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Internal skeletal analysis of the colonial azooxanthellate scleractinian *Dendrophyllia cribrosa* using microfocus X-ray CT images: underlying basis for its rigid and highly adaptive colony structure

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Abstract

Dendrophyllid Scleractinia exhibit a variety of colonial morphologies, formed under the strict constraints on (1) budding sites, (2) orientations of the directive septa of offsets, (3) inclination of budding direction, and (4) those constraints in every generation. *Dendrophyllia cribrosa* exhibits a sympodial dendroid form, characteristically large coralla, and occasional fusions of adjacent branches within the
same colony. Adjacent corallites are bound and supported by coenosteum skeleton. This study examined the inner skeletal structures at the junctions of fused branches using a non-destructive microfocus X-ray computed tomography (CT) imaging approach, and considered the reasons for the large colonial sizes and their adaptive significance.

Three-dimensional reconstructions of two-dimensional X-ray CT images reveal that individual corallites are not directly connected in fused parts. Additionally, no completely buried individuals were found within fused skeleton. When adjacent branches approach one another, constituent corallites change their growth directions to avoid collisions between the branches. The adjacent branches fuse without a reduction in the number of constituent corallites, leading to the establishment of reticular and rigid colonial structures. In addition, a nearly even distribution of individuals on the colony surface facilitates efficient intake of nutrients. Thus, the growth of large *D. cribrosa* colonies involves avoidance of collision between constituent individuals, the reinforcement of colonial structure, and efficient uptake of nutrients. These observations provide insights on the dynamics of interrelationships between colony-making mechanisms and the adaptive strategies required under habitat conditions such as specific current activities.
Introduction

Scleractinian corals, especially zooxanthellate species, are common and well-known components of the calcifying biota of modern oceans. They mostly reproduce by asexual processes, such as budding and division, forming colonies. In reef environments, biological interactions between scleractinians and other clonal organisms are an important aspect of their behaviour, and control many aspects of their morphology and the development of reefs.

Genetically identical clones often fuse together, whereas non-genetically identical clones may or may not fuse, depending on their histocompatibility (Ronald et al., 2011). Experimental research on reactions between mature coral branches from different colonies has been conducted chiefly to investigate the nature of competitive interactions and histocompatibility (Potts, 1976; Collins, 1978; Neigel and Avise, 1983).

The growth and survival strategies of clonal organisms are markedly different from those of solitary multicellular organisms, and a variety of clonal algae, sponges, corals and bryozoans play dominant roles as frame-builders in both living and fossil reefs (Fagerstrom and West, 2010). However, few studies have examined the behaviours of adjacent individual corallites and their fusion within a colony.
An understanding of the internal anatomies and types of budding in extant corals is crucial for understanding the biology and ecology of these organisms (Stasinska, 1969; Nowinski, 1976). Techniques of X-radiography are commonly used in studies of extant corals (e.g., Logan and Anderson, 1991; Roche et al., 2010; Veal et al., 2010), and during the past two decades, the application of X-ray microtomography has become increasingly popular in palaeontology (e.g., Hamada et al., 1991; Tafforeau et al., 2005; Henderickx et al., 2006; Sutton, 2008; Bosselaers et al., 2010). No special preparation of samples is required prior to X-ray scanning. Furthermore, a great advantage of X-ray computer tomography (CT) technology is that it is non-invasive and non-destructive (Zapalski and Dohnalik, 2013). The technique provides a full 3-D representation that can be inspected from arbitrary viewpoints, so that a variety of internal features can be observed.

Extant species of the family Dendrophylliidae are distributed worldwide at water depths of 0–2165 m (Cairns, 1994). The family includes both zooxanthellate (e.g., Turbinaria) and azooxanthellate (e.g., Dendrophyllia) forms, which allows it to exploit a wide range of habitats. Even in the case of the same sympodial growth species, D. boschmai (40–165 m) is different from D. cribrosa (7–40 m) in habitat depth. It is highly probable that these differences result from habitat segregation, depending largely on
the physical properties of sea water such as wave and current intensities (Sentoku & Ezaki, 2013). According to Cairns (1994), the Dendrophylliidae comprises 29 genera and 364 species, of which 20 genera and 166 species are extant; it is the third largest family in the Scleractinia in terms of Holocene species richness (12.6% of the total) and the fourth largest in terms of Holocene genus richness (9.0% of the total). The earliest known fossil record is from the Early Cretaceous (Barremian) of Serbia (Cairns, 1994).

