Inner Structure of Cretaceous Fossil Flower Revealed by X-Ray Microtomography (XRMT)

Flowering plants (angiosperms) consisting of more than 350,000 living species dominate the vegetation of most terrestrial ecosystems. The origin and early evolution of angiosperms had remained as an abominable mystery in evolutionary biology in the last twenty-five years. Recent paleobotanical studies of the early fossil history of angiosperms have, however, been revolutionized with the discovery of small and well-preserved three-dimensional fossil flowers (mesofossils) from Cretaceous between 125 and 65 million years before present. The mesofossils that are usually preserved as charcoal from ancient forest fires provide unrivalled insights into the structure, biology and evolutionary relationships of ancient angiosperms. The paleobotanical studies have greatly increased the quantity and quality of information available about the structure and relationships of Cretaceous flowers [1].

There are usually one or a few specimens available for the paleobotanical studies of mesofossils in Cretaceous, and synchrotron radiation X-ray microtomography (SRXTM) is a useful new tool for obtaining details of the internal structure without the need for destructive analysis. The SRXTM at beamline **BL20B2** is a powerful tool for obtaining a new level of detail from fossil angiosperm flowers from Cretaceous.

The Kamikitaba plant mesofossil assemblage, the first and unique record of mesofossil preservation from eastern Asia, was isolated from carbonaceous, black, poor-sorted sandy siltstone (Late Cretaceous; early Coniacian, *ca*. 89 million years before present) in Fukushima Prefecture, Japan [2]. After drying in the laboratory, the sedimentary rock samples were disaggregated in water and sieved through a 150 μm mesh. The carbonaceous debris recovered was then cleaned of adhering mineral material by treatment with hydrofluoric and hydrochloric acids, thoroughly rinsed in water, and dried in air. Individual specimens were separated by picking under a dissecting microscope. The assemblage includes well-preserved angiosperm flowers, fruits, seeds, leaf fragments and wood [2].

During the study of the Kamikitaba assemblage, a new specific and well-preserved fossil flower, less than 3 mm in diameter, was recovered from the assemblage [3]. The fossil flower was observed in detail with FE-SEM (field emission scanning electron microscopy) and then exposed to an 8-keV X-ray at beamline BL20B2 for microtomography analysis to characterize the internal structures. The three-dimensional microtomography (micro-CT) images of the fossil flower were reconstructed with Tri3DVOL software by Ratoc System Engineering Co. (Tokyo). These reconstructions also allowed the electronic removal of the androecium (male organ) to better reveal the structure of the gynoecium (female organ).

The structure of the fossil flower revealed by SRXTM at beamline BL20B2 is as follows. The fossil flower is small, pedicellate, bisexual, and actinomorphic (Fig. 1). The floral receptacle is flattened and disk-shaped, with a perianth consisting of a small number of tepals, born in at least two cycles, around the rim. The androecium (male organ) comprises ~90-100 stamens that are curved toward the center of the flower. Stamens are stout, with no clear differentiation into anther (pollen organ) and filament. The thecae (pollen sacs) are lateral to abaxial in position, extended for most of the length of the stamen, and have latrorse-extrorse dehiscence. The connective is expanded above the thecae into a prominent, flattened, sterile apical appendage. The gynoecium (female organ) is composed of ~100-120 free carpels born on a small, conical projection in the center of the receptacle (Fig. 2). SRXTM now provides new information on important organizational features of the fossil flower and therefore allows a more thorough discussion of the phylogenetic position of the fossil flower. On the basis of comparison with extant plants, the fossil flower is established a new fossil species of Annonaceae (Custard-Apple family)

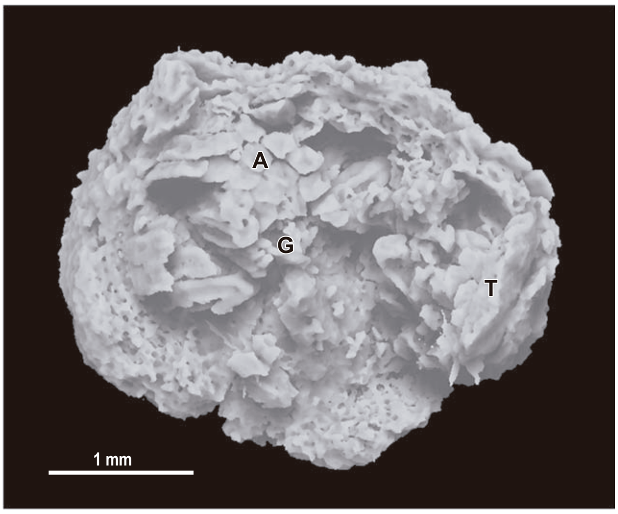

Fig. 1. XRMT image of fossil flower of *Futabanthus asamigawaensis* Takahashi *et al.* (Annonaceae) from the Futaba Group (89 Ma, Late Cretaceous) of northeastern Japan. Scale bar = 1 mm. T: tepal (perianth), A: androecium (male organ), G: gynoecium (female organ).

