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Three-dimensional imaging of fossil cheilostome bryozoans in Eocene chert by Synchrotron Radiation Micro-Computed Tomography

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ABSTRACT: The goal of this project is to describe the application of Synchrotron Radiation Micro-Computed Tomography (SRμCT) for three-dimensional imaging of fossil cheilostome bryozoan colonies. The technology is applied to the challenging problem of 3D visualization of silicified bryozoan fossils in Eocene cherts from southern Western Australia. The reconstructed colonies from Aboriginal chert artifacts and chert nodules from well cuttings allowed assignment to the following genera: *Quadricellaria*, *Siphonicytara*, *Trigonopora*, and *Reteporella*. The benefits of SRμCT include effective imaging of bryozoans in dense cherts non-destructively and the integration of 2D and 3D internal zooecium- and colony-level morphologic characters. The disadvantages of SRμCT include large data handling and analysis, relatively high cost, and low resolution in chert.

1 INTRODUCTION

It is a challenge to image species-diagnostic internal morphology of fossil bryozoans when they are embedded in rock, and even more so when they are in valuable cultural artifacts. An analysis of the three-dimensional (3D) morphology is a necessary part of species identification and typically requires the destructive process of thin sectioning which involves cutting, grinding, and polishing the rock to expose a bryozoan colony (Nye *et al.* 1972). The final product is a two-dimensional thin slice through the rock (i.e. a thin section) mounted on a glass slide that can be imaged with a microscope. Thin sections can yield high resolution images. Acetate peels are made similarly. After polishing, the colony is etched with acid, so any internal structures stand out in relief. Then a sheet of acetate is partially chemically dissolved onto that surface with acetone (Boardman & Utgaard 1964; Wilson & Palmer 1989). The acetate is then pulled off the colony and can be imaged with a microscope. Acetate peels allow image resolutions almost as good as thin sections, but they fall short in two areas. 1) They do not have the longevity that thin sections have. 2) Skeletal wall structure is not as well resolved as in thin sections.

Both techniques have always been destructive and labor-intensive (Boardman 2008). Intersecting a bryozoan colony inside the rock is not guaranteed. With both of these methods, accurately oriented sections (i.e. mutually perpendicular longitudinal, transverse, and tangential) are hard to achieve (Wyse Jackson & Buttler 2015). Fortunately, both methods allow for serial sectioning which can then be used to make three-dimensional reconstructions (Key *et al.* 2011). The grinding needed for serial sectioning is destructive and results in the complete loss of the specimen. The spacing is coarser with thin sections than acetate peels, and computer stitching of digital 2D slices is labor-intensive (Sutton *et al.* 2001).

The latest technique for three-dimensional imaging is laboratory-based X-ray Micro-Computed Tomography (μCT). By laboratory-based, we mean it is an accessory for an SEM or a small stand-alone cabinet-based system. X-ray

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absorption is the main source of contrast in μCT imaging. The ability to differentiate between morphological features depends on variations in mineral composition and/or void space/porosity, which have a bearing on X-ray attenuation through a colony. The first use of μCT was by Taylor *et al.* (2008) who imaged the internal morphology of Recent and fossil cyclostomes. Since then there have been dozens of publications using μCT 3D imaging technology in bryozoology (Key & Wyse Jackson 2022) . For example, the μCT lab at the Paleontological Institute of the Russian Academy of Sciences in Moscow has been very productive (Koromyslova & Pakhnevich 2016). Roughly twice as many of these studies are on fossil bryozoans, probably in response to the labor involved in making serial thin sections and acetate peels to create 3D reconstructions (e.g. Snyder 1991).

μCT 3D imaging works best when the mineralized skeleton and zooidal cavities have very different X-ray absorption properties. For example, Matsuyama *et al.* (2015) produced beautiful images of the autozooid chambers, suboral avicularian chambers, orifices, and frontal pores in Recent cheilostomes from marine colonies off Mauritania. Schwaha *et al.* (2018) used μCT to create 3D renderings of the various parts of cyclostome polypides, sampled from marine colonies off New Zealand. It is more of a challenge to obtain contrast for fossils in limestone when both the specimen, surrounding sediment matrix, and infilling cements are all composed of the same carbonate mineral and therefore share more similar X-ray attenuation (Keklikoglou *et al.* 2019; Sutton 2008). This homogenous carbonate mineralogy is often the case with a calcite bryozoan colony in a limestone (Key *et al.* 2005). If the densities of the bryozoan skeleton and infilling cements are too similar, imaging fails (Buttler *et al.* 2012).

