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ARTICLE

Visualizing Complex Anatomical Structure in Bamboo Nodes Based on X-ray Microtomography

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ABSTRACT

In recent years, bamboo has been widely used in a broad range of applications, a thorough understanding of the structural characteristics of bamboo nodes is essential for better processing and manufacturing of biomimetic materials. This study investigated the complex anatomical structure for the nodes of two bamboo species, *Indocalamus latifolius* (Keng) McClure and *Shibataea chinensis* Nakai, using a high-resolution X-ray microtomography (μCT). The results show that the vascular bundle system in the nodal region of *I. latifolius* and *S. chinensis* is a net-like structure composed of horizontal and axial vascular bundles. Furthermore, the fiber sheath surrounding metaxylem vessels tended to be shorter in the tangential direction. This structure of bamboo nodes facilitates the tangential and axial transport of moisture and nutrients. The anatomical structure of *I. latifolius* and *S. chinensis* nodes has obvious differences, especially in the arrangement of vascular bundles. Vascular bundle frequency was significantly higher in *S. chinensis* nodes than in *I. latifolius* nodes. These findings indicate that μCT is a non-destructive three-dimensional imaging method that can used to examine the anatomical structure of bamboo nodes.

KEYWORDS

Anatomical structure; bamboo nodes; *I. latifolius*; *S. chinensis*; X-ray microtomography (μCT)

1 Introduction

Bamboo is one of the most important forest resources, especially in Asian countries. With its excellent mechanical properties, fast-growth rate, high-yield, and renewable nature, bamboo is widely used for various products, such as paper, furniture, and structural composites. A bamboo culm consists of a linear series of hollow internodes and solid nodes [1]. In the internode region, all cells are strictly aligned in the axial direction, resulting in the bamboo culms being easily split by force [2–4]. In the nodal region, some horizontal vascular bundles appear on the diaphragm [5,6]. The presence of horizontal vascular bundles not only strengthens the uprightness of bamboo culms [3,7] but also plays a key role in the transversal transport of water and nutrients [5,6,8,9]. The unique anatomical structure of bamboo is well-known to determine its physical and mechanical properties. Therefore, a better understanding of the structural characteristics of bamboo, especially the bamboo nodes, will lead to a better understanding of its function and performance, and inspire the design of biomimetic materials.

Due to the complex arrangement of vascular bundles in the nodal region, the anatomical structure of the nodes has been less studied than that of the internode. Bamboo nodes consist of a sheath scar, diaphragm, nodal ridge, and intra-node. Grosser et al. first investigated the anatomical characteristics of bamboo nodes. This study reported that the most significant difference between the internodes and the nodes occurred in the horizontal vascular bundles of the nodes [10]. Since, several other studies have focused on the complex arrangement of vascular bundles in the nodal region. These studies showed that most vascular bundles that passed into the diaphragm were derived from the inner part of the bamboo culm, and the other part came from the periphery [3,11,12]. Also, Ding et al. [6] reconstructed a three-dimensional (3D) image of the nodal region based on the serial sections of a sample to visualize the internal structure of the nodes. In the above-mentioned studies, the structural characteristics of bamboo nodes were studied by serial sectioning methods. However, such serial sectioning methods have disadvantages, e.g., they are invasive and time-consuming, thus non-invasive and high-resolution approaches are needed.

X-ray-computed microtomography (μCT), a high-resolution non-invasive imaging technique, has been increasingly used in wood and bamboo research to explore internal structure [9,13−15], density [16,17], knots [18,19], and fracture characteristics [20,21] of plant culms. Peng et al. [5] first used μCT to examine the complex vascular system in the nodal region [3]. Palombini et al. [9] presented one of the first 3D microstructural reconstructions of the complex nodal vascular system of monocots, identifying its arrangement within the node and tracking the lateral movement of the vascular bundles. The above-cited studies found that branching types were a major factor that strongly influences the anatomy structure of bamboo nodes. However, there has not been research on the anatomical structure of different branch types in the nodal region.

The study presents 2D and 3D characterization of the morphology and the structural analysis of a single-branched type (*Indocalamus latifolius* (Keng) McClure) and multiple-branched type (*Shibataea chinensis* Nakai) bamboo nodes using a high-resolution μCT. *I. latifolius* is a shrubby bamboo species, up to 2 m tall and 0.5–1.5 cm diameter, commonly distributed in eastern China. Its culm is used as raw materials for chopsticks and penholder. The species *S. chinensis* is one ornamental bamboo, up to 1 meter height and 2–3 millimeter diameter, mainly distributed in Jiangsu, Anhui, and Fujian province, China. This study will provide critical data for further construction of a bamboo nodes database.

2 Materials and Methods

2.1 Material

To characterize the anatomical structure of the bamboo nodes, samples of *I. latifolius* and *S. chinensis* were collected from a bamboo plantation of Taiping Lake Base in Anhui Province, China. *I. latifolius* culm belongs to the single-branched type, and *S. chinensis* culm belongs to the multiple-branched type [22]. Mature bamboo plants (3-year-olds) were used for all experiments (Fig. 1a). The complete specimen of bamboo nodes was cut from the 10th nodes of the bamboo culm and then brought back to the laboratory. The air-dried specimens were mounted on the holder with an aluminum tube as an adapter (Fig. 1b) and stored at room temperature until the imaging experiments were carried out.

