Axial canal regulates the processes of coral branching and calcareous transportation in *Acropora*

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ABSTRACT
In *Acropora*, the complex canals in a coral colony connect all polyps into a holistic network to collaborate in performing biological processes, while axial canal is the largest canal amongst the network and distributes at the center of a coral branch. However, previous studies indicated that, in the non-radial symmetry transport system of *Acropora*, axial canal do not play a major role in the transport of hydroplasm, and the action of axial canal in coral growth is still obscure. In this study, we reconstructed six *Acropora muricata* samples by high resolution micro-computed tomography to investigate the growth patterns of axial canals during the processes of new branch forming and truncated branch rebuilding. We found that the axial canal of a new branch is transformed from a calice and the polyps in the new branch are budded from the polyp in the axial canal. Meanwhile, the axial canal can transport the calcareous skeletons to rebuild the tip of a truncated branch, which represents as the change in the diameter of axial canal and calcareous deposition/reduction in it. This work indicate the regulation of axial canal in the growth processes including budding, branching, and mineralising of an *Acropora* colony.

KEYWORDS
Axial canal; reef-building coral; high resolution micro-computed tomography; *Acropora muricata*; calcareous transportation

Introduction

Coral reef is a highly diverse underwater ecosystem characterized by reef-building corals (1). Reef-building corals are key for maintenance of biodiversity and ecological function of coral reefs (2). Among the major reef-building corals, *Acropora* species are responsible for forming the immense calcium carbonate substructure, which is the core of a reef and supports its thin living skin (3). There are various types of canals including calice, axial canal and other internal canals supporting the canal network in a coral colony, supporting the physiological processes in coral growth (4). In the coral canal network, axial canal is a unique and the largest canal along the branch centre in an *Acropora* colony, and its extension reveals growth directions of coral branch. However, according to experiments of the non-radial symmetry transport system in *Acropora* branches, the axial canal do not play a major role in the transport of hydroplasm (5). The role of axial canal in an *Acropora* colony and its structural transformation during coral growth are still obscure, as literature on this subject is scarce (6). The non-transparent skeleton influences direct observation of the distribution, parameter and relationship among canals insight into coral colonies. Experiments with traditional biological methods provided very limited and circumstantial evidence about how axial canal participates in coral growth.

In this study, we used high resolution micro-computed tomography (HRCT) to reconstruct six representative samples of *Acropora muricata* (7), which is common and frequently a dominant species. We created 3D reconstructions of the axial canal and calice inside coral skeleton to obtain information related to coral growth during the processes of new branch forming and truncated branch rebuilding. Both of these processes cover and visualize the pattern regulation of axial canals in reef formation, revealing their shape, size, location, distribution, content and transport characteristics. Thus, the regulations of axial canal in *Acropora* could be determined.

Results

Axial canals in the 3D reconstructions of *A. muricata*

Three-dimensional skeletal structures of six *A. muricata* samples were reconstructed by HRCT, including both surface morphology and the internal structural characteristics, helping us to study the structural pattern of skeletons around the axial canal in the processes of coral branch formation (Fig. 1).

