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Three-dimensional imaging of the Jurassic radiolarian *Protunuma* ? *ochiensis* Matsuoka: an experimental study using high-resolution X-ray micro-computed tomography

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Key words: three-dimensional imaging, X-ray micro-computed tomography, laminated object manufacturing, Radiolaria, *Protunuma* ? *ochiensis*.

Abstract. To evaluate the utility of high-resolution micro-computed tomography (micro-CT) in observing radiolarian fossils, we examined the skeleton of the Jurassic radiolarian fossil *Protunuma* ? *ochiensis* Matsuoka using a micro-CT device. Although this species is a closed Nassellarian with a thick exterior wall, important taxonomic characters on the interior and exterior of the shell were represented almost perfectly in the acquired three-dimensional computer graphic images. These characters include those documented in the original description, such as the height and width of the skeleton, the outline, the number of segments, the pore arrangement and other features. In addition, the structure of the initial spicule of this species was revealed newly. An enlarged plaster radiolarian model printed using the laminated modelling method was useful for detailed observation. Nondestructive omnidirectional observation is an advantage of this method, which is not possible with scanning electron microscopy or optical microscopic investigations. Micro-CT technology would become an effective observational tool for radiolarian studies in the near future.

INTRODUCTION

X-ray computed tomography (CT), which is commonly used for medical and industrial purposes, is a nondestructive method of visualising and measuring the internal geometries of opaque objects. Micro-CT is specialised for visualising the structures of micrometre- to centimetre-sized objects with greatly increased resolution. This developing technolo-

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gy is being applied to a wide range of geological and palaeontological investigations (*e.g.* Ketcham, Carlson, 2001).

To evaluate the potential of micro-CT imaging technology in investigating microfossils, the authors have experimentally examined the skeletons of foraminifers, ostracodes, diatoms and radiolarians. For example, Matsuoka *et al.* (2012) reported the exact pore number of a specimen of the genus *Pantanellium* and represented its pore distribution on a spherical shell based on three-dimensional (3D) scanning data. Furthermore, Yoshino *et al.* (2014) presented a method that uses 3D information to automatically determine the pore numbers of spherical radiolarian skeletons.

In this study, we present the results of 3D scanning of the Jurassic radiolarian *Protunuma* ? *ochiensis* Matsuoka using a high-resolution micro-CT scanner. Although closed Nassellarians, including this species, are commonly recovered from Jurassic sediments, conventional methods cannot be used for nondestructive observation of a combination of both their surface textures and their internal structures. Observation using high-resolution micro-CT overcomes this difficulty. This study introduces the observation of radiolarian fossils using 3D imaging methods and emphasises the utility of high-resolution micro-CT for palaeontological investigations.

METHODS AND MATERIALS

MICRO-CT DEVICE

A micro-scanning system using a micro-CT device (Micro-CT in SEM; SkyScan, Belgium) was tested in the Department of Mechanical Engineering, Faculty of Science and Engineering of Setsunan University for this study (Fig. 1). This device consists of several modules attached to a scanning electron microscope (SEM) (JSM-6510; JEOL, Japan), with a high-resolution X-ray detector (format: 512×512 pixels; highest resolution: 600 nm/pixel) capable of imaging millimetre- to micrometre-sized structures.

STUDY SAMPLE

The specimens of *Protunuma* ? *ochiensis* Matsuoka used in this study were recovered from rock sample HKW-bk01 using dilute (5%) hydrofluoric acid. The sample is part of a tuffaceous mudstone boulder from the Hikawa Formation, which is an Upper Jurassic (Oxfordian to Tithonian) trench-slope basin deposit outcropping in the Kanto Mountains, Tokyo Metropolis, Japan (Ishida, 2004, 2011). Radiolarian fossils from HKW-bk01 such as *Kilinora spiralis*, *K. hemicostata*, *K. tecta* and *Striatojaponocapsa conexa* correlate with the characteristic radiolarian assemblage in the lowest part of the

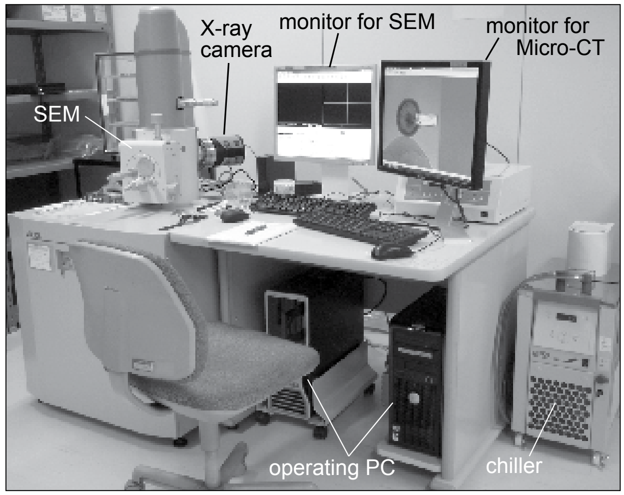

Fig. 1. Overview of the three-dimensional scanning device (Micro-CT) used in this study

