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## Deciphering the inner structure of *Cycloseris vaughani*

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**Abstract.** Reef-building corals are the primary component of coral reefs and are significant for marine ecosystems. However, currently coral reefs are declining globally due to El Niño, ocean acidification and overexploitation. To maintain coral populations and reduce the impact of ecological threats, it is crucial to understand the structures and formation processes of coral reefs. Large-scale microtomography can reveal the structures of entire coral reefs at the resolution of micrometres, providing an effective and innovative way to study the formation of coral reefs. Here, we use this technique to capture the entire structure of a reef-building coral in the *Fungiidae* from the South China Sea, *Cycloseris vaughani*, a reef-building coral of high ecological and economic value. In this study, we investigate its distinctive skeletal structure and growth rings. Reconstructed results exhibit microporous tunnels in the central mouth area and radiant rib-shaped skeletons surrounding the mouth. Our work promotes an in-depth understanding of coral structure while also providing a novel research approach for the protection of coral reefs. It also shows that large-scale microtomography is an effective method in tracking the growth process of reef-building corals, facilitating the understanding of coral biological characteristics, and filling the gaps in current coral research.

### 1. Introduction

Reef-building corals (Scleractinia) are key coral reef species [1], providing complex three-dimensional (3D) niches for various species [2–5]. However, coral reefs are currently declining globally due to climate change [6–8], especially the intensification of El Niño [9–11], water quality deterioration [12,13] and overexploitation [14,15]. Studies of the biochemical processes governing coral reef ecological health under environmental change are therefore understandably in the spotlight now [16].

The *Fungiidae* (mushroom corals) is an important family of large-polyp stony corals, and *Cycloseris vaughani* is a common species in this group [17]. It is widespread in the Indo-Pacific area from eastern Africa to the west coast of America in shallow waters to a maximum depth of over 100 m. Due to its tolerance of turbid waters, *C. vaughani* usually inhabits lower reef slopes and areas between reefs with soft sediments.

*C. vaughani* is mostly pale mottled brown, usually with a pale-coloured mouth. However, when living in environments with good water quality, it displays multiple colours, such as green and orange, making it an indicator of water quality. There is typically only one polyp in each coral, the largest



among the reef-building corals found so far. The polyp is circular and generally dome-shaped, with a flat undersurface. Costae resembling imperforate corallum walls alternate at the polyp margin. *Cycloseris* species are considered to be the most basal taxa of coral owing to their costal spines and septal dentations, the fossil record of which can be traced back to the Cretaceous.

As the main component of many coral reefs and coral islands, *C. vaughani* is important for the reconstruction of coral reefs around the world and in global marine ecological biological management. Research on the biology and ecology of this coral is, however, still limited at this stage. In light of the distinctive features of its skeleton, we decided to uncover the mystery of *C. vaughani* growth, beginning with a study of its structural biology.

Most current studies of coral structures are based on small samples at low resolution of *Pocillopora damicornis* or *Acropora vaughani* [18–24]. These studies are limited by the tools used: optical microscopes [18], scanning electron microscopes (SEM) [19], and general computed tomography (CT) [20–24]. These techniques do not provide a precise or comprehensive overview of coral structures because they are not capable of achieving the required resolution or scale. In order to study coral growth patterns, traditional micro-CT has been used in several studies. These results produced partial coral growth patterns and limited information about the internal skeleton. However, as micro-CT technology was not mature when these studies were done, the reconstructed images revealed only the exterior of coral samples and could not show the internal pore canals.

Recently, microtomography technology has received increased attention in paleontological biology due to its high resolution, non-destructive nature and large-scale applicability [25–28]. This technique has been able to provide more detailed structural information about fossil fishes, and the samples can be used again after imaging because the process does not destroy their original structure [25–32]. However, this exquisite technique had not been applied in neontological biology. Here, for the first time, we use the latest-generation large-scale high-resolution microtomography (X-ray microfocus computed tomography system) to reconstruct the 3D structure of *C. vaughani*. In this work, we investigate the structural characteristics of a living sample of *C. vaughani*, focusing on its growth rings and home skeleton.

