikos Member, pinnacle buildups developed on the platform margins (21). These pinnacle comprises microbial mounds made up of a combination of columnar or encrusting stromatolites and columnar or massive thrombolites. Both Cloudina and Namacalathus can be found in the fill between microbial buildups and in clinoformal grainstones. Namapoikia is found encrusting the walls and tops of microbial buildups in decimeter-wide neptunian dykes (Fig. 1 D and E), which are shallow fractures in the reef that opened to the seafloor.

Individual Namapoikia can be up to 1 m wide and up to 0.25 m tall (13, 23). Namapoikia specimens comprise regularly spaced, millimeter-thick structural elements, here referred to as “partitions.” These elements split and merge in transverse and longitudinal directions (see Fig. 2 for a visual reference to the spatial conventions used in this work). In certain specimens, partitions have been said to be intersected by perpendicular tabulae (13). A detailed survey of the pinnacle reef at Driedoornvlakte reveals that Namapoikia is rare, occurring in 3% of observed locations (Fig. 1C). To the best of our knowledge, Namapoikia has not been described in other, contemporaneous rocks in Namibia or, for that matter, anywhere else on Earth.

Fig. 1. Field observations. (A) Location of the study area in Namibia. The red square is Driedoornvlakte Farm, while the light gray fill depicts the geographic extent of Nama Group rocks. (B) View of the reef complex at Driedoornvlakte Farm, with the study area marked by the yellow rectangle. (C) Interpolated survey data showing the occurrence of Namapoikia in the reef. The contour interval is 5 m; contours were generated from a drone imagery-derived digital surface model (11). (Bottom) Pie charts depicting the percent of observed locations exhibiting a feature (presence of feature denoted in black). (D and E) Field photographs of in situ Namapoikia. Map coordinates in B and C are with reference to WGS84 UTM 33K.
We reconstruct and measure two specimens of *Namapoikia* (referred to as sample A and sample B) from the pinnacles at Driedoornvlakte Farm. Both samples comprise white, calcified partitions surrounded by a matrix of fine-grained, black micrite fill (Fig. 2 A and B). The fill contains no evident syndepositional sedimentary structures. Blocky calcite spar occurs throughout both samples and appears to replace fill between the partitions (Fig. 2 A and B). Sample A includes a small amount of yellow dolomite (approximately 1.6% by volume, occurring predominately along fracture planes). Sample B is bounded by microbial textures (Fig. 2B). A petrographic thin section of sample A reveals that the fossil and matrix phases are recrystallized.

When reconstructed, partitions meander, branch, and merge in transverse and longitudinal sections with no evidence of tabulae (Fig. 2 C and D) ([SI Appendix, Fig. S1F and Movies S1 and S2](#)). Partition thickness and spacing vary between the two samples. In sample A, partitions have a thickness of 640/1,030/1,393 µm (25th/50th/75th percentiles; this convention is used through the remainder of the text), while the interpartition voids have a thickness of 1,559/2,402/3,547 µm (Fig. 3B). In sample B, partitions have a thickness of 539/831/1,122 µm, and the interpartition voids have a thickness of 735/1,221/1,881 µm. A two-sample Kolmogorov–Smirnov test of partition thicknesses rejects the null hypotheses that the populations are from a single distribution ($P < 0.001$, $D = 0.19$; $D$ refers to the magnitude of the Kolmogorov–Smirnov statistic). The same test on interpartition thicknesses also rejects the null hypothesis ($P < 0.001$, $D = 0.41$).

Notably, both samples A and B contain large, irregularly distributed voids. To test whether the presence of these voids contributes to the differences in partition and interpartition thicknesses between the two samples, representative subvolumes—comprising regularly spaced partitions and referred to as subvolume A and subvolume B—are chosen and measured (Fig. 3D). Within these selected regions, disparities between the two samples persist. In subvolume A, partitions have a thickness of 430/856/1,204 µm, and interpartition voids have a thickness of 1,285/1,811/2,377 µm, while, in subvolume B, partitions have a thickness of 566/781/1,020 µm, and interpartition voids have a thickness of 671/1,005/1,435. A two-sample Kolmogorov–Smirnov test of partition thicknesses rejects the null hypothesis that the populations are from the same distribution ($P < 0.001$, $D = 0.14$), as does a test of interpartition thicknesses ($P < 0.001$, $D = 0.44$).