Attachments for micro-computed tomography scanning, rotational stage and X-ray imager were added to the scanning electron micrograph (SEM)

K. spiralis Zone of Matsuoka (1995) and indicate a latest Middle Jurassic (Late Callovian) age (Ishida, 2015). *Protunuma* ? *ochiensis* is a four-segmented closed Nassellaria that has been recorded from Middle to Upper Jurassic sediments in Japan and the Tethys region (*e.g.* Baumgartner *et al.*, 1995; O'Dogherty *et al.*, 2005). This species was first described by Matsuoka (1983) based on observations with a biological microscope and an SEM.

SCANNING AND POST-HANDLING

SAMPLE SETTING AND SCANNING

A unique sample holder for the microfossils was created for this study (Fig. 2A). An uncoated specimen of *Protunuma* ? *ochiensis* was mounted with woodworking glue on the top of the holder under a stereomicroscope (Fig. 2A). The holder was inset on a rotating stage that was attached to a specimen chamber in place of the standard SEM stub (Fig. 2B).

After evacuation, an SEM electron beam set at 30 kV and 0.085 μA hit a brass target to produce X-ray radiation. A projected shadow of the specimen was acquired by the X-ray detector (a cooled back-illuminated charge-coupled device, or CCD) positioned on the flange on the side of the SEM specimen chamber (Fig. 1). Magnification of the shadow was controlled by the distance between the X-ray emission point and the specimen. The X-ray source was 167.25 mm from the detector, and the distance between the source and the specimen was 4.30 mm.

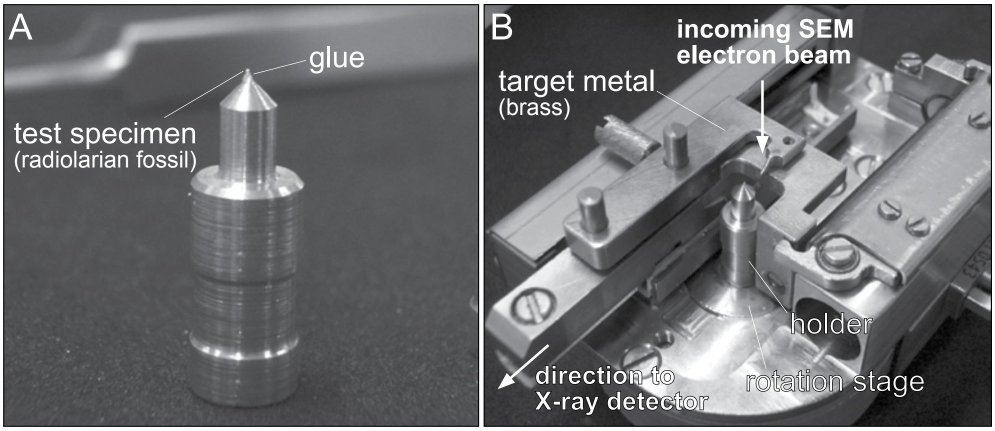

Fig. 2. Sample setting for X-ray scanning

A. A radiolarian specimen was mounted on a sample holder using woodworking glue. Height of the holder is 17.5 mm. **B.** The holder was mounted on a rotational stage near the brass target producing X-ray radiation

Before scanning began, parameters to improve the signal-to-noise ratio of the images, like the exposure time, number of composite images and gain of images, were adjusted. Two-dimensional (2D) X-ray images of the projected shadow were sequentially recorded in 16-bit Tag Image File Format (TIFF). In total, 410 X-ray images were taken in half-rotation (180° rotation) mode with a 0.45° angular step; scanning took 62 minutes. The internal structures are visible through the thick silica wall in the acquired images (Fig. 3).

RECONSTRUCTION

The set of X-ray images was "reconstructed" into cross sections along the rotational axis of the specimen by the equipment image processing software (Fig. 4). Computer graphic (CG) images synthesised from these cross sections were displayed using viewing software, and morphometric features such as length, volume and angle were analysed.