## 2. Materials and methods

### 2.1 Ethics

All coral samples collecting and processing were performed according to the local laws governing the welfare of invertebrate animals and were approved by the Southeast University (SEU) ethical committee.

### 2.2 Specimen collection

The *C. vaughani* in this study was collected from the South China Sea in 2018 (Figure 1). The coral sample was kept whole and housed in our laboratory coral tank, where all conditions simulated its habitat in the South China Sea. *C. vaughani* polyps are brown and shaped like a mushroom approximately 50 mm in diameter and 12 mm high. The coral skeleton is round with a short and deep fossa in the centre, where the solitary polyp grows. Its oral surface is convex and its aboral surface is concave. Thin, sharp ribs radiating from the centre of its oral surface resemble the gills of a mushroom. The coral polyp grows outward from the central mouth area.

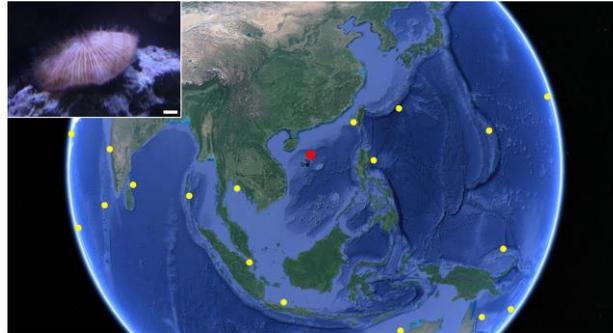


Fig. 1. Distribution of *Cycloseris* in the eastern hemisphere. The *C. vaughani* in this study was collected from the Xisha Islands (latitude 15°40'–17°10' north, longitude 111°–113° east), shown as a red dot at the centre of the map. The yellow dots represent other distributions of *Cycloseris* in the Indo-Pacific area. Scale bar: 1 cm.

### 2.3 Experimental system for maintaining live corals

The *C. vaughani* is cultured in a standard RedSea® tank (redsea575, Red Sea Aquatics Ltd) (Figure 2). The temperature is kept at 25° C and the salinity (Red Sea Aquatics Ltd) is 1.025. The culture system is maintained by a Protein Skimmer (regal250s, Reef Octopus), a water chiller (tk1000, TECO Ltd), three coral lamps (AI®, Red Sea Aquatics Ltd), two wave devices (VorTech™ MP40, EcoTech Marine Ltd), and calcium reactor (Calreact 200, Reef Octopus) etc.

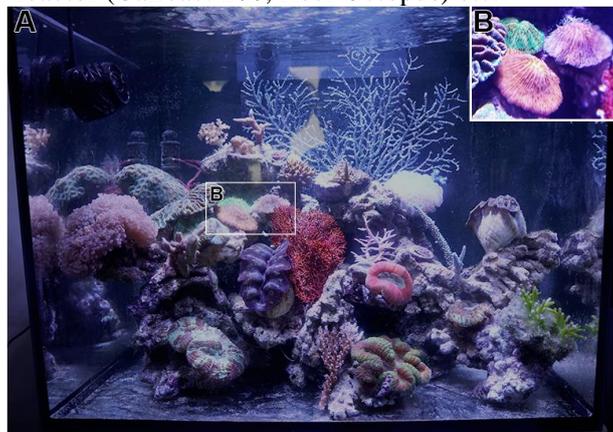


Fig. 2. Experimental system for *C. vaughani*. (A) The standard RedSea® tank where we culture *C. vaughani*. (B) *C. vaughani* samples exhibiting colour variations.