Fortunately, in some carbonate sedimentary rocks, there is often enough variation between the fossil skeletons, the surrounding sediment matrix, and the secondary cements that sufficient contrast is achieved with μCT. For example, Viskova & Pakhnevich (2010) used μCT to image soft bodied Jurassic ctenostome colonies boring in mollusc shells. Wyse Jackson & McKinney (2013) applied this technology to a study of type material of a Mississippian fenestrate bryozoan in limestone to resolve the nature of its polymorphs along with the help of thin sections and SEM images. The application of μCT technology has not been limited to visualizing internal taxonomic characters. David *et al.* (2009) used this technique to document marine bryozoans encrusting Recent sea urchin spines from Antarctica. Heřmanová *et al.* (2020) used μCT to reveal commensal ecological relationships in Ordovician bryozoans encrusting conulariids and hemispherical colonies bored by sponges.

This problem of 3D imaging of fossilized bryozoans using μCT is accentuated when the fossilized remains have been replaced by new minerals. For example, Zhang *et al.* (2021) used μCT to show the first Cambrian bryozoans were soft-bodied. They were preserved through phosphatization which revealed bilaminate autozooids with possible interzooidal connections through the mesotheca. The challenge of 3D imaging of bryozoans is most difficult when there is not enough X-ray contrast to distinguish skeletal features from cement infilling. This is especially true in two situations. First, in Paleozoic palaeostomes, there is normally not enough contrast to identify features required for taxonomic identification where there are calcified walls and calcite infilling of zooecial chambers. Second, imaging is also a challenge when the original calcite or aragonite bryozoan skeleton has been replaced by silica during the diagenetic process creating chert (Maliva & Siever 1988; Murray *et al.* 1992). If the contrast between the bryozoan skeleton and its infilling cements and surrounding sediment matrix is too low, synchrotron phase-contrast imaging may be a better solution for 3D visualisation than μCT (Sutton *et al.* 2014).

Following the suggestion by Schmidt (2013), Ward *et al.* (2019a) were the first to try to image silicified fossil bryozoans in chert using Synchrotron Radiation Micro-Computed Tomography (SRμCT). SRμCT possesses some advantages over standard μCT. A synchrotron source provides a higher-flux, higher-intensity X-ray beam, allowing acquisition of potentially higher-resolution 3D images with a higher signal-to-noise ratio (Salomé *et al.* 1999). A synchrotron is ~100 m diameter dedicated facility that produces many times more flux than a laboratory-based μCT instrument, so the exposure times are smaller, and therefore data collection is faster (Betz *et al.* 2007). Because of the higher flux/brightness, the technicians can tune the energy (i.e. discard certain energy ranges leaving potentially complimentary energies to the sample chemistry) to try to obtain better contrast in the samples. Because of the faster scan times, multiple fields of view of larger/thicker samples can be acquired in a realistic timeframe. This is a key advantage over laboratory-based systems. Without the high-flux we could never have imaged the bryozoans in these cherts due to the size of the samples.

The goal of this project is to document the application of SRμCT technology for 3D imaging of bryozoans. In particular, we focus on silicified fossil bryozoans in Eocene cherts. The cherts came from well cuttings and from irreplaceable Australian Aboriginal prehistoric artifacts that prohibit destructive serial sectioning. One of the challenges of using fossil bryozoans to source artifacts is the need to image the internal morphology of the colonies to identify the exact

species. This is typically done through destructive analysis in two dimensions from thin section and SEM images of the rock containing the fossil bryozoan (Wyse Jackson & Buttler 2015). Here we demonstrate that SRμCT technology offers a viable alternative.