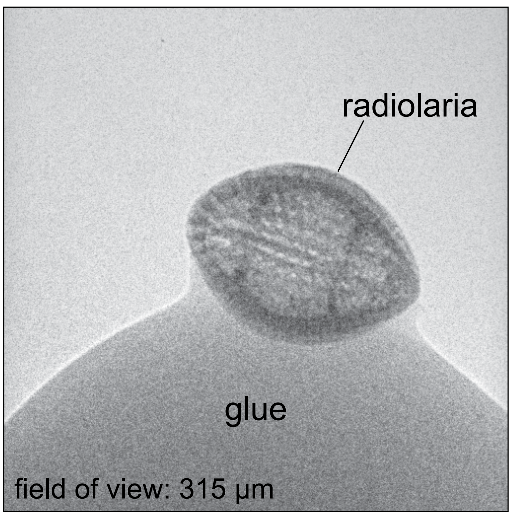

Fig. 3. Example of an X-ray photograph of a specimen of *Protunuma* ? *ochiensis* taken using the micro-computed tomography device.

A sequence of 410 images was recorded during a single scanning

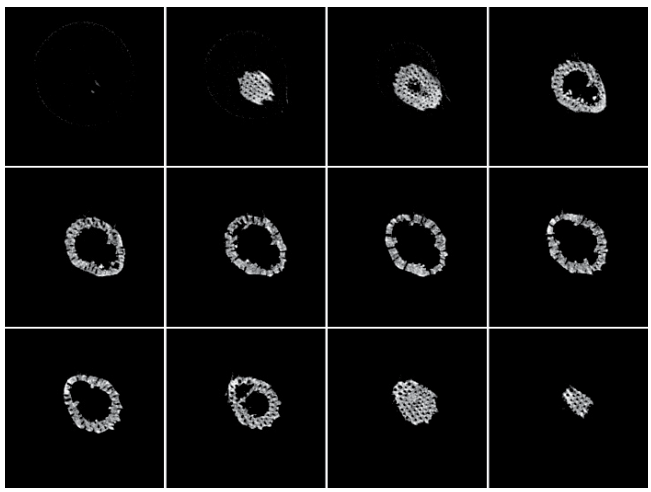

Fig. 4. Cross-sectional images along the rotational axis of the specimen reconstructed from the sequence of X-ray images

SOLID MODELLING

The CG images were saved in Standard Triangulated Language (STL) format for solid modelling. A plaster model of the specimen was printed with a 3D printer using powder laminated manufacturing (ZPrinter 450; 3D Systems, USA). The layer thickness of one lamina was *c.* 0.1 mm. The total height of the plaster model of *Protunuma* ? *ochiensis* is 15.6 cm and is *c.* 1600 times larger than the original.

RESULT AND DISCUSSION

UTILITY FOR PALAEONTOLOGICAL APPLICATION

The CG images of this specimen acquired using this technique can be viewed omnidirectionally on a computer screen (Fig. 5). Although this species has a thick wall (*c.* 10 μm) (A-2 of Fig. 6), the internal structures could be visualised through the wall. The palaeontological characters of this specimen observed in the CG and the plaster model are described as follows.

The specimen has a spindle outline without an aperture. The software calculated the total height and maximum width of the specimen as 97 and 78 μm, respectively. The 18 specimens measured by Matsuoka (1983) ranged from 118 to 183 μm (mean 148 μm) in height and 85 to 126 μm (mean 105 μm) in width; therefore, this specimen is particularly small. This specimen also lacks an apical horn.

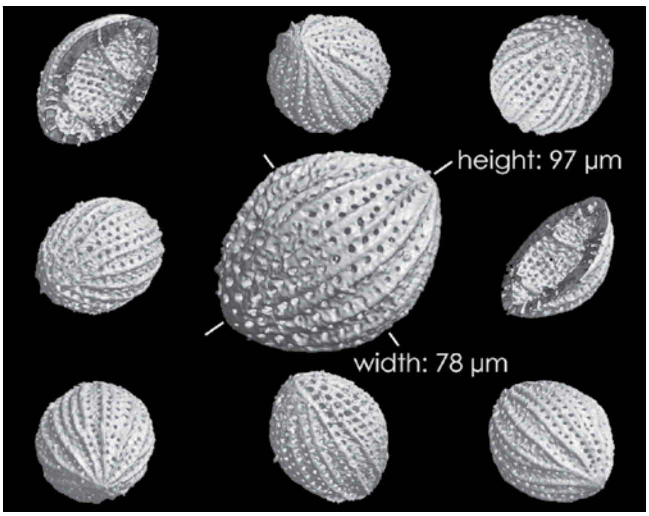

Fig. 5. Omnidirectional observation of *Protunuma* ? *ochiensis*

These computer graphic images were synthesised from the X-ray cross-sectional frames shown in Figure 4

Longitudinal plicae are visible on the surface of the specimen and run continuously through the segments (A-1 of Figs 6, 7A). Twenty-one plicae were counted on the equatorial plane (A-3, 4 of Fig. 6). Some of these converge with neighbouring plicae near the proximal and distal portions of the specimen. One to three rows of small circular pores without pore flame are aligned between adjacent plicae. The pores around the distal end are larger than those in the upper and middle part.