Structure of skeletons in the apical region of coral branch are porous. Complex skeletons form a net-like external surface around the axial canal, while skeletal protrusion with the characteristic of hexactin can be found in the cavity of axial canal (Fig. 1A). In stark contrast, the skeletons around the cavity of calice, the area in which a coral polyp lives, is relatively smooth and nearly nonporous (Fig. 1B). We also obtained three steps of the transformation in skeleton and cavity structure at the tip of a newly formed branchlet (Fig. 1C-E). From step 1 to step 3, the morphological characteristics gradually change from calice-like to axial canal-like along with the growth process. As to the tip rebuilding process in truncated branches, which perform an extreme growth regulation of coral colony, an unusual phenomenon appears in the cavity of axial canal (Fig. 1F-I). The structure of a truncated branch is similar to the normal branch at day 0 (Fig. 1A,F), and when the new tip is rebuilt at the truncated area at day 14, irregularly shaped calcareous skeletons appear in the cavity of axial canal (Fig. 1G). Till day 21, the axial canal in the rebuilt tip is nearly filled with calcareous skeletons (Fig. 1H). However, all those sediments disappear after the rebuilding process at day 28 (Fig. 1I), and the branch shape returns to the status
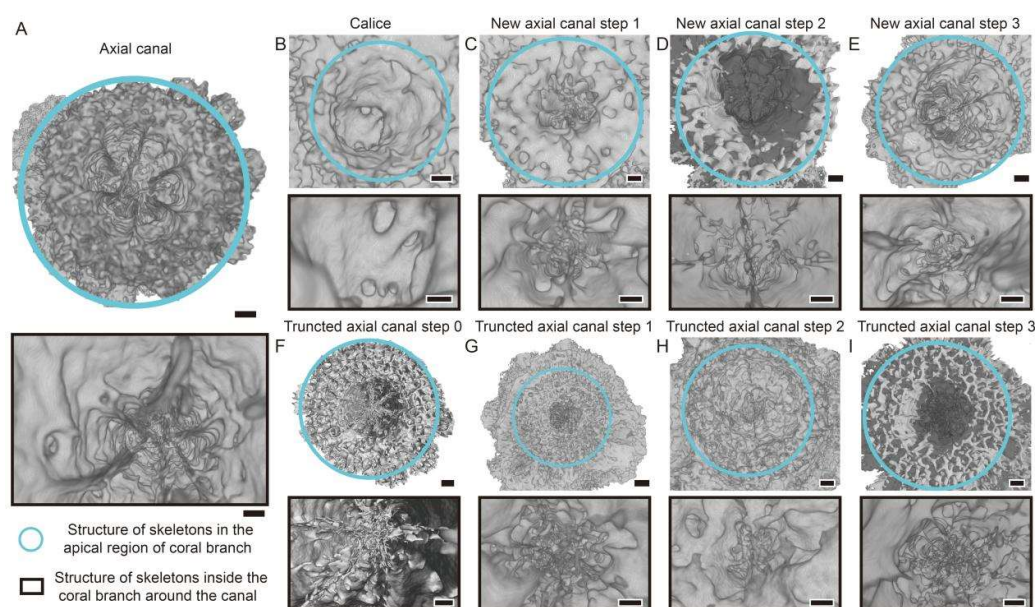
shown in Fig. 1A.

Fig. 1 | Structure of the skeleton surround axial canal during the processes of new branch forming and truncated branch rebuilding in *A. muricata*.
(A) Axial canal in a healthy and mature coral branch. (B-E) A calice transfers into an axial canal of the new branch. (F-I) The rebuilding process of the truncated branch. Scale: 1mm.

Canal reconstructions reveal the role of axial canal in coral growth

In *A. muricata*, the polyp network is complex because multiple canal types are involved. A large amount of internal canals connect the axial canals and all polyp calices into a holistic network to collaborate in performing biological processes in a single coral colony. To illustrate the role of axial canal hidden in skeletal structures, we reconstructed the canals inside sample skeletons to visualize the growth processes with axial canal formation, including colony branching and truncated-branch rebuilding.

In the *A. muricata* branch, the distance among adjacent calices is similar, and the calices circle the axial canals along the growing direction. The distances from each calice bottom to the axial canal are also similar, and complex internal canal links them together (Fig. 2). When an *A. muricata* colony branches, the axial canal reveals the branch growth direction, and the new axial canal appears in the centre of the newborn branchlet (Fig. 2A). The newborn axial canal first appeared at the stage shown in slice 1 (Fig. 2B). At that time, its cross section was approximately circular, like that of a common calice. The distance between the bottom of the new axial canal and that of the old axial canal was approximately 1.5 mm, and the distance to adjacent calices was approximately 2 to 3 mm (Fig. 2B). The shape, location, and distribution of the axial canals were similar to those of the calices at this stage. Coming to slice 2, the cross section of the new axial canal started to present a hexactinal shape like that of an old axial canal, and two new calices emerged close to this younger one (Fig. 2C). At the stage of slice 3, the cross section of the new axial canal approached a hexactinal shape further (Fig. 2D), and a piece of skeleton appeared inside its axial canal. Also, the skeleton outline of the new branchlet tended to be patterned. In slice 4, new