2 MATERIALS

The samples in this study come from an ongoing geoarcheology project using fossil bryozoans to determine the source of Aboriginal prehistoric chert artifacts from Western Australia (Key *et al.* 2019; O'Leary *et al.* 2017; Ward *et al.* 2019a, 2019b, 2021). Most of the artifacts were chert cutting tools. The SRμCT technology was tested on fossil cheilostome bryozoans in Eocene cherts. Colonies of *Quadricellaria* were identified in Eocene chert Aboriginal artifacts (UWA-74610 Pinnacles and UWA-74781 Mandurah) collected on the Swan Coastal Plain around Perth and archived in the John Glover collection at the University of Western Australia museum. Colonies of *Siphonicytara*, *Trigonopora*, and *Reteporella* were identified from a 60.10 g chert nodule from well cuttings from 180 m deep in well FOR004 (Key *et al.* 2019). Well FOR004 (latitude 31.28008°S, longitude 128.55396°E) was drilled as part of the Eucla basement stratigraphic drilling program (Spaggiari & Smithies 2015). Stratigraphically, the well cuttings came from the Eocene Wilson Bluff Limestone (Key *et al.* 2019) which is a likely source of the chert for the artifacts (O'Leary *et al.* 2017; Ward *et al.* 2019a, 2019b). The size range of the chert samples ranged from 10–40 mm in maximum dimension. In all cases, samples that had bryozoans visible at or near the surface were deliberately targeted to increase the chances of finding embedded fossils. The specimen numbers and repositories are listed in Ward *et al.* 2019a).

3 METHODS

SRμCT requires a synchrotron that produces intense light. It does this by accelerating high-energy electrons in a circular orbit inside the synchrotron's tunnels by 'synchronized' application of strong magnetic fields (Betz *et al.* 2007). The light is channeled down beamlines to experimental workstations where it is used for research. We used the Australian Synchrotron which is located outside of Melbourne. It has nine beamlines. We used enclosure 3B of the Imaging and Medical BeamLine (IMBL) which provides a sample view area of up to 30 x 40 mm. In contrast, a typical laboratory-based μCT system can handle specimens up to 2 mm. Newer medical μCT systems can handle human skull-sized specimens. The IMBL delivers the world's widest synchrotron X-ray beam which allows high contrast 3D X-ray imaging at high resolution so as to hopefully reveal minute differences at the interfaces of the fossil bryozoan skeleton, sediment matrix, and secondary cements.

The resolution of each voxel (3D pixel) was 9.7 μm x 9.7 μm x 9.7 μm. A typical bryozoan zooecium is on the scale of 1 mm^3 (Ryland 2005), so a 9.7 μm resolution will allow up to 100 slices through a typical-sized zooecium. Each scan consisted of 1800 projections acquired as the sample is rotated at 0.1° steps through 180°. A standard filtered back-projection algorithm coupled with ring artifact removal was used to reconstruct the X-ray projection images into a stack of 2D slices, creating an isotropic voxel. Each voxel has a specific 16 bit grey value representing the linear X-ray attenuation coefficient of the composite material, which is dependent on density and chemical composition.

The first steps of image processing, such as flat-field correction, noise suppression, stitching and CT reconstruction were performed using the ASCI high performance cluster at the Australian Synchrotron. The subsequent 3D volumes could then be rendered for visualization and undergo subsequent post-processing and analysis. For further details on μCT scanning see Ngan-Tillard & Huisman (2017).

The synchrotron images were numerous and data rich. The number of synchrotron image files for each sample ranged from 340 to 6050. The size of each image file ranged from 0.64 to 20.3 MB. For the samples imaged, the total data set was > 800 GB. Hence, the first step involved reducing the data set to a more manageable size by cropping to specific regions of interest (ROIs) (i.e. bryozoan colonies) using Fiji (ImageJ) software (Schindelin *et al.* 2012). The ROIs were then analyzed using Avizo 9.2.0 (Avizo 2016) and Drishti 2.6.2 (Limaye 2012) software to assess presence or absence of individual bryozoan colonies. The xyz-position of individual colonies were recorded and examples of different bryozoans were selected to render in 3D in the Avizo software. Other 3D modelling software (e.g., Dragonfly) and freeware (e.g. Seg3D, 3D Slicer) are also available. Each voxel's X-ray attenuation coefficient (i.e. contrast value) was then color-coded to enhance visualization. This color coding (a.k.a. segmentation/labelling) is the most complex and labor-intensive aspect of the process. Owing to the sheer volume of data, only a small number of key fossil bryozoans from each region of interest were chosen for full 3D visualization. Normally, the more you look the more you find. Searching 3D data using slices can mean that, because of the oblique angles of the cuts through randomly oriented samples and the shape of the colony, much can be missed.