The median thickness of partitions varies with respect to height in each sample (Fig. 3E). When applying a best-fit line...
Fig. 3. Measurements made on reconstructions of Namapoikia specimens. (A) The size of various skeletal elements (and voids) from four different hypercalcified sponges discussed in this study. For V. crypta, A. seunesi, and G. discoforma, bars represent the range of values as reported in literature (refs. 14, 31, and 40, respectively), sometimes from multiple specimens. In the case of A. perforata (SI Appendix, Fig. S2 A and B), bars represent the 25th and 75th percentile bounds of measured data from a single specimen, while the white dots depict median values. (B and C) Histograms illustrating the thickness of partitions and interpartition voids in samples A and B, respectively. The thickness of interpartition voids can be considered equivalent to spacing between partitions. The blue highlighted area depicts the region shown in A. (D) A comparison of partitions and interpartition void thicknesses from subvolumes selected for their regularly spaced partitions (as described in the text). (E) Median partition thickness, mean subtracted, versus height in both samples A and B. Stratigraphic up is in the direction of increasing height, which also is interpreted to be the direction of growth. For each sample, two best-fit trends are shown as dashed lines: in black, the fit takes into account all data, while, in light blue, the fit excludes outliers. Trends are similar even after excluding outliers, so only the r² of the dotted black line is denoted. (Inset) A box plot showing the range of median partition thicknesses (i.e., as calculated at each sampled height) in both samples.

To the data, the two specimens exhibit different trends, with partitions thinning toward stratigraphic up in sample A and partitions thickening toward stratigraphic up in sample B. In addition to exhibiting low coefficients of determination (Fig. 3E), the absolute change of these trends is small relative to a sample’s median partition thickness and interpartition spacing. In sample A, the change is 117 µm (or 11% and 6% of median partition and interpartition thicknesses, respectively), while, in sample B, the change is 131 µm (or 16% and 13%).
Discussion

Researchers increasingly believe sponges to be a monophyletic group that—in terms of evolution and phylogeny—should be placed at or near the base of the metazoan tree (24). This group diverged in the Cryogenian, and the last common ancestor (LCA) of modern sponges likely was thin walled, with a single layer of spicules (Namapoikia hypothesis is debated, however; see ref. 25 for a counterpoint). From a morphological standpoint, Namapoikia is unlike the proposed LCA or even the thin-walled sponges of the early Cambrian [e.g., the Archaeocaythids (18)]. Namapoikia does bear a passing resemblance to hypercalcified sponges in the rock record, such as Vaceletia crypta and various Inoxoa (SI Appendix, Figs. S1 and S2). However, 3D reconstructions of Namapoikia, with its sheet-like partitions and lack of tabulae or chambers (SI Appendix, Fig. S1 C–F), make it clear that any apparent morphological similarities are superficial (see SI Appendix for a quantitative comparative analysis).

Given the lack of morphological similarities between Namapoikia and other described sponges, it may be argued that the fossil represents a stem group poriferan. While exact poriferan synapomorphies are debated, traits unique to Porifera include a branching aqueous system that moves water from pores known as ostia, through choanocyte-bearing chambers, and out one or more osculi (26). Specimens of Namapoikia lack any remnants of a clear aqueous system, ostia, an osculum, and/or any spongion, tissues, or fibers. As a result, a poriferan assignment for Namapoikia is doubtful.