calices appeared between the new and old axial canals, while the connection between the newborn branchlet and the old branch consisted of only a piece of skeleton and an internal canal (Fig. 2E). Up to slice 5, the new axial canal was surrounded by more calices, and its cross-sectional shape was the same as that of the old one (Fig. 2F). The new branchlet separated from the old one and its axial-canal formation was finished. This pseudotime process from slices 1 to 5 shows the transformation of a newborn axial canal from a calice type to a mature state and the birth pattern of a new branchlet. Meanwhile, the metamorphosis from calice to axial canal explains why leading polyps are distributed in *A. muricata* branch tips and indicates the budding process in a new branchlet (Fig. 3).
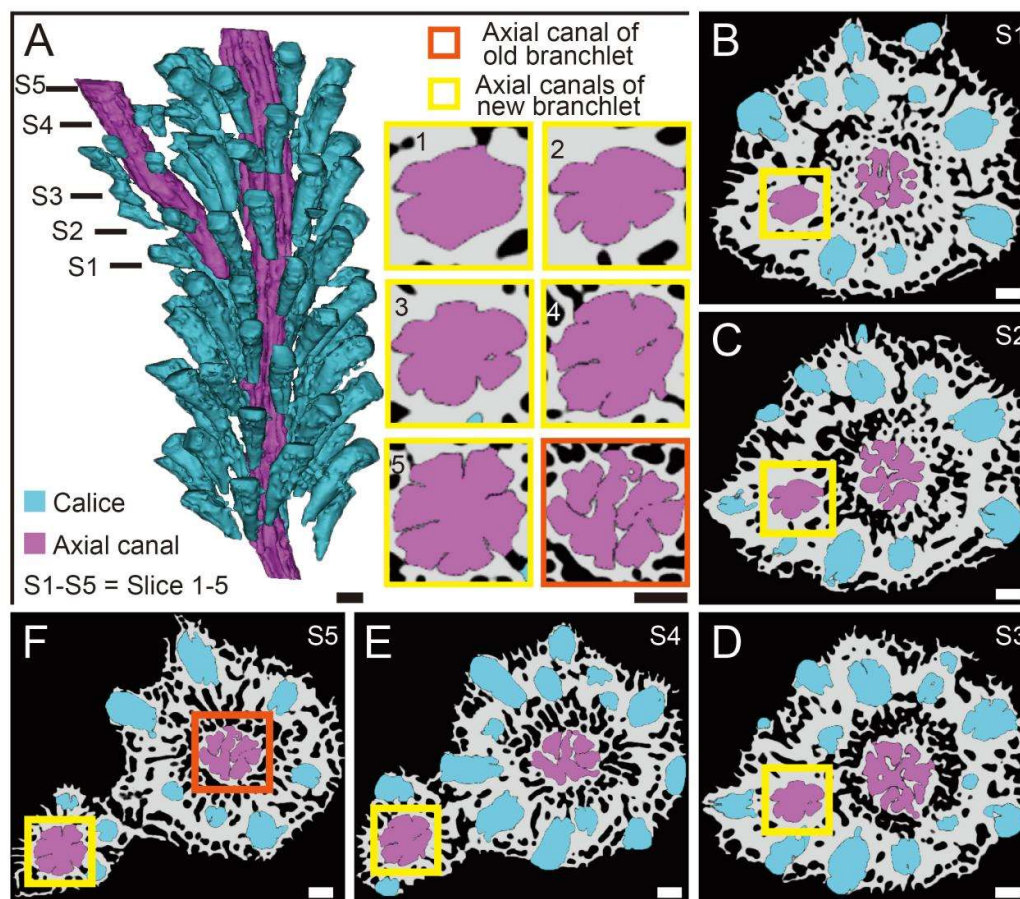

Fig. 2 | 3D canal reconstruction visualizes the axial canal formation during branch-born process in *A. muricata*.
(**A**) An *A. muricata* branch with new-born branchlet. (**B,C,D,F**) The S1-S5 cross-sections reveal the calice-axial canal transformation during the birth of a new branchlet. Scale bars: 1 mm.
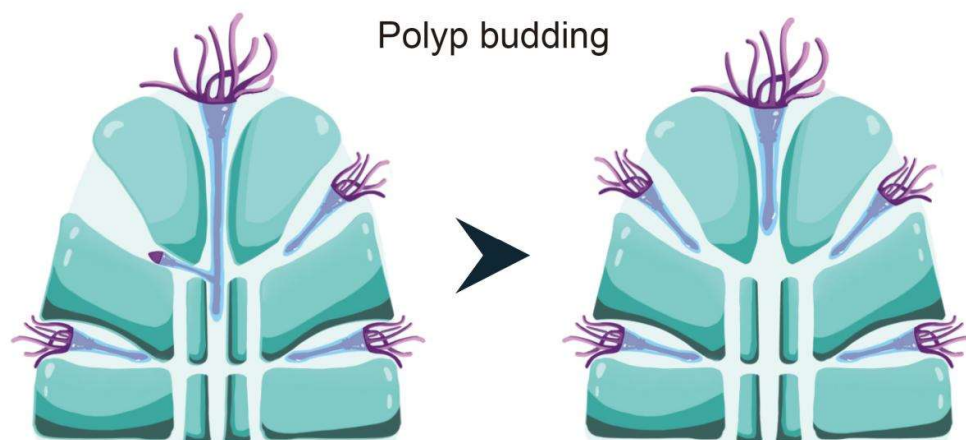
Fig. 3 | Polyp budding visualization according to the canal network reconstructions.
The polyp buds after the mineralization of new skeletons and canals, and the newly budded polyp migrates to its new calice through the internal canal, which connect axial canal and calices.