Dendrophyllid Scleractinia exhibit a variety of colonial morphologies, formed under the strict constraints on (1) budding sites, (2) orientations of the directive septa of offsets, (3) inclination of budding direction, and (4) those constraints in every generation (Sentoku and Ezaki, 2012a–d). Given these regularities, *D. cribrosa* grows helically by budding at a particular site (Sentoku and Ezaki, 2013). Regular budding is defined by budding sites at two or four lateral primary septa, the orientations of directive septa of lateral corallites (nearly perpendicular to the growth directions of parent corallites), and the inclination angles of budding (diagonally upward).

Importantly, these regularities persist and remain valid in every generation during growth of the colony. The only differences occur in relation to the budding sites: in *Dendrophyllia cribrosa* (Fig. 1A–B), offsets occur around either lateral primary septum on one side of corallite; the resultant individuals thus show a definite polarity with
respect to the directive septa, and only when branching dichotomously offsets occur around both primary lateral septa.

Fig. 1. *Dendrophyllia cribrosa* (OCU 6662 and 6672). A, Side view of a colony. Scale bar = 5 cm. B, Living colony surrounded by orange-coloured coenosteum tissue. Scale bar = 5 cm.

Given these regularities, *D. cribrosa* grows helically (clockwise and anticlockwise) by budding at particular sites and develops stout branches by secreting coenosteum skeleton around the internal spiral-forming individuals (Fig. 2; also see Sentoku and Ezaki, 2013), forming colonies that are up to 30–50 cm in size. When adjacent branches of *Dendrophyllia cribrosa* approach one another, they grow nearly parallel or fuse together. However, little is known about the behaviours and internal structures of constituent corallites in the fused sections of branches.
Fig. 2. Schematic diagrams of the spiral architecture of *Dendrophyllia cribrosa* (red bars, directive septa; green bars, four lateral primary septa; triangles, budding sites; red circles, polarity; arrows, growth directions; white lines, plane of bilateral symmetry). A, Budding sites of individual corallites (triangles). Note that offsets appear either site of the first order of septa on one side of parent corallites. B, One round of spiral architecture, which is made up of three individuals. The angle between parent and daughter corallites is ~120°. C, Growth directions of the individuals with each corallite budded clockwise. D, Schematic view of the internal structure showing constituent corallites and coenosteum skeleton. E, Budding sites and growth directions of individual corallites. Rectangle, dichotomous branching of colony. F, Top view. G, Lateral view. Notably, given these regularities, *D. cribrosa* grows helically by budding at particular sites. In addition, *D. cribrosa* inevitably changes the directions of rotation at right and left branches after branching due to the presence of developmental constraints on polarity.

In this study, we meticulously observed the adjacent or fused corallites in three dimensions, using X-ray CT images. Finally, we consider the underlying basis for the large size of *D. cribrosa* colonies, through analyses of the assembly patterns of individuals and the related efficiency of nutrient uptake.
**Materials and Methods**

We examined 46 corolla of *Dendrophyllia cribrosa* (Fig. 1A, B) collected at water depths of 7–40 m offshore of Minabe (Wakayama Prefecture), Sakai (Tottori Prefecture), Amakusa (Kumamoto Prefecture), and Minamiise (Mie Prefecture), southwest Japan. Of these, three large corolla were selected for internal skeletal analyses of the fused parts of coralla using microfocus X-ray CT morphometric images.

