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X-ray micro-CT observation of the apical skeleton of Japanese white coral *Corallium konojoi*

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ABSTRACT

Precious corals within Japanese waters have long been commercially traded as valuable marine resources. To manage and recover coral resources toward sustainable use, the accumulation of scientific knowledge including coral biology and ecology is essential. This study aimed to obtain biological and structural information on the white coral *Corallium konojoi*, a Japanese precious coral, using a synchrotron X-ray micro-computed tomography. Tomographic images of a high spatial resolution and edge-contrast showed morphological features of not only the skeletal structures, axial skeleton, and sclerites but also of living tissue, leading to the identification of polyps (autozooids and siphonozooids) and gastrovascular canals. A coenenchyme with a multilayered structure of the dispersed sclerites and the gastrovascular canals were also observed. External and internal forms of the axial skeleton suggested that skeleton formation at an apical part was in combination with incorporations of the sclerites and a biomineralization through a skeletogenic epithelial layer surrounding the axial skeleton.

1. Introduction

Precious corals are valuable living marine resources and have been used in jewelry, medicines, and other valuable products (Tsounis et al., 2010). These corals belong to the genera *Corallium* and *Paracorallium* (subclass Octocorallia; Order Alcyonacea; Family Coralliidae), and eight species in total are known as precious corals. In waters near Japan, four species of precious corals, namely white coral (*Corallium konojoi*), pink coral (*Corallium elatius*), Japanese red coral (*Paracorallium japonicum*), and miss coral (*Corallium sulcatum*) are mainly distributed on rocky bottoms at depths of 80–450 m (Iwasaki et al., 2009; Nonaka et al., 2004; Seki, 1991; Tu et al., 2012). Harvesting of the Japanese precious corals began during the 19th century (Ogi, 2010). It is commonly suspected that historical overfishing caused population decline and the subsequent extinction of certain precious coral resources (Iwasaki and Suzuki, 2010). Thus, to achieve a sustainable use of these corals, it is necessary to develop appropriate management and recovery strategies. For this purpose, scientific information in various fields, such as biology and ecology is required. In recent years, various studies on the Japanese precious corals have been conducted (Debreuil et al., 2011b; Hasegawa et al., 2012; Iwasaki et al., 2012; Luan et al., 2013, 2014; Nonaka et al., 2012; Perrin et al., 2015; Tamenori et al., 2014; Uda et al., 2011, 2013).

Precious corals are characterized by two types of skeletal structures, namely the axial skeleton and sclerites (Iwasaki and Suzuki, 2010). The sclerites are small granules with a size of ca. 60 μm and are dispersed in the coenenchyme. The main component of the axial skeleton and the sclerites is magnesium calcite, which is closely packed with small amounts of organic compounds. For the Mediterranean red coral, *Corallium rubrum*, growth mechanisms of the axial skeleton have long been debated, and two processes have been proposed (Allemand and Grillo, 1992; Allemand, 1993; Allemand and Bénazet-Tambutté, 1996; Debreuil et al., 2011a, 2012; Grillo et al., 1993; Lacaze-Duthiers, 1864; Marschal et al., 2004, Perrin et al., 2015; Vielzeuf et al., 2008, 2010). One of the proposed processes is that the sclerites are incorporated into the axial skeleton. The other is the biomineralization process occurring at the skeletal surface where a skeletogenic epithelial layer of coenenchyme is in contact. Allemand et al. (Allemand and Grillo, 1992; Allemand, 1993; Allemand and Bénazet-Tambutté, 1996; Debreuil et al., 2011a, 2012; Grillo et al., 1993) have shown that the nascent skeleton is formed by the former process, allowing the axial skeleton to elongate and then the centrifugal growth to proceed during the latter process.

X-ray computed tomography (CT) is a promising tool that can be used to visualize external and internal structures of materials in three dimensions without damages to the samples. By utilizing a synchrotron radiation source, CT experiments with a micrometer range resolution, so-called X-ray micro-CT, were readily achieved (e.g., Rack et al., 2008; Uesugi et al., 2001). Recently, it has become popular to use X-ray CT in studies of reef-building corals (e.g., Caroselli et al., 2011;

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Helmle et al., 2000; Roche et al., 2010; Sentoku et al., 2015; Veal et al., 2010). Fully digitized and spatially accurate image data can be used to estimate these surface areas and skeletal architectures, which facilitates the understanding of biological processes of the skeletons. In the present study, synchrotron X-ray micro-CT was applied to a branch tip of axial skeleton of *C. konojoi*. From the three-dimensionally visualized morphology, the internal structures of the branch tip and the skeleton formation at an apical part are discussed.

2. Material and methods

2.1. Sample preparation

A colony of *C. konojoi* (Fig. 1A) was collected from a depth of 290 m off Iriomote Island, Okinawa, southern Japan on October 23th, 2011 using a remotely operated vehicle "Hakuyo 3000". Water temperature at the bottom was 19.7 °C. After collecting the colony, it was immediately dried at room temperature, and a branch tip of the axial skeleton (Fig. 1B) was cut from the colony and used as a sample for the X-ray micro-CT experiment. The sample was desalted by immersing it in distilled water, and then dried at room temperature. This desalting procedure was carried out twice before the experiment.