We also investigated the rebuilding process of a branch and its axial canal in *A. muricata* by a truncation experiment and a 3D canal reconstruction (Fig. 4). The columnar axial canal in the day 0 group had a smooth surface with few skeletal structures scattered inside it, and its cross-sectional diameter was approximately 2.0 mm (Fig. 4A). Obvious changes appeared in the structure of the axial canal beginning at day 14 (Fig. 4B). The amount of inner skeletal structure within the axial canal increased, and the surface of the axial canal became rough with more concave structures, indicating that the skeleton around the axial canal was proliferating inward. The cross-sectional diameter of the newborn part of the axial canal was approximately 1.5 mm thinner than its previous diameter (Fig. 4B). By day 21, the coral branch entered the peak period of the rebuilding process, and its axial-canal structure had changed the most (Fig. 4C). The inner skeletons had been connected into many long column-like structures and penetrated the axial canal deeply, occupying nearly half of the axial canal space. The cross-sectional diameter was approximately 1.0 mm, half of the diameter of the day 0 group. Amazingly, the cross-sectional diameter of the previous axial canal reduced to 1.5 mm, suggesting that a long-distance branch skeleton was also concerned in this rebuilding process (8). In the 28-day samples, fewer inner skeletons were left in the axial canals, and the cross-sectional diameter of an axial canal was similar to that of a day 0 group (Fig. 4D). The surface of the axial canal was smooth again, indicating that the rebuilding process of the branch was almost complete. The rebuilding process was a kind of extreme growth pattern, and related 3D reconstructions suggest that the axial canal plays an important role in this course, implying that the axial canal holds the position in calcium transportation during self-healing process, and a canal network can connect the polyps in one branch into a polyp network to regulate the process of coral growth.
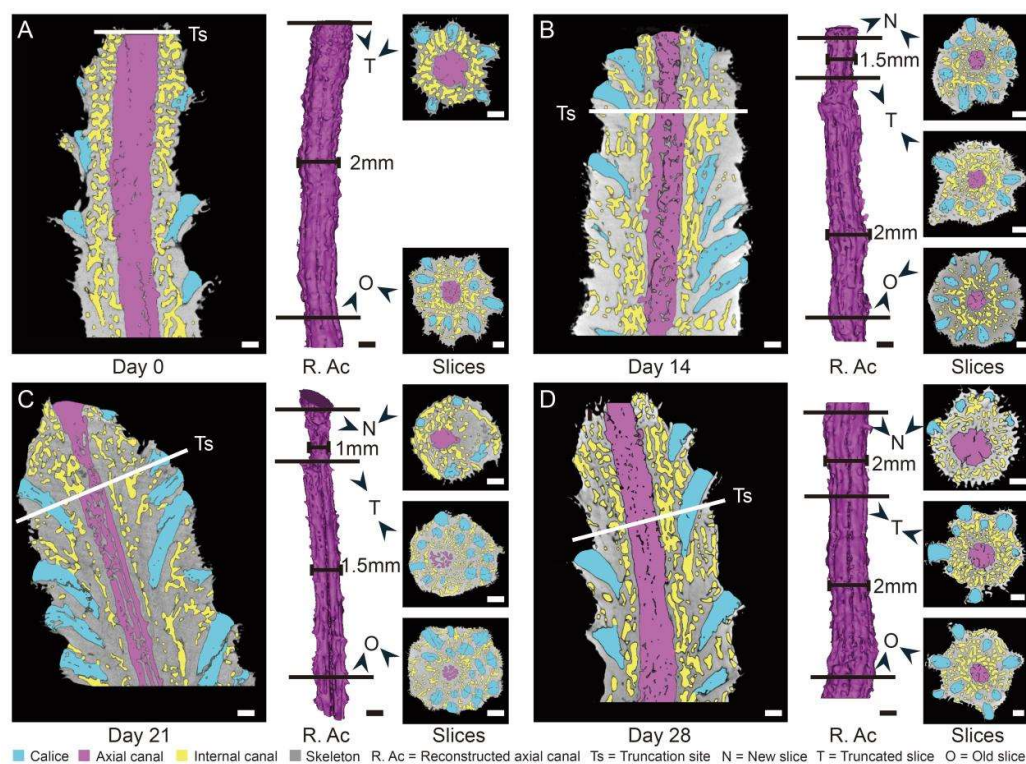
Figure 4 | 3D canal reconstructions reveal how axial canal regulates truncated-branch rebuilding process in *A. muricata*.
(**A**) In 0-day samples, the columnar axial canal has few skeletal structures scattered inside it, and its cross-sectional diameter is about 2.0 mm. (**B**) In 21-day samples, the amount of inner skeletal structure within axial canal is increased. The cross-section diameter of the new-born part of axial canal is about 1.5 mm, thinner than its existed part. (**C**) In 21-day samples, the inner skeletons increase and occupy nearly half of axial canal space. The cross-section diameter of axial canal is about 1.0 mm in new-born part and 1.5 mm in existed part. (**D**) In 28-day samples, there is less inner skeletons left in axial canal, and the cross-sectional diameter of axial canal is similar to day 0 group. Scale bars: 1 mm.