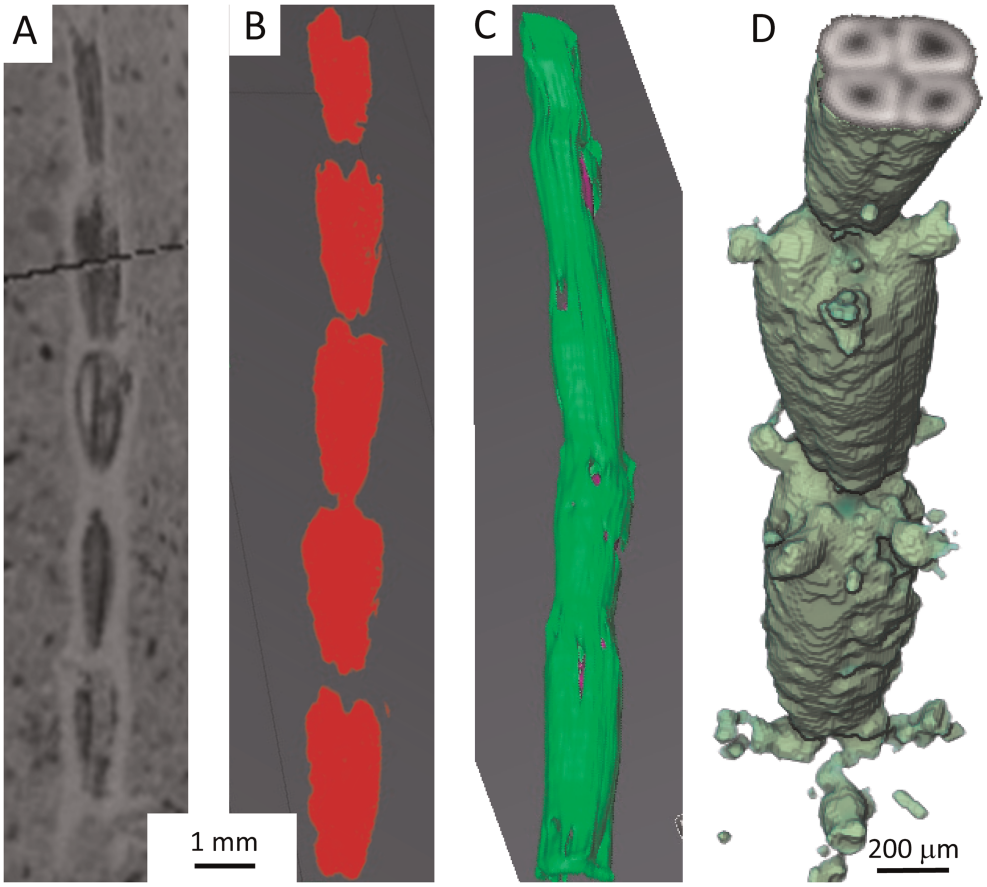

Figure 1. SRµCT images of the cheilostome bryozoan *Quadricellaria*. A, 2D grayscale image showing brighter skeletal walls surrounded by darker sediment matrix and even darker cement infilling zooecial cavities. B, 3D reconstruction of zooecial cavities in red. C, 3D reconstruction of exterior frontal walls of zooecia in green with opesia in purple. D, close up of B with exterior surface of zooecial cavities in green and a transverse 2D section at the top through the colony in grayscale.