The greater calicular diameter (GCD; *sensu* Cairns 1994) was the diameter in a direction parallel to the two directive septa (Fig. 3A–C). The maximum GCD observed in our specimens of *D. cribrosa* was 6.0 mm, and the maximum lesser calicular diameter (LCD; *sensu* Cairns 1994) was 5.1 mm. *D. cribrosa* develops branches by secreting coenosteum skeleton among the individuals (Fig. 3D, E).
Fig. 3. Side, calicular, and transverse views of *Dendrophyllia cribrosa*, and a schematic illustration of an individual corallite (OCU 6637). A, Side view of a dichotomous branch. Scale bar = 10 mm. B, Enlarged calical features shown in the rectangle in A, indicating the distinct Pourtalès plan of septa. C, Schematic drawing of B, showing the two opposite directive septa, the greater calicular diameter (GCD), and the lesser calicular diameter (LCD). Numbers indicate the cycles of septa: circled numbers indicate the first cycle of septa, and shaded circles indicate the two directive septa. D–E, Images obtained by micro-focus X-ray computer tomography. Note that individual corallites are connected by the coenosteum skeleton.

To assess the morphometric parameters of constituent individuals and the fused parts of branches, we measured the following features: (1) GCD, (2) LCD, (3) length of lateral corallites, and (4) height of the entire coralla. We photographed relevant coralla at various angles and magnifications to determine: (1) the angle of branching, and (2) the thickness of the branches. When necessary, measurements were obtained using image-processing software (Adobe Photoshop) and an electronic caliper. The studied
specimens are registered in the Department of Geosciences, Graduate School of Science, Osaka City University (OCU 6669-6688), and in the Tottori Prefectural Museum (TPM 10505), Japan.

**Microfocus X-ray CT scan analysis**

Computer tomography was chosen for the analysis because it is direct, non-invasive, and non-destructive, and because it gives the spatial distribution of bodies with different densities and shapes internal to the skeleton and fused parts of the coral colonies. We used an HMX225·ACTIS+3 (TESCO Corporation) micro CT system, at the Kochi Core Center, Kochi University/JAMSTEC, Japan. The spatial resolution of CT images is determined principally by the size and number of detector elements, the size of the X-ray focal spot, and the source object–detector distance. Smaller samples can be set nearer to the X-ray source and projected larger in the detector plane, supposing that the distance between X-ray source and detector plane is fixed. Thus, the spatial resolutions of X-ray images tend to be finer for smaller samples. The finer the X-ray image resolution is, the smaller the scale of reconstructed 3D voxels can be.

Specimens were scanned perpendicular to their long axes (Fig. 4) on 11 July 2012, using the microfocal subsystem with X-ray settings at 120 kV and 30 mA. A total of 617
1024 × 1024 slices were obtained, with slice thicknesses and inter-slice spacings of 0.039 mm, and a field of reconstruction of 24.02. Subsequently, the images were imported into Image J software to visualize 2D cross-sections (Fig. 5C–F, I–L) and 3D visualizations (Fig. 5A, B, G, H). A series of cross-sections equivalent to classical transverse thin sections is presented in Fig. 5E, F, J, K.

Fig. 4. Fused parts of branches analysed by using microfocus X-ray computer tomography. A and C, Fused parts of branches. Dotted arrows indicate growth directions of branches. Scale bar = 10 mm. B and D, Enlarged fused parts shown in the rectangles in A and C, respectively. Scale bar = 5 mm.
Fig. 5. Images of the fused parts of *Dendrophyllia cribrosa* obtained by micro-focus X-ray computer tomography (CT). A–F, Internal skeletal features at the fused sections of branches observed in Fig. 4D. G–L, Internal skeletal features at the fused sections of branches observed in Fig. 4B and D. A and B, 3D reconstruction with semi-transparent versions of images shown in Fig. 4B and D, respectively. C–F and I–L, Spatial distributions of corallites in fused parts (obtained by CT); white dotted lines indicate the section planes of C–F and I–L, respectively.

**Results**

In *Dendrophyllia cribrosa*, budding occurs near one of the lateral primary septa on one side of a corallite. As a result, individual corallites are arranged helically (Fig. 2).

In addition, individuals are distributed in a helical as indicated above manner, with budding occurring at nearly equal spacings along the branches (Fig. 5E, F, J, K).