Discussion

Axial canal regulates budding and branching process

In this study, we reconstructed the axial canal and the skeletons around its cavity through HRCT to investigate the role of axial canal in budding, branching, calcium transporting and self-healing processes of coral growth (Fig. 1,2,4).

Canal network, which makes up an apparent polyp network, is the basic foundation of coral growth (4,9). As the largest canal in the network of *A. muricata* colony, axial canal is transformed from specific calice (Fig. 1B-E, Fig. 2). The chosen calice transform into the axial canal of new branch following the regulation of canal network in branching process (Fig. 2). Meanwhile, the canal network reconstructions reveal that in the newly formed branchlet, new calices distribute near their axial canal may only obtain the polyps from the new axial canal (Fig. 2A,E,F), which also suggests the budding patterns in the new branchlet (Fig. 3). Although how coral colony selects specific calice and induces its transition to a new axial canal during

coral branching is still unclear, the visualization of this process provides a basis for studies toward this area.

Axial canal regulates calcium transportation

Calcium can be carried over considerable distances inside the coral colony toward the zones of maximum growth and calcification (8). In truncated branches, the most active area of calcification is the rebuilding tip at truncated region, thus, calcium would be deliver to rebuilding area through the transport system in a colony. Although axial canal does not play a major role in the transport of hydroplasm in *Acropora* (5), extreme growing process, like truncated branch rebuilding, may change the role of axial canal in the transport system of *A. muricata* (Fig. 1F-I, Fig. 4). A large amount of calcium is transported through the axial canal to the truncated region to rebuild coral branch, and the increase of calcium content in the axial canal also leads to the deposition of calcareous skeletons in the cavity, represents as a decrease in the diameter of the axial canal cavity (Fig. 4B,C). After the self-healing of coral colony, this calcium transportation stops and the deposit calcareous skeleton reduce for the decrease of calcium content in the axial canal, and the structure in the cavity returns back to what it was (Fig. 4D).

However, this phenomenon does not appear in coral branching (Fig. 1B-E, Fig. 2), during which the formation of a new branchlet will also lead to calcium transport in the colony. This indicates that the calcium transportation through axial canal may only happens during the self-healing process in coral colony, and the mechanism deserves further study. This truncated-branch rebuilding experiment on *A. muricata* also suggest that the polyp network of the canal system makes coral branch growth a kind of integral behaviour.

Methods and Materials

Ethics

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

Sample collection

All six *A. muricata* samples in this study were collected from the South China Sea in 2018. The coral samples were kept whole and housed in our laboratory coral tank, where all conditions simulated their habitat in the South China Sea. These samples were kept in the tank for about one to three months before the HRCT test. Among these *A. muricata* samples, one of them is a colony and the other five are coral branches.

Coral culture system

Our coral samples were cultured with the laboratory auto calibration balance system (10) in a standard RedSea® tank (redsea575, Red Sea Aquatics Ltd) following Berlin Method. 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^{TM} MP40, EcoTech Marine Ltd), and calcium reactor (Calreact 200, Reef Octopus) etc.

We put about 20kg of live rocks, which were also collected from the South China Sea, in the coral tank. These live rock provide the growth environment and some necessary microorganisms. We also regularly add additives to the tank,

including Mg, Ca, KH, K, I, and Fe.