2.2 Methods

As a non-invasive and non-destructive detection method, μCT can obtain a 2D slice imaging of a sample based on the attenuation of the radiation. The scans were performed with a laboratory scaled X-ray-microscope (Xradia510 Versa; Carl Zeiss (Shanghai) Co., Ltd., Shanghai, China) entailing geometric and optical magnification. It was possible to place the specimens entirely within the equipment's field of view because of their relatively small sizes. The 510 Versa has a sealed transmission source that reaches 160 kV and 10 W, with a voxel resolution ranging from 3 μm to 20 μm, depending on the size of the region of interest (ROI). The detailed parameters of scanning for the bamboo nodes are listed in Tab. 1. The sample was mounted on a stage and rotated 360° around its central axis to produce a series of XY-, YZ-, and XZ-projected images. After scanning, the background image was subtracted from the projected image. Then, the tomographic image was obtained by using an FDK reconstruction algorithm which is based on the GPU acceleration (Fig. 1c). Finally, the tomographic images were imported into ZEISS XM 3DViewer software for 3D reconstruction (Fig. 1d).

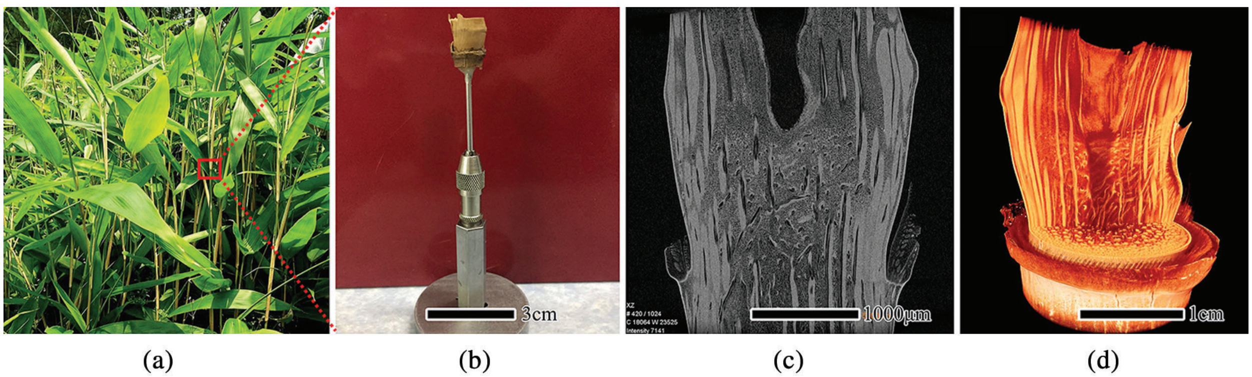

Figure 1: The sample preparation process of bamboo nodes for μCT, mature bamboo stem (a), the dried specimens of the bamboo nodes (b), μCT image of axial section (c), 3D reconstruction image (d)

Table 1: Scanning parameters for the bamboo nodes

Bamboo species	Voxel resolution (μm)	Field of view (mm)	Voltage/power (kV/W)	Current (μA)	Exposure time (ms.)
I. latifolius	15.7	Φ15.7 × 15.7	50/4	80	4
S. chinensis	3.3	Φ3.3 × 0.3	40/3	75	1

The number of vascular bundle in the bamboo nodes was counted using the image-processing software Image J. The vascular bundle frequency was calculated according the following formula, where VBF is the vascular bundle frequency (No. mm^2), N is the total number of vascular bundle in the inner, the middle, and the outer of bamboo culm, and A is the transverse-sectional area of the inner, the middle, and the outer of bamboo culm:

$$VBF = N/A$$

3 Results and Discussion

3.1 Structure of I. latifolius Nodes

Fig. 2 shows the scanning area and the transverse section μCT slices of *I. latifolius* nodes. The nodes of *I. latifolius* are convex with an undeveloped branch on the outside of the culm (Fig. 2a). In the sheath scar, the unshed sheath leaf encloses the bamboo culm. The vascular bundles within the sheath leaf were arranged in 1–2 rounds with a larger fibrous cap near the dorsal side of the sheath leaf (Fig. 2b, white arrow). According to the vascular bundle classification method proposed by Liese in 1998 [1], the typical vascular bundle in the internodes of *I. latifolius* was classified as double broken-waist type, which consists of a central vascular strand and two fiber strands (outside and inside the central vascular strand) [22]. However, similar typical vascular bundles were not observed in the nodal region. The morphology of the vascular bundle in the nodal region was observed at high voxel resolution (Fig. 2c). It was found that the fiber sheath close to intercellular space was longer in the radial direction, while the fiber sheath surrounding metaxylem vessels tended to be shorter in the tangential direction. This finding is consistent with Grosser and Liese (1971) [10]. In the diaphragm, the frequency of vascular bundle (Tab. 2) and the proportion of fiber surrounding vessels decrease from the outer to the inner of the culm, while the size of the vessel becomes larger. Many horizontal tubular vascular bundles were observed in the transverse section (Fig. 2d, white arrow). In the nodal ridge, the thickness of the bamboo wall and the size of the vascular bundle at the nodal ridge were larger than those at other parts of the node (Fig. 2e). Such behavior is consistent with the description of bamboo nodal structure reported by Shao et al. [4].