### 2.4 X-ray microtomography

We analyzed a living *C. vaughani* specimen from the South China Sea using 3D models constructed with the 230 kV latest-generation X-ray microfocus computed tomography system (Phoenix v|tome|x m, General Electric Company (GE)) at Yinghua Nondestructive Testing (NDT), Shanghai, China. It was scanned with a beam energy of 120 kV and a flux of 100  $\mu$ A at a detector resolution of 13  $\mu$ m per pixel, using a 360° rotation with a step size of 0.225°. A total of 1,600 transmission images were reconstructed in a 3,900  $\times$  4,000 matrix of 2,000 slices in a two-dimensional reconstruction software developed by GE.

### 2.5 Anatomical reconstruction

The 3D reconstruction was created with the software Datos (version 2.3). The images of the reconstruction were exported from Datos and the restoration was carried out in VGSTUDIO Max 3.2.

### 3. Results

#### 3.1 General morphological structure of *C. vauhani*

Here we reconstruct a monolithic 3D structure of the skeleton of a living *C. vauhani* reef of about 40 cm<sup>3</sup> using large-scale micro-CT (Figure 3). The image completely captures a macroscopic view of the original coral skeleton. The surface bumps, folds and polyp mouth area are visible, which is of great help for the study of the whole coral skeleton (Figure 3D,E,F). Morphologically, *C. vauhani* resembles a flattened bun with a depression at the mouth. On the oral surface, costal spines and septal dentations—ridge-shaped parts of the skeleton—alternate at the polyp margin, protecting the elliptically-shaped central mouth of the polyp (Figure 3B). The aboral surface is flatter (Figure 3C) and covered with veins corresponding to each rib that radiates from the centre to the edge of the coral. No part of the surface of the *C. vauhani* skeleton is smooth: neither the mouth area, the aboral surface, nor the rib-shaped parts of the skeleton. The surface is covered with short, conical spikes (Figure 3D,E,F) that help the polyp secure itself to the skeleton, allowing it to draw its tentacles in and the polyp is able to stick to the skeleton so firmly that wave action cannot pull it off.

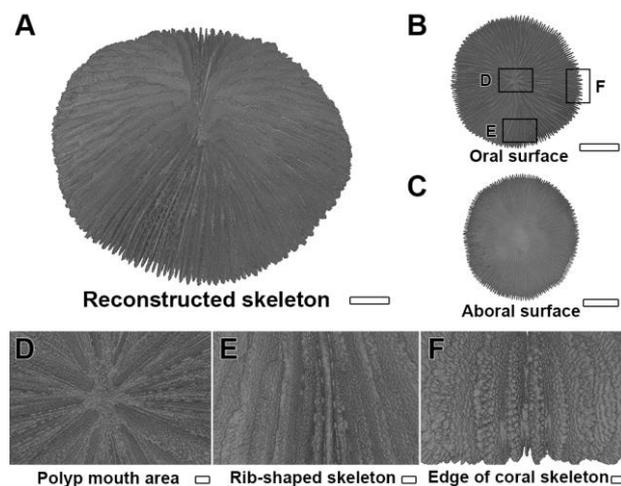


Fig. 3. Large-scale high-resolution micro-CT reconstruction analysis of *C. vauhani*. (A) Reconstructed model image of the whole skeleton of the *C. vauhani* sample, oral surface. (B) Oral surface of *C. vauhani*. (C) Aboral surface of *C. vauhani*. (D-F) Surface texture of coral skeleton. D. Polyp mouth area of *C. vauhani*. E. Rib-shaped skeleton of *C. vauhani*. F. Edge area skeleton of *C. vauhani*. Scale bars: (A) 1 cm; (B,C) 2 cm; (D-F) 1mm.

On the oral side, a crack along the central axis of *C. vauhani* (Figure 4A), divides the whole coral in two. The coral splits along this crack when it engages in asexual reproduction. The mouth of the coral is located at a depression at the centre of the crack. We captured a longitudinal section of the reconstructed coral structure (Figure 4B) along the frontal plane perpendicular to the central axis, showing the internal skeletal structures of the crack and coral mouth area. This section shows the site from which the *C. vauhani* polyp grows, that is, the mouth area at the centre of the crack. The upper part of the crack is narrow and the lower part is wide. The crack is widest at the centre and decreases as it approaches the edges of the coral. A short, ribbed area of skeleton on either side of the crack protect the indentation at the centre from predators such as fish and sea stars. The skeleton in the mouth area has a complex pore canal structure distinct from the dense skeleton on either side.