HRCT test

We analyzed six *A. muricata* samples from the South China Sea using three dimensional models constructed with the 230 kV latest-generation X-ray microfocus computed tomography system (Phoenix v|tome|x m, General Electric (GE)) at Yinghua NDT, Shanghai, China. Two-dimensional image reconstructions of each specimen from matrices of scan slices were assembled using proprietary software from GE.

Acropora colony one was scanned with a beam energy of 150 kV and a flux of 180 µA at a detector resolution of 37 µm per pixel, using a 360° rotation with a step size of 0.18°. A total of 2,000 transmission images were reconstructed in a 3,990 × 4,000 matrix of 2,000 slices. *Acropora* branch one was scanned with a beam energy of 130 kV and a flux of 60 µA at a detector resolution of 6 µm per pixel, using a 720° rotation with a step size of 0.18°. A total of 1,500 transmission images were reconstructed in a 2,800 × 4,000 matrix of 4,000 slices. *Acropora* branch two was scanned with a beam energy of 120 kV and a flux of 115 µA at a detector resolution of 12 µm per pixel, using a 720° rotation with a step size of 0.18°. A total of 2,400 transmission images were reconstructed in a 1,980 × 2,000 matrix of 4,000 slices. *Acropora* branch three was scanned with a beam energy of 130 kV and a flux of 60 µA at a detector resolution of 6 µm per pixel, using a 720° rotation with a step size of 0.18°. A total of 1,500 transmission images were reconstructed in a 2,800 × 4,000 matrix of 4,000 slices. *Acropora* branch four was scanned with a beam energy of 130 kV and a flux of 100 µA at a detector resolution of 9 µm per pixel, using a 720° rotation with a step size of 0.18°. A total of 2,500 transmission images were reconstructed in a 1,985 × 2,000 matrix of 4,000 slices. *Acropora* branch five was scanned with a beam energy of 160 kV and a flux of 70 µA at a detector resolution of 9 µm per pixel, using a 720° rotation with a step size of 0.18°. A total of 1,600 transmission images were reconstructed in a 1,500 × 4,000 matrix of 4,000 slices.

Internal canal reconstruction

Slice data derived from the scans were then analyzed and manipulated using VG software. The 3D reconstructions were created in the Mimics (v20.0) software and VG Studio Max (v3.3.0) following the method as previously described (4). The images of the reconstructions were exported from Mimics and VG Studio Max, and finalized in Adobe Photoshop CC 2019 and Adobe Illustrator CC 2019.

Truncation experiment

We select four groups of *A. muricata* branches with similar size and shape to truncate their tips at same position and culture them in same environment, and take the truncated samples at day 0, 14, 21 and 28 (*A. muricata* branch 2-5) to do HRCT detection.

Data Availability Statement

The HRCT data that support the findings of this study are available to share. You may download the HRCT reconstruction data through following links.

Acropora muricata colony: doi:10.5061/dryad.wdbrv15nm
https://datadryad.org/stash/share/rWeAOhUx1u__18sUEdkAIPAtTq3UGbxsYwO95ktjWhs

Acropora muricata branch 1: doi:10.5061/dryad.ghx3ffbnh
https://datadryad.org/stash/share/7YfZPthkA9VP6Djw0OPRyKGhXAP6L-Bb5XYMYVA4ZVQ

Acropora muricata branch 2: doi:10.5061/dryad.p2ngf1vq4
https://datadryad.org/stash/share/V3iXkB8Fk2M9nvELjGCyOJsRzpNFyBBrKo34oGdwu7M

Acropora muricata branch 3: doi:10.5061/dryad.08kprr524
https://datadryad.org/stash/share/8SPvv12ifZv_wEf2Uy61PauSkL0oX8tF1iR91f74DIM
Acropora muricata branch 4: doi:10.5061/dryad.8w9ghx3mb
https://datadryad.org/stash/share/ZTV11TYS1f5Kgqnfo1UZBdS8KabYJSPrrL7U6jg6Jv0
Acropora muricata branch 5: doi:10.5061/dryad.pnvx0k6m8
https://datadryad.org/stash/share/e94f4E80T8ZLfC-YX0ACLdMoPMc14AfVLwt6xdLvNb8

Author Contributions

Y. L., C. H. and Z. L. conceived the project. Y. L. wrote the paper. Y. L. reconstructed the images and performed the biological analyses. Y. L. produced the figures. Y. L. and T. H. uploaded the data to the public database. Y. L., C. H. and Z. L. edited the paper. All authors discussed and commented on the data.

Acknowledgments

Declaration of Interests

The authors declare no competing interests.

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