An additional challenge to a poriferan affinity for Namapoikia comes from the scale of the partitions and voids in observed specimens. While the skeletons of calcareous sponges are not necessarily canal systems, their dimensions are controlled by the scale of living tissue. Both the size and spacing of skeletal elements may be impacted by diagenetic processes (e.g., thickening of calcareous elements at the expense of interpartition void space), the effects of which can be difficult to determine in reconstructions. That said, the combined thickness of partition and interpartition void in Namapoikia—a metric which negates the effects of postmortem diagenesis—speaks to a structure that is anomalously large when compared to other poriferans. Sponges typically produce small-diameter, high-density canal systems to deal with the diffusion processes required for gas exchange and nutrient capture (27). In order to effectively pump water (and overcome resistance/ frictional losses), small, densely populated choanocyte chambers are thought to function as peri-staltic pumps within sponges (28, 29) (see also ref. 30). Structures built by calcifying sponges generally have features that are submillimeter to a millimeter thick. For example, in the recent hypercalcified demosponge V. crypta, which has been proposed to share morphological and biological properties with Namapoikia (13), walls and void spaces have a combined thickness of 650 μm to 950 μm (with the walls being 50 μm thick and tissue-bearing chambers ranging from 600 μm to 900 μm in diameter; Fig. 3A and ref. 14). Gigantospongia discoforma, an exceptionally large hypercalcified sponge (31), has a combined skeletal and canal thickness ranging from 1,000 μm to 2,200 μm (with walls between 300 μm and 1,000 μm thick and tubular canals between 700 μm and 1,200 μm in diameter; Fig. 3A).

In our reconstructions, Namapoikia partition thicknesses range from 200 μm to 1,650 μm (5th to 95th percentiles for both samples combined), and, even when only examining subvolumes that exclude large voids, Namapoikia interpartition thicknesses range from 200 μm to 2,905 μm (5th to 95th percentiles for both samples combined). The combined partition and interpartition thickness of spicules (24) (this prediiming is debated; see ref. 25 for a counterpoint. Considering subvolumes only: 300 μm to 4,452 μm) is up to 2.9 times as large as the combined skeletal and canal thickness of G. discoforma, one of the largest known sponges.

In addition to producing large and widely spaced structures, Namapoikia samples also exhibit significant variance in both partition and interpartition void thicknesses that cannot be explained by selective calcification, even when controlling for irregular voids (Fig. 3 B–D). In contrast, sponges (and indeed, all metazoa) produce regularly spaced and sized structures. For example, measurements of voids in V. crypta and Acanthochaetetes seunei (another proposed Namapoikia analog), both made on single specimens, show a total range of only 600 μm (31). The source of the variation seen in Namapoikia (Fig. 3A). Taken together, our observations of scale suggest that Namapoikia likely created structures too large to be choanocyte bearing (and therefore capable of actively pumping and filter feeding) and too variable to be a regular metazoan construction.

Given our reconstructions, which lack the regularity expected of sponges or, more generally, animals, we suggest that Namapoikia was not a metazoan. Namapoikia’s morphological expression, which can be summarized as widely spaced, meandering partitions that split and merge both transversely and longitudinally (SI Appendix, Fig. S1F), likely lacked the structural integrity to stay upright without external support (i.e., much like trying to stand playing cards up on their sides). As a result, Namapoikia probably had low emergent synoptic relief (Fig. 4) illustrates this proposed characteristic. We suggest that such a morphological expression can be explained by the growth of partially or totally microbiolally mediated structures.

Microbiolally mediated sedimentary constructions, including the stromatolites and thrombolites that make up the reef at Driedoorblakte Farm, are the result of incremental growth, aggregation, and calcification. The morphologies of such bioconstructions partly are controlled by environmental conditions, including water depth, light levels, and sediment flux (32). In the case of Namapoikia, the observed intersample differences in partition and interpartition thicknesses could be explained by variations in local environmental conditions through time and space on the reef. Consistent with our hypothesis is the observation that morphological expressions of microbial constructions often are regional (33). This property would account for why Namapoikia is not found cooccurring with Cloudina and Namacalathus assemblages on other paleocontinents (5, 34, 35).

Both stromatolites—comprising fine laminations—and thrombolites—made up of clogged fabrics—can produce branching forms. Certain thrombolites, such as Favosamaceria cooperi (33), also create calcified vertical curvatures (referred to as “macé- riae” for their likeness to the walls of garden mazes; see SI Appendix, Fig. S3 and Movie S4 for our reconstruction). It follows that a microbiolally mediated construction could produce the morphologies expressed by Namapoikia. Fabrics in thrombolites characteristically are millimeter-to-centimeter thick and much less regular in size than metazoaons. Thrombolites also contain large, irregularly distributed voids like Namapoikia (36). The scale and variance of partitions and interpartition thicknesses, especially within individual Namapoikia specimens, are consistent with these attributes of thrombolites.