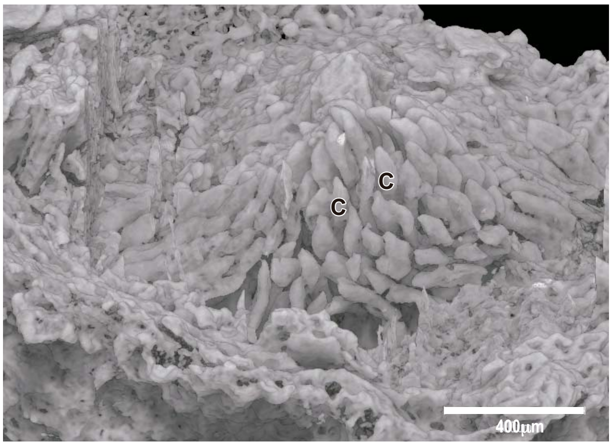

Fig. 2. Lateral view of closely packed carpels revealed after stamens are removed from the XRMT image. Scale bar = 400 µm. C: carpel.

under a scientific name, *Futabanthus asamigawaensis* Takahashi *et al.* (Fig. 3) [3].

The extant Annonaceae is a large family of chiefly tropical trees and shrubs, comprising ~128 genera and ~2500 species. The family is the most diverse group of basal angiosperms, some of which are used for their fruits. A most striking feature of the *Futabanthus* flower, compared with flowers of extant Annonaceae, is its very small size and the corresponding small size of the stamens and carpels. The small size of the fossil flower fits the consistent pattern observed when many other Late Cretaceous flowers are compared with flowers of their extant relatives. The fossil provides the earliest record of the family and documents the presence of Annonaceae in eastern Eurasia during the middle part of the Late Cretaceous. Evidence that Annonaceae were already differentiated in the Late Cretaceous of Asia is also interesting with respect to the biogeography of the family. Recognition of probable crown-group Annonaceae in the middle of the Late Cretaceous (Coniacian, 89 Ma) in Japan extends significantly the age of the family and indicates an earlier presence in eastern Eurasia than might have been expected. The results have been spectacular and promise to open up new levels of insight into the evolution of flowers and flowering plants from the age of Cretaceous.

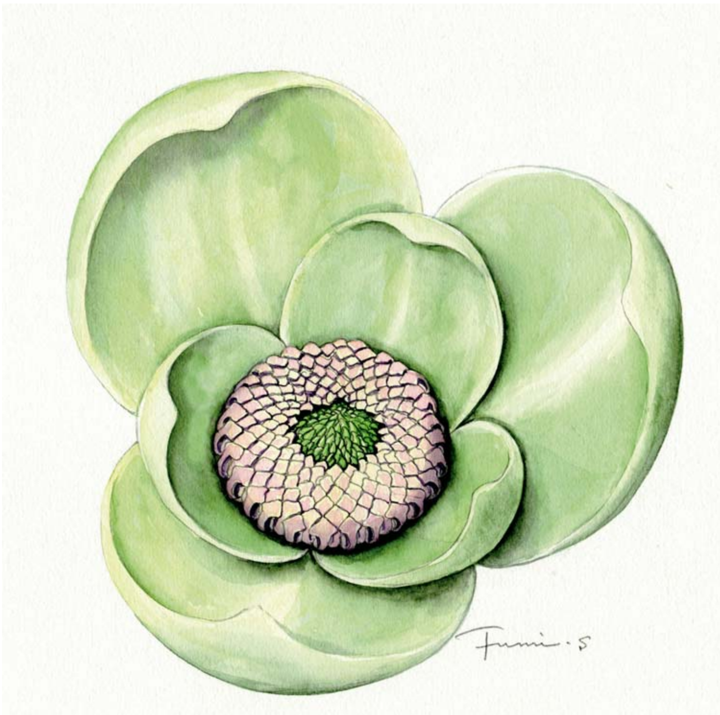

Fig. 3. Reconstructed illustration of Cretaceous fossil flower, showing two trimerous perianth cycles, numerous stamens, and numerous carpels.

Masamichi Takahashi

Graduate School of Science and Technology, Niigata University

E-mail: masa@env.sc.niigata-u.ac.jp

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