Four segments were observed internally (A-2 of Fig. 6 and Fig. 7B). The first segment, namely cephalis, is round, poreless and thickly walled. An initial spicule at the base of cephalis (A-5 of Fig. 6) is composed of median bar (MB), vertical spine (V), dorsal spine (D), right lateral spine (Lr) and left lateral spine (Ll), and has a cruciform structure surrounded by a collar ring (CR) (A-6 of Fig. 6). This cephalic skeletal structure corresponds to that of the "*Arcanicapsa*-type" of Takemura (1986). Two platy ring-shaped septa located in the upper and lower parts partition the inner space. The inner edges of the septa are rimmed by a slight bulge (Fig. 7B).

The taxonomic characters, particularly those documented in the original description such as outline, number of segments and pore arrangement were represented almost perfectly in the CG and plaster models. In addition, the structure of the initial spicule was elucidated newly. Thus, the micro-CT device is suitable for the palaeontological description of this species.

ADVANTAGES OVER CONVENTIONAL METHODS

We prepared several other specimens of *Protunuma* ? *ochiensis* for observation using an SEM and a biological microscope for comparison. Both methods have conventionally been used for the description of radiolarian skeletons, including the original description of *Protunuma* ? *ochiensis*. Some specimens were laid out on a stub with gold evaporation coating for SEM and others were mounted on glass slides for biological microscope observation.

The SEM showed the surface of the specimens as 2D images in good detail. Some taxonomic characters such as shell outline, pore arrangement and surface ornamentation were clearly visible (B-1, 2 of Fig. 6), but the internal structures cannot be observed using this method without destroying shells. In contrast, the biological microscope using transmitted light clearly showed internal structures like the number, location and shape of segments (C-1 of Fig. 6), as well as shell characters like thickness and pore arrangement (C-2 of Fig. 6). However, faint textures on the shell surface and cephalic skeletal structure were barely visible. In both methods, specimens are fixed and the angle of view is restricted; there-

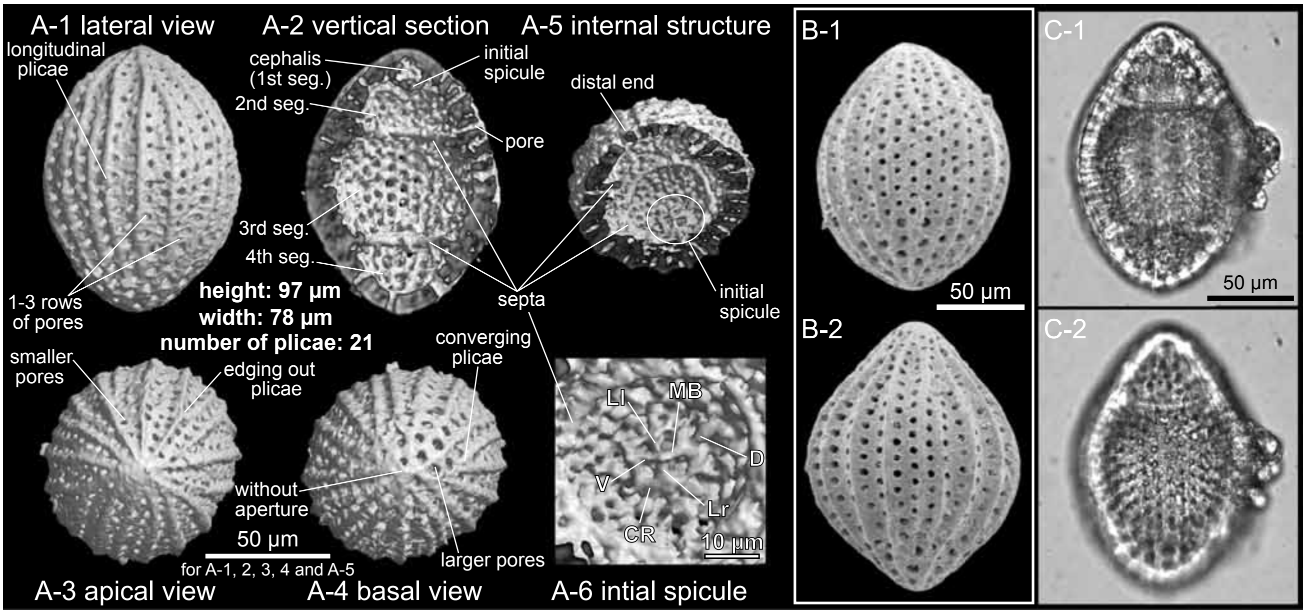

Fig. 6. Computer graphics of *Protunuma* ? *ochiensis* showing taxonomic characters of this species