During growth, the GCD remains constant at ~5 mm. We measured a large-sized colony (40 × 30 × 50 cm) in detail (Fig. 1A); branching occurred ~180 times, resulting in ~300 branches consisting of ~4460 individuals. The total length of the branches was approximately 10.57 m, and the colony included 7 parts that were fused by the coenosteum skeleton. Two patterns of fusions were recognized. 1) Pattern A is a fusion between peripheral branches at the distal parts of colony (Fig. 6X, Y). 2) Pattern B is a fusion between proximal branches at the basement of colonies (Fig. 6Z). In the first
pattern, fusion occurs when branches meet at obtuse angles in the distal parts of a colony (e.g., Figs 4, 6B, X, Y). In the second pattern, *D. cribrosa* develops stout and mechanically strong branches by secreting coenosteum skeleton between the spirally budding individuals. The old branches in the basal part of the colony are particularly stout, and fusion in this part of the colony occurs only occasionally (Figs 1A, 6C).

Fig. 6. A, Schematic view of the placement of branches within a large colony. Older branches are shown by dark colours, and younger branches by pale colours. X–Z, Fused parts of branches. B, Fused parts of branches at distal locations (X, Y). C, Fused parts of branches at a proximal part (Z).

The images in Fig. 5D and E are from an X-ray CT scan. Figure 5A, B, G, H shows 3D reconstructions based on 2D X-ray CT images. The inner skeletal structures at the sites of fused branches were observed from various directions (Fig. 5C–F, I–L). The numbers and spatial relationships of individual corallites in the 3D reconstructions were the same as those observed visibly on the surface, which indicates that individual
corallites do not collide with one another at the apparently fused parts. Additionally, no immersed individuals were found within fused skeleton. When adjacent branches approach one another, the constituent corallites maintain a certain distance from one another, without colliding, and they change their growth directions rapidly to avoid collision, even though each corallite is laterally connected to adjacent corallites by the coenosteum skeleton (Fig. 5D, E, J, K).

**Discussion**

The 3D reconstruction of 2D X-ray CT images of *Dendrophyllia cribrosa* colonies reveals that fused corallites are not directly connected in the fused sections. Additionally, no buried individuals are found in the fused sections of the colony. When adjacent branches approach one another (Fig. 7, 8A), constituent corallites rapidly change their growth directions in advance of a possible collision (Fig. 5D, E, J, K). Our observations of corallites indicate that clonal individuals in a colony avoid fusion by regulating their branching frequency and growth direction. Fusion of individual branches, which occurs without a reduction in the number of constituent corallites (Fig. 8B), leads to the establishment of a rigid reticular colony structure, which allows the colony to thrive in strong current environments.
Fig. 7. Living colony of *Dendrophyllia cribrosa* surrounded by orange-coloured coenosteum tissues and tentacles. Note that tentacles spread and extend from coenosteum tissues.

Fig. 8. Schematic diagram showing the behaviours of tentacles and individual corallites in *Dendrophyllia cribrosa*. A, A vertical branch is approaching a horizontal branch. B, The branches in (A) are fused. Note that the constituent corallites change their growth directions (red arrows) before collision, lest they become buried into the coenosteum skeleton. C, Branches arranged in parallel.

It is thought that individual corallites sense and maintain their distance from surrounding corallites by extending their tentacles. The tentacles assist corals to acquire nutrients (Fig. 7), and the outward reach of tentacles is about twice that of the GCD. Branches in the colony are approximately parallel (Fig. 8C), and the interval...
between branches is maintained so as to minimize interference, which occurs by sensory recognition of tentacles. Branching does not occur in the neighbourhood of adjacent corallites, suggesting that the growth directions of individual branches are adjusted locally, by mutual recognition between corallites and its neighbours via the tentacles.

In addition, the density of individual corallites in areas of fused branches is lower than that in other areas (Fig. 5E, F), owing to increased amounts of coenosteum skeleton in these areas. Importantly, even when collisions between individuals are imminent, constituent individuals take evasive action to avoid approaching corallites, and are thus distributed nearly evenly throughout the colony, which enhances the efficient intake of available nutrients. The growth of large colonies of *Dendrophyllia cribrosa* is possible because individuals avoid collisions with nearby individuals, the colony structure on the whole is strengthened, and food uptake under strong currents is optimized. These findings contribute to an understanding of the dexterous relationship between mechanisms of colony construction and adaptive strategies under specific habitat conditions.

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study of methods for surface area and three dimensional shape measurement of coral


**Figure captions**

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