2.2. X-ray micro-CT

The X-ray micro-CT experiment was performed at Hyogo prefecture beamline BL08B2 in SPring-8. The X-ray beam from a bending magnet source was monochromatized at 20 keV by a Si(111) double-crystal monochromator. The sample was mounted on a high-precise rotation stage. Transmitted X-ray images were taken by an indirect X-ray imager. The transmitted X-rays were converted into visible light by a single-crystal LuAG:Ce ($Lu_3Al_5O_{12}$:Ce) scintillator with thickness of 25 μm. The visible-light image projected on the scintillator was magnified to 2.1 times by tandem-lens optics (Ai Nikkor 50 mm f/1.2S and Ai Micro-Nikkor 105 mm f/2.8S, Nikon Corp.), and the magnified image was recorded by scientific CMOS camera (Orca-Flash2.8, Hamamatsu Photonics K.K.). In this setup, the effective pixel size of the imager was 1.73 μm. The transmitted images were taken at 0.12° intervals with a rotation of the sample from 0° to 180°. Exposure time of a single projection was 0.8 s. After a flat-field correction was applied to every transmitted image, the corrected images were reconstructed into tomographic images by a convolution-back projection algorithm. ImageJ software (Schneider et al., 2012) was used for three-dimensional representation and analyses of the tomographic images.

3. Results

Fig. 2 depicts volume renderings of the reconstructed *C. konojoi* branch tip. An enormous number of granules with protuberances completely covered the surface of the branch tip (Fig. 2A). Their average size was 58.0 μm ± 5.2 μm (number of sample: n = 40) in length. The granules had the same linear attenuation coefficient (LAC), corresponding to a material-dependent conversion factor, as the axial skeleton lying center of the branch tip. Judging from these observations, the granules were assigned to sclerites. In the tomographic images, organic component parts of the coenenchyme were obscure because of a low signal-to-noise ratio. This was because the organic matrix composed of light elements showed low LACs compared with those of the axial skeleton and the sclerites. However, the edge-enhancement effect of synchrotron X-ray imaging (Baruchel et al., 2006; Kagoshima et al., 1999; Yagi et al., 1999) led to a visualization of boundaries between the air and the coenenchyme. In addition, the dense dispersing of the sclerites allowed the coenenchyme form to be recognized. As a consequence, the morphological features of the living tissues, such as polyps (autozooids and siphonozooids) and gastrovascular canals, were apparent. These living tissues were identified with reference to the histological examinations of Nonaka et al. (2012).

The largest cavities corresponded to the autozooids. There were four autozooids in the tomographic images. The autozooids were of hemispherical shape with an average size of 0.83 mm in height and 1.0 mm in width. Their surfaces were covered with a thin layer of coenenchyme, and coenenchymal mounds with mouth openings were formed. The second largest cavities corresponded to the siphonozooids. Some of the siphonozooids existed near the surface and had openings (Fig. 2B and E). The siphonozooids formed various shapes, e.g., a spherical shape in Fig. 2B and a conical shape in Fig. 2E, and ranged in size from 0.22 mm to 0.50 mm (n = 7) in spherical approximation. The gastrovascular canals, which connected the autozooids and the siphonozooids, are indicated by white arrows in Fig. 2. Their average diameter was 0.10 mm ± 0.01 mm (n = 10). The gastrovascular canals could often be found at the middle and at the axial skeleton side of the coenenchyme (Fig. 2B, C, E, F). The canals at the axial skeleton side were parallel to the axis. On the other hand, the canals at the middle ran in longitudinal and concentric directions to the axial skeleton, similar to a net. These findings indicated that the coenenchyme formed a four-layer structure: 1) the outer surface that was densely filled by the sclerites; 2) the canal network layer; 3) the intermediate layer of the dispersed sclerites; and 4) the canal layer at the axial skeleton side.

The axial skeleton was an irregular form with a Y-shape cross section (Fig. 2B, C, E, F) and contained many micro-pores (dark spots of the axial

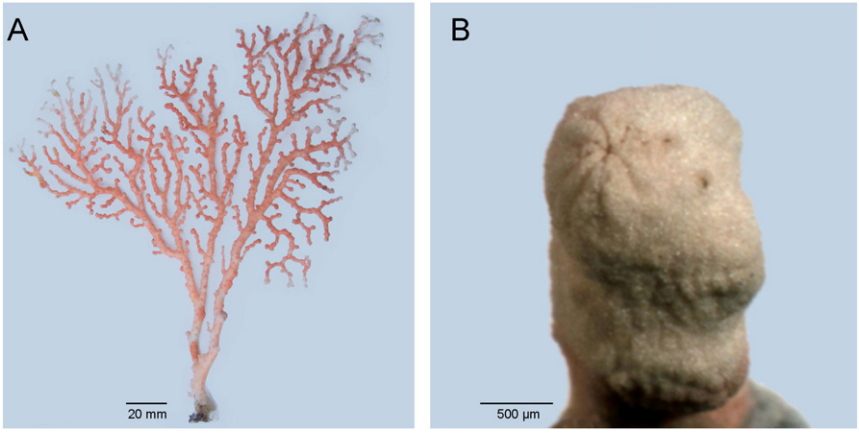

Fig. 1. Photographs of *Corallium konojoi*. A) A colony after drying at room temperature. B) A branch tip cut from the colony for X-ray micro-CT measurement.