There is, to the best of our knowledge, no other exactly equivalent microbiolally mediated structure to reconstruct and compare to Namapoikia. Given the influence that environment has on morphological expression of microbial construction, this lack of formal twin is not surprising.

The fractures in which Namapoikia is found provided a unique ecological niche in which Namapoikia could grow with a distinct morphological expression (Fig. 4). In our model, Namapoikia would, at any given moment in time, appear as a series of protruding ridges, which would respond to changing conditions (i.e., light, nutrient, and/or sediment flux) via migration and branching (Fig. 4 B, i–iii). Namapoikia would exhibit low synoptic relief, and the space between partitions would be filled with baffling cement and/or sediment (Fig. 4), so as to provide support to microbial structures that otherwise would deform or topple easily. Thus, the character of longitudinal and transverse branching is indicative of the shape evolution of microbial ridges (Fig. 4B) and not the result of coalescing metazoan skeletal walls. Simply put, the final expression of Namapoikia represents a collection of multiple, incremental events through time.

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Fig. 4. Diagrams illustrating the proposed model for Namapoikia. (A) Cartoons depicting the presence of Namapoikia in the reef at Driedoornvlakte Farm. Namapoikia grew incrementally in syndepositional fissures, interacting with the thrombolites and enrusting stromatolites that made up the reef. (B) A model of incremental growth, with only several partitions illustrated. (i) Illustration of partitions and void fill at some time 0, with the partitions having low emergent synoptic relief. (ii) A second moment in time, where a partition has grown upward and split and migrated. The locations of splits are marked in red. Transverse migrations and meanders of partitions lead to drift in longitudinal cross-section, such that sheet-like partitions may not be perfectly vertical. (iii) Partitions have both merged and split. The location of the merger is marked in yellow.

Our analyses of Namapoikia demonstrate that, in order to assign organismal affinity of problematic fossils based on morphology, 3D observations are required. Quantitative measurements made on reconstructions can both elucidate the presence or absence of structures for use in identification (e.g., testing for tabulae and/or chambers; SI Appendix, Fig. S1 C–F) and give clues about functional morphology (e.g., the suggestion that Namapoikia grew with low synoptic relief). The 3D data provide insights about basic organismal form and function that cannot confidently be extracted using 2D observations alone.

Materials and Methods

Survey Data. To map the spatial extent of various facies, a detailed survey of the pinnacle reef at Driedoornvlakte Farm was conducted. Using a handheld Trimble GeoXH6000 GPS unit (excluding the external antenna), a total of 1,254 GPS points, arrayed on an orthogonal grid with 10 × 20 m spacing, were collected. At each point, a set of discrete keywords—corresponding to visible sedimentological, lithological, and/or physical characteristics—were recorded. The GPS data were differentially corrected using the GPS Pathfinder Office software package at Princeton University. Differential corrections were made with data from the TrigNet Springbok base station, located 654 km from Driedoornvlakte Farm. The corrected data have a mean horizontal accuracy of 0.596 m (SD = 0.126 m) and a mean vertical accuracy of 0.706 m (SD = 0.256 m).

Next, the corrected data—along with associated field observations—were examined for the presence or absence of Namapoikia. Points with no evidence of Namapoikia were filtered out, and then multiple modeled semivariograms were fit to the resulting empirical (culled) dataset. The best-fit semivariogram (in this case, an exponential with range = 127.69 m, sill = 0.14, and nugget = 0.10) was used to perform indicator kriging, which resulted in a map of the spatial distributions of Namapoikia.

Sample Collection and Serial Grinding. Two Namapoikia-bearing samples, referred to as A and B, were collected at Driedoornvlakte Farm for 3D reconstruction. In the case of sample A, the rock’s location was recorded with a handheld Trimble GeoXH6000 GPS unit, and its field orientation (i.e., bedding plane direction up) was denoted with arrow markings on multiple faces.