4 RESULTS

What follows are the more successful 3D imaging attempts. Figure 1A shows a *Quadricellaria* colony with the skeletal walls in the grayscale 2D image clearly brighter than the surrounding sediment matrix and the cement infilling the zooecial cavities. In the resulting 3D reconstruction, the zooecial cavities are colored red (Figure 1B). Most of the exterior frontal walls of the zooecia (except for what remains of the opesia (colored purple in Figure 1C) have been covered by secondary calcification (colored green in Figure 1C). This allows us to infer that the the imaged fragment is from the proximal part of a colony because in some anascan cheilostomes, secondary calcification occurs in the older proximal parts of colonies (Sandberg 1983). In a close up of Figure 1B, the exterior surface of the zooecial cavities is colored green, a transverse 2D section through the colony is in grayscale (Figure 1D). The protuberances on the zooecial cavities (Figure 1D) are interpreted as opesia.

Figure 2 shows a 3D rendering of a branching *Siphonicytara* colony. The colony surface is colored in grayscale with orifices in green (Figure 2A) and the whorled zooecial cavities in green revealing opesia (Figure 2B). One of the zooids in the right-hand branch (just above the bifurcation) appears to show an orifice and latero-oral avicularium (Figure 2B arrow).

Figure 3 demonstrates the usefulness of the SRµCT technology by integrating traditionally oriented 2D sections with a 3D reconstruction of a *Trigonopora* colony. The colony surface is colored yellowish-brown with darker orifices (Figure 3A) with standard longitudinal (Figure 3B) and transverse (Figure 3C) sections in grayscale with skeletal walls brighter than surrounding sediment matrix and infilling cements.

The most challenging 3D rendering was a colony of *Reteporella*. The reticulate zoarial habit made it hard to isolate the target colony from other colony fragments in the sediment matrix