This high-resolution 3D skeletal reconstruction provides us with more details than ever before of the large polyp of this reef-building coral, revealing more detail than studies of coral skeletons cleaned with bacterial digestion (Figure 5). Our study therefore can serve as a solid foundation for further study of *C. vauhani*.

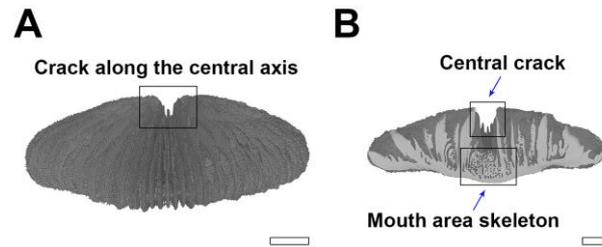


Fig. 4. Image of the crack along the central axis of *C. vauhani*. (A) The crack along the central axis of *C. vauhani* is where the coral splits during asexual reproduction. (B) Longitudinal section of reconstructed coral skeleton, showing the internal structures of the central crack and coral mouth area. Scale bars: (A,B) 1 cm.

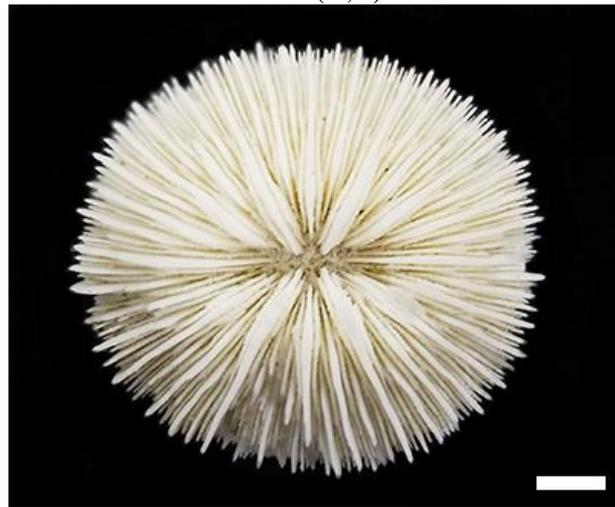


Fig. 5. Coral skeleton of *C. vauhani* prepared using bacterial digestion. Scale bar: 1 cm.

### 3.2 Internal skeleton structure of *C. vauhani*

We examined multiple sections of the reconstructed model of *C. vauhani* to study its growth patterns in microscopic detail (Figures 6–8), revealing the details of its internal structure for the first time.

The mouth area of the *C. vauhani* skeleton is obviously different from the rest of the skeleton. Growth of these two parts differ owing to the pattern of asexual reproduction in *C. vauhani* (Figure 6), in which the coral splits in two along its central axis. The internal skeleton is complex and interconnected, showing a maze-like pattern with complicated pore canals (Figure 7). The pore canals extend from inside the skeleton up through the mouth area and into the crack along its central axis, providing a connection between the interior of the polyp and the sea water. This connection permits the polyp to sense changes in the marine environment around it and make adaptive adjustments in response. This structure therefore contributes to *C. vauhani*'s adaptability to diverse environmental conditions, including its resistance to the acidification of sea water and the rise of sea surface temperatures.