Samples A and B both were slabbed and then mounted on to steel plates using epoxy adhesive. Each sample then was serially sectioned and imaged (final ground dimensions for samples A and B: 41.0 × 57.9 × 19.7 mm and 126.9 × 116.8 × 29.4 mm, respectively). Sample A was processed using the Grinding, Imaging, and Reconstruction Instrument (GIRI) at Princeton University (11). GIRI comprises a computer numerical control surface grinder that has been retrofitted with misting, wiping, and imaging stages. The imaging stage is made up of an 80-megapixel Phase One IQ180 digital back equipped with a 120-mm Schneider Kreuznach macro lens. This imaging system is positioned vertically so as to attain a 1:1 reproduction ratio at a resolution of 5.73 µm per pixel. For sample A, GIRI programmatically 1) ground away 30 µm of material from the sample surface, 2) wiped off any excess coolant, 3) took an image, 4) evaluated image quality, and 5) then repeated the grinding process a total of 658 times.

Sample B was processed at Massachusetts Institute of Technology (MIT) using a manual serial grinding and imaging procedure. After removing 100 µm of material with a surface grinder, the sample was placed (polished side down) on an EPSO flatbed scanner, and data were recorded at 600 dots per inch resolution (corresponding to a per-pixel resolution of 42.33 µm). This method was repeated 319 times.

For comparative analysis, an A. perforata specimen (originally collected by Rigby and Senowbari-Daryan (37)) and an F. cooperi specimen were selected for 3D reconstruction. The A. perforata specimen was processed with GIRI, following the same grinding and imaging procedures as for sample A, with the only difference being a smaller step size (i.e., 20 µm as opposed to 30 µm). A total of 1,624 images of the A. perforata specimen were collected, and approximately 1 mm of the sample was preserved and redeposited at the Smithsonian National Museum of Natural History. The F. cooperi specimen also was processed with GIRI, following the same grinding and imaging procedures as for sample A (with a step size of 30 µm). A total of 391 images of the F. cooperi sample were collected.
Image Processing. GIRI outputs data in a high-resolution image file format (IIQ), which must be converted to 16-bit RGB TIFF files before further processing. With the exception of applying the same white balance value to all images, the raw data were not adjusted before conversion. Sample B images, which were created as 8-bit JPEG files, required no additional conversion before processing.

Prior to 3D visualization and analysis, images were segmented into distinct classes (e.g., matrix, calcified elements, blocky calcite, and dolomite). Two different neural networks were leveraged for this classification task. In the case of sample A, a hidden layer neural network—operating on superpixels or pixel values—was generated on the basis of color and texture (SB) was used. For sample B, A. perforata, and F. cooperi, a convolutional neural network was applied.

In all instances, the neural network had to be trained prior to classification. First, a number of representative images—three for sample A, A. perforata, and F. cooperi—and, due to variations in image quality throughout the grinding process, five for sample B were selected. Next, training data were compiled using a series of custom scripts written in Matlab. For sample A, superpixels were calculated for each image, after which a user selected and assigned superpixels to one of a set of predefined classes via a graphical user interface. Upon completion, a collection of statistics (i.e., the mean, SD, and covariance of Red, Green, and Blue channel values, as well as an entropy term) for each chosen superpixel were calculated and stored in a database. The choice of sample B, A. perforata, and F. cooperi, pixels were painted by a user, thereby marking them as belonging to a given class. For each painted pixel, a square neighborhood (11 × 11 for sample B and A. perforata and 33 × 33 for F. cooperi) was extracted and then stored as a TIFF in a directory corresponding to its assigned class. For both networks, training was accomplished by 1) initializing each neuron within the network with a random weight, 2) running training data through the network to produce a prediction, and 3) updating neuron weights (via stochastic gradient descent with momentum) with the intent of improving network accuracy.

Following training, images of sample A, sample B, A. perforata, and F. cooperi were run through their respective neural network to produce probability maps, which then were thresholded to create classified TIFFs. These TIFF files then were loaded into Avizo, a software package designed for visualization and analysis of volumetric datasets. In particular, thickness values were generated using the Thickness Map module within Avizo. The module, which implements local thickness as defined by ref. 39, calculates, at each volumetric pixel (or voxel), the diameter of the largest sphere that can be fit in the object (i.e., partition or void) and includes that voxel.

All raw image data are available upon request. The computational source code used to process data in this paper is located in a public repository at https://github.com/giriprinceton/namapoikia.

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