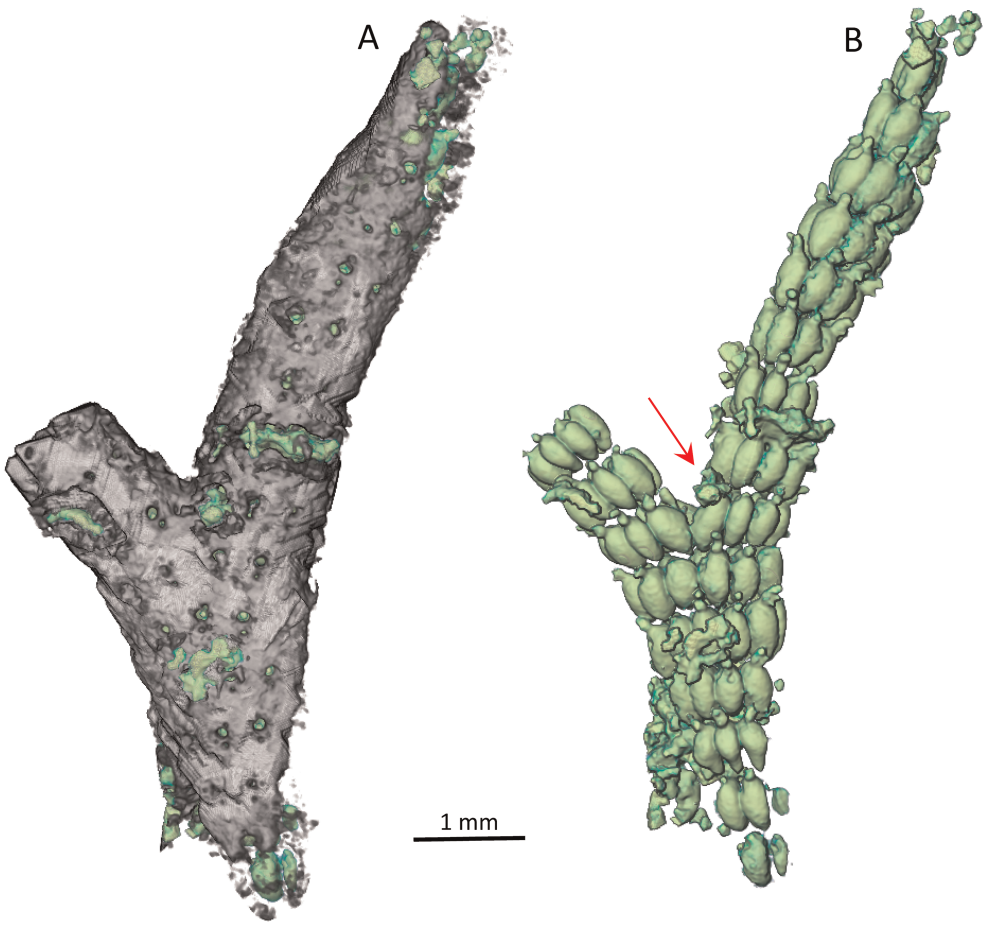

Figure 2. SRμCT 3D renderings of a branching colony of the cheilostome bryozoan *Siphonicytara*. A, colony surface is colored in grayscale with orifices in green. B, whorled zooecial cavities in green revealing opesia. One of the zooids in the right-hand branch (just above the bifurcation) appears to show an orifice and latero-oral avicularium (arrow).

(Figure 4A). The target colony surface is colored yellow with green orifices (Figure 4B) connected to the systematically arranged zooecial cavities in green (Figure 4C).

5 DISCUSSION

There are several benefits to SRμCT imaging of bryozoan fossils embedded in chert compared to more traditional thin sectioning and acetate peel technologies:

- It is non-destructive. Archaeologists, Traditional Owners, and museum curators of type specimens are reluctant to allow any destructive analysis of irreplaceable chert artifacts and type material such as by thin sectioning or acetate peels.
- The data processing makes creating 3D reconstructions of bryozoan colonies equally labor-intensive as serial sectioning, but the advantage is that you have a fully digital serial sectioned volume to explore in one dataset.
- A digital volumetric dataset can be sectioned and explored in any orientation non-destructively, something that is virtually impossible with thin sections. Therefore, it is easier to rotate the bryozoan colony into exact orthogonal section orientation (i.e. longitudinal vs. tangential vs. transverse) than with thin sections or acetate peels.
- It is easier to relate 2D images to 3D colony renderings as the technology provides both stand-alone 2D images and integrated 3D rendered colonies.
- Can make useful fly-through videos which facilitate relating 2D thin section views to 3D morphology and makes the results more useful for teaching and more accessible for other interested parties.
- Can easily distinguish skeletal walls and zooecial cavities.