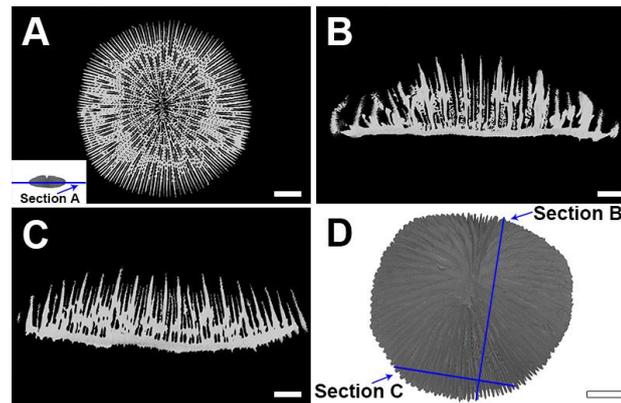


Fig. 6. Three cross-sections and the 3D view that can be observed simultaneously while analyzing the reconstructed skeleton in VGStudio MAX 3.2. (A-C) Three different sections of the reconstructed *C. vaughani* skeleton. (A) Transverse section. (B) Lateral section. (C) Longitudinal section. (D) 3D reconstructed skeleton. Scale bars: (A) 1 cm; (B) 5 mm; (C) 2 mm; (D) 1 cm.

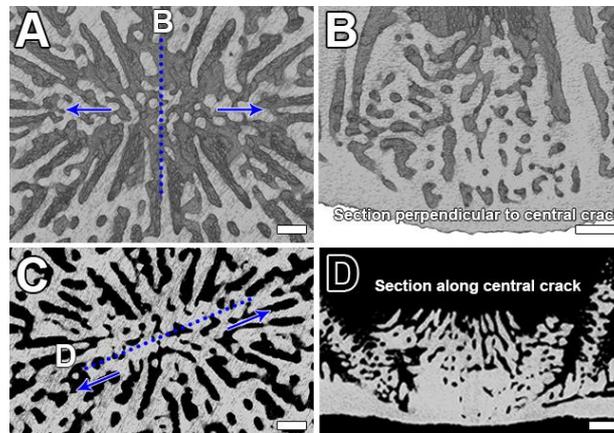


Fig. 7. Mouth area of *C. vaughani*, different sections through 3D reconstructed skeleton. (A) Transverse section of coral mouth area. The arrows show the direction of coral growth during asexual reproduction. (B) Longitudinal section of coral mouth area. The section corresponds to the dotted line in A. (C) Transverse section of coral mouth area. Arrows show the direction of coral growth during asexual reproduction. (D) Longitudinal section of coral mouth area. The section corresponds to the dotted line in C. Scale bars: (A) 2 mm; (B) 1 mm; (C,D) 2 mm.

The skeleton everywhere other than the mouth area is relatively nonporous. There are several interconnected structures between adjacent areas of the skeleton, resulting in multiple pores arranged in rings in transverse section (Figure 8A). The connections between laterally adjacent pores form concentric rings (Figure 8B). *C. vaughani* grows from its centre, so the longer it survives, the more rings it produces, giving us an easy way to calculate its lifespan—the determination of which is quite difficult using other techniques. By observing the number and size of the growth rings in transverse sections, we can effectively compare the lifespans of different individual colonies of this species.

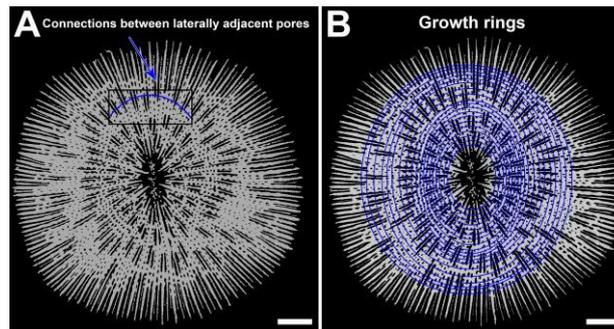


Fig. 8. Visualization of the growth rings of *C. vauhani*. (A) A transverse section of the reconstructed *C. vauhani* skeleton. (B) *C. vauhani* growth rings shown in the section. Visualization of the growth rings on a transverse section of the reconstructed skeleton is an important tool for studying the growth pattern of this coral on the scale of years. Scale bars: (A,B) 1 cm.