A-1 – lateral view; **A-2** – vertical section; **A-3** – apical view; **A-4** – basal view; **A-5** – internal structure; **A-6** – close-up view of initial spicule: MB – median bar, V – vertical spine, D – dorsal spine, Lr – right lateral spine, Ll – left lateral spine, CR – collar ring. Micrographs of other specimens of *Protunuma* ? *ochiensis* taken with a scanning electron microscope **B-1, 2** and a biological microscope **C-1, 2** are shown for comparison

fore, neither method can be used to observe the complete suite of internal and external characters for a single specimen.

The micro-CT method was demonstrated to show taxonomic characters at a comparable level of resolution to conventional methods. In addition, an overwhelming advantage of this new method is the simultaneous observation of the internal and external structures of a single specimen, including closed radiolarian groups.

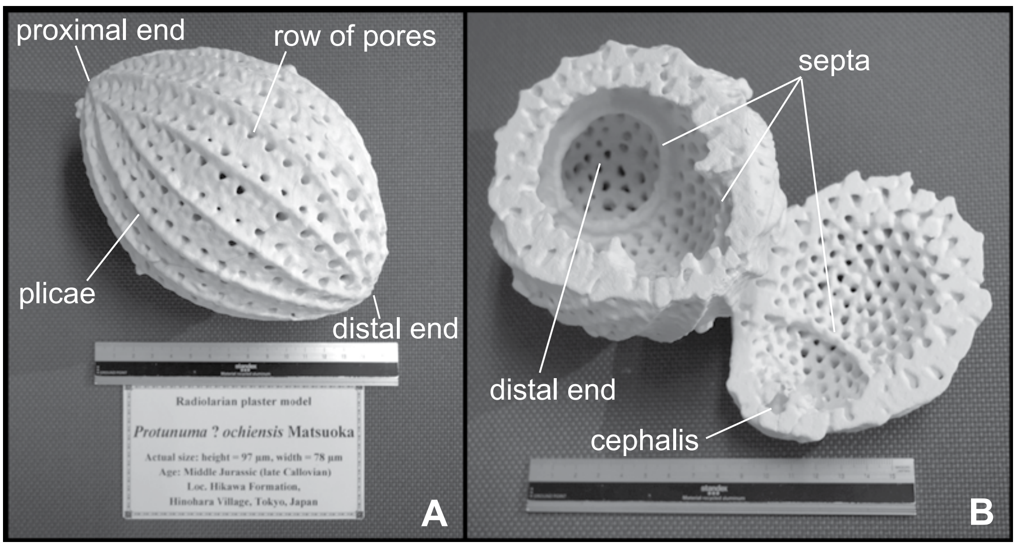

Fig. 7. An enlarged plaster model of the specimen that was printed using a three-dimensional printer

A – external view of the model. Spindle outline, rows of pores and plicae connecting the proximal and distal ends are represented; total height of this model is 15.5 cm. **B** – internal side of the half split model. The cephalis and two septa are visible

CONCLUDING REMARKS

Excellent 3D data for *Protunuma* ? *ochiensis* were acquired using a high-resolution X-ray micro-CT device in this experimental investigation. Computer graphics and a plaster model created from the 3D data showed almost all of the taxonomic characters of this thick-walled closed-shell species, including the height and width of the skeleton, outline, number of segments, pore arrangement and cephalic skeletal structure. Although a few minor matters such as the strict calibration of distortion and threshold value settings for data-processing remain to be resolved, the technical procedure for radiolarian examination has been established. Adequate resolution for radiolarian observation has been established for this technique because we have succeeded in scanning more delicate radiolarian structures such as the initial spicule of recent Nassellarians (Ishida *et al.*, 2013). Moreover, the 3D imagery is anticipated to provide accurate geometric models for skeletons of microfossils that have been presented previously (Yoshino *et al.*, 2009, 2012). Thus, 3D imaging using high-resolution micro-CT would become an effective method for radiolarian investigations in the near future.

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