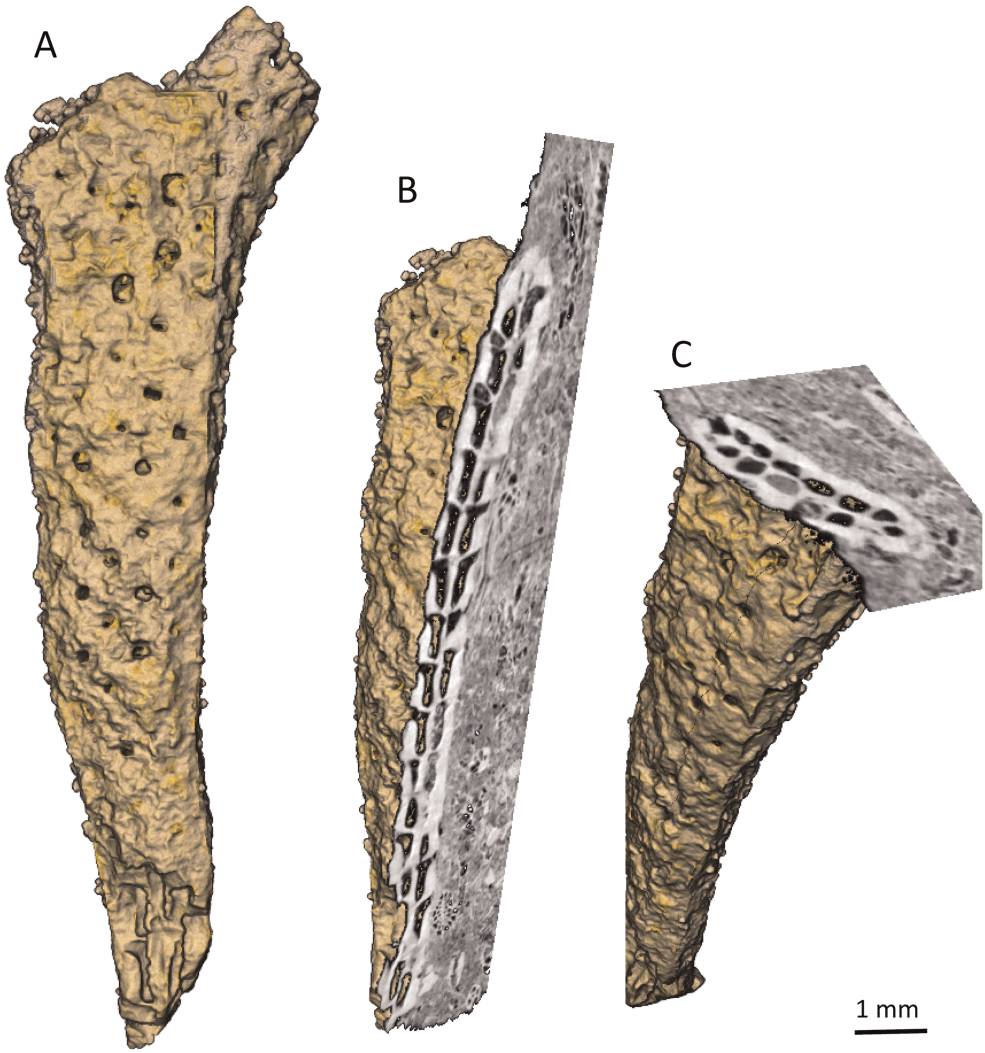

Figure 3. SRμCT 3D renderings of a blade-like colony of the cheilostome bryozoan *Trigonopora*. A, colony surface is colored yellowish-brown with darker orifices. Longitudinal (B) and transverse (C) 2D sections in grayscale with skeletal walls brighter than surrounding sediment matrix and infilling cements.

- 3D imaging allows easier inclusion of species-diagnostic internal zooecial morphology into taxonomic descriptions. Such characters are generally absent in bryozoology studies due to the lack of access to the zooecial interiors.
- 3D imaging allows easier inclusion of colony morphology characters into taxonomic descriptions. In fossils, the 3D colony morphology of a colony is rarely preserved due to burial, compaction, and fragmentation (Key *et al.* 2016). For example, the anastomosing branches of a net-like erect rigid fenestrate (reteporiform) colony of *Reteporella* would not be preserved in a fossil unless it was preserved on a bedding plane after burial (e.g. Suárez Andrés & Wyse Jackson 2015, fig. 3B).
- Compared to laboratory-based μCT systems, SRμCT 1) can typically image specimens an order of magnitude larger, and 2) image acquisition is much faster due to the greater X-ray flux.

Compared to more traditional thin sectioning and acetate peel technologies, there are several disadvantages to using SRμCT technology to image bryozoan fossils embedded in chert:

- Access to SRμCT technology is more geographically restricted (i.e. only ~90 synchrotrons globally). For example, not all artifacts can be