## 4. Discussion

### 4.1 Growth patterns of *C. vauhani*

We have captured the micro-scale structure of the skeleton of a living *C. vauhani* reef with an X-ray microfocus computed tomography system, enabling us to be the first to reconstruct a high-precision *C. vauhani* 3D structural model. The resulting ways of visualizing the reconstructed skeleton provide practical results that can lead to innovative and important studies of coral growth patterns.

During the process of coral growth, new layers of skeleton are laid down successively from the centre to the periphery. When the central polyp forms a new layer, the macroscopic result is a growth ring. Therefore, *C. vauhani* has a growth pattern expressed in growth rings at the macro scale, displayed in the form of a large number of tiny holes (pore canals) arranged in rings. The fundamental cause of this growth pattern is that the neighbouring rib-shaped skeletal projections share a linking-skeleton, so that each growth ring can maintain a synchronized growth rate. This reconstructed model is the first to visualize growth ring production in a member of the *Fungiidae* in reef-building coral research and is of great significance to studies of coral growth on the scale of years.

Through our analysis, we found that the skeletal structure of the mouth area of *C. vauhani* is loose and porous, while the costal spines and septal dentations are dense and thick. This finding demonstrates that the coral polyp can adaptively mineralize its skeleton into different structures according to its needs as it grows. In its early years, the polyp first mineralizes the area around its mouth, which skeleton is suitable for the polyp to attachment, providing a good environment for its early growth. After that, during a long growth process, the polyp continues to mineralize skeletal ribs, which are hard and dense, to protect itself from predation. This reflects the adaptability of *C. vauhani*.

In addition, we also found that the skeletal region under the *C. vauhani* mouth is narrow and elliptical, and both this and the rib-shaped projections of the skeleton show extremely obvious asymmetry between frontal plane and profile plane sections (Figures 6–7) due to the bifurcation of *C. vauhani* along its central axis during asexual reproduction. During this process, it grows faster in the directions of the arrows in Figure 7 (profile plane), resulting in the asymmetry of the coral skeleton in the direction of the arrow (profile plane) and the plane perpendicular to the direction of the arrow (frontal plane). Understanding these biological characteristics plays an important role in further research in ecobiology, protection and breeding of *Fungiidae*.

### 4.2 Advantages of X-ray microfocus computed tomography in reef-building coral research

In this research, we present a novel use of high-quality research technology for studying the skeletons of reef-building corals. X-ray microfocus computed tomography systems can non-destructively capture the appearance and internal structure of corals. Compared with traditional biological techniques, such as SEM and grinding sections, it has multiple advantages. First, it does not require

complicated and potentially destructive preparations such as pickling, grinding or fixing, which can easily damage or even destroy coral skeletons. It can even be used directly on living corals. Secondly, traditional biological techniques are unable to visualize the internal structure of reef-building corals, not to mention skeletal growth patterns, which can be easily analyzed using reconstructed 3D skeletons at resolutions up to 13  $\mu\text{m}$  for samples of 40  $\text{cm}^3$ . Finally, since microtomography can capture all coral reef structural information in detail at once, we can observe any position and section of a reef as needed, saving coral resources and eliminating the burden of multiple measurements while deriving a complete analysis of a sample. Our results therefore suggest that microtomography technology can be used to characterize the development and progress of coral skeleton growth, filling in the gaps in current coral studies and making significant contributions to marine ecobiology.

## 5. Conclusions

We captured information about the entire structure of a *C. vaughani* reef with an X-ray microfocus computed tomography system, and visualized its growth rings. The detailed images we captured of its internal skeleton allowed us to visualize the internal features of the coral mouth area for the first time ever, demonstrating the origins of its asymmetrical growth during asexual reproduction. These results led us to a deeper understanding of the skeleton structure and growth patterns of *C. vaughani* and lay the foundation for further structural studies of reef-building corals.

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