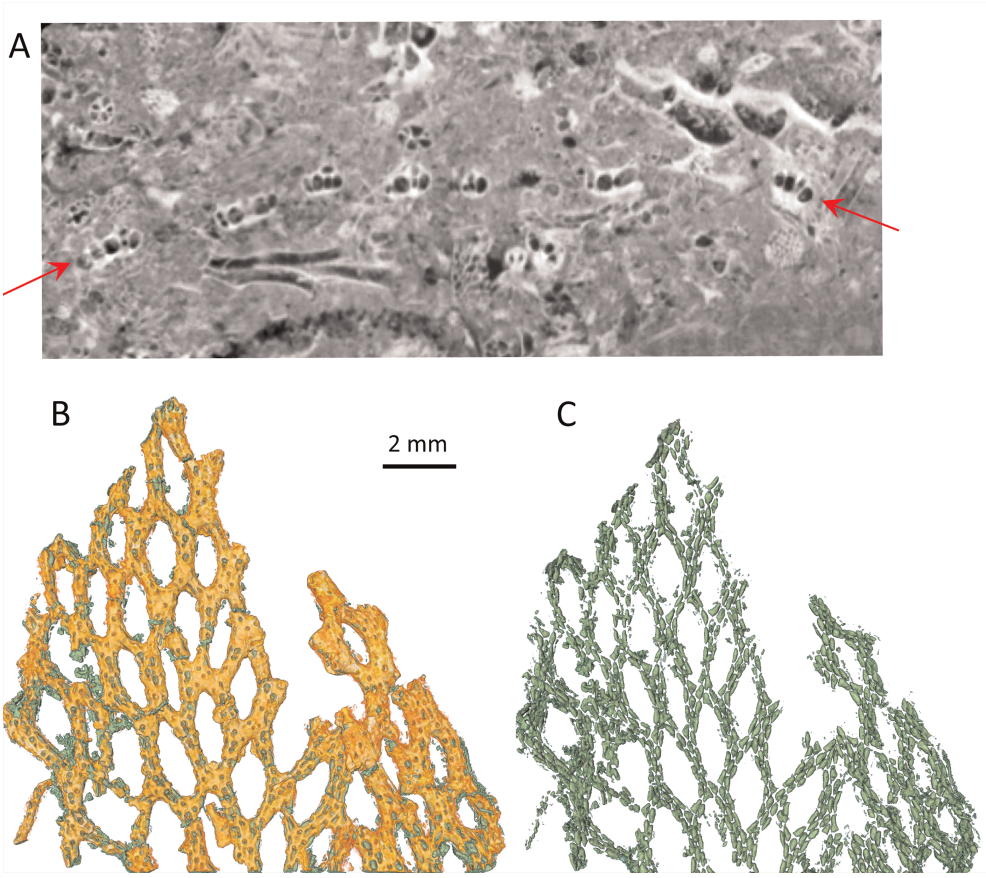

Figure 4. SRμCT images of the cheilostome bryozoan *Reteporella*. A, reticulate zoarial habit in cross section indicated by red arrows at each end of the colony. Brighter skeletal walls are surrounded by darker sediment matrix and even darker cement infilling zooecial cavities. B, 3D rendering with colony surface colored orange. C, systematically arranged zooecial cavities in green.

transported interstate or even overseas, without traditional owner permissions.

- Not all synchrotrons have beamlines suitable for imaging relatively large and dense objects such as chert artifacts with embedded bryozoans. The Australian synchrotron IMBL can handle specimens up to 30 x 40 mm.
- It is expensive, although access grants are often available. For example, the Australian synchrotron costs $800 AU ($575 US) per hour with a four-hour minimum usage.
- The post-processing of 3D tomographic data is time consuming due to the large amount of data. It may be as time consuming as thin sectioning and is best done by experts. Fortunately, this processing is getting faster due to machine learning and semi-automatic segmentation methods.
- Poor preservation of the fossils themselves is still a potential problem.
- The resolution is still not as good as thin section photographs (Ward *et al.* 2019a, fig. 10) or SEM images (Martha *et al.* 2019), which is required for species level identification. Higher resolution sub-micron X-ray CT scanning facilities exist as both synchrotron and laboratory-based X-ray sources and could provide greater spatial resolution, thereby providing more detailed taxonomic identification of embedded bryozoan fossils (Huisman *et al.* 2014). However, most laboratory-based μCT sources have limited flux, making the imaging and analysis of large/dense samples impractical. Sub-micron scale X-ray CT beamlines are now available. The one at the Australian Synchrotron is under construction and is planned to be available from 2022. Another solution is to scan samples before thin sectioning and combine the data from both into a single 3D data set.

6 CONCLUSIONS

This is a useful technique for 3D imaging of bryozoans in artifacts when destructive methods are not an option due to their cultural value. This study provides only the second known non-destructive synchrotron imaging of bryozoan fossils preserved in chert. SRμCT 3D imaging is sufficient for some genus level identification, but not for the species level. The resolution should be better in non-silicified fossil bryozoans where the colony skeleton has not been replaced by secondary minerals as in this study. There are pros and cons to using SRμCT technology to image 3D fossil bryozoans. In total, the benefits outweigh the disadvantages.

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