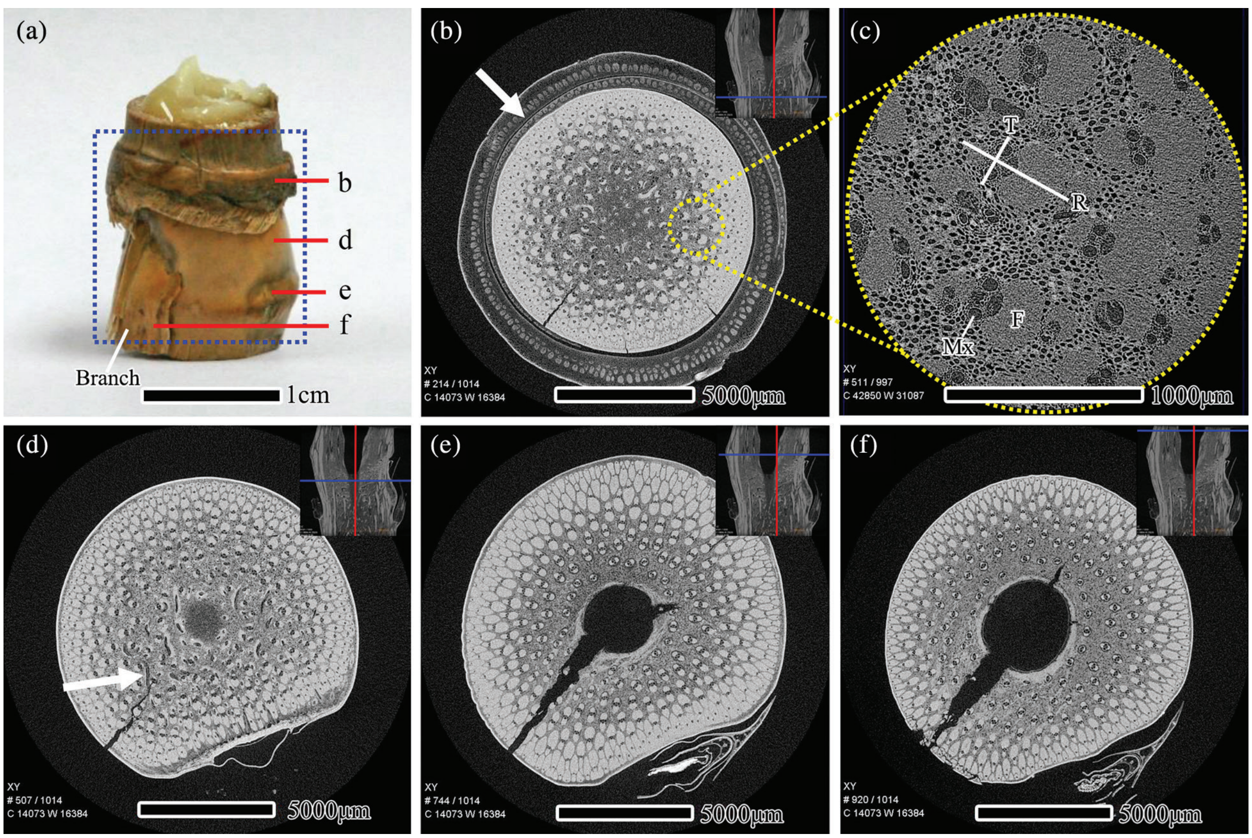

Figure 2: The scanning area and the transverse section μCT slices of *I. latifolius* nodes, (a) scanning area of the specimen, scale bars = 1 cm. μCT slices is located at the sheath scar (b), diaphragm (d), nodal ridge (e), the top region of the nodal ridge (f), scale bars = 5000 μm. (c) The vascular bundles in the nodal region with high magnification, scale bars = 1000 μm. Mx: Metaxylem, F: Fiber, R: Radial direction, T: Tangential direction

The axial slices of the *I. latifolius* node are depicted in Fig. 3. The horizontal and curved vascular bundles (Fig. 3a, white arrow) were observed at the inner of the bamboo culm along the bottom of the sheath scar, which illustrated that the vascular bundle in the nodal region begins to bifurcate below the sheath scar [6]. Therefore, the study of node anatomical structure cannot be limited between the nodal ridge and the sheath scar. Vascular bundles originating in the leaf sheaths insert into the outer area of the culm, merging with the vascular bundles in the outer area (Fig. 3a, dark arrow). In the diaphragm, the vascular bundles are numerous and larger near the periphery, and tended to be sparser and smaller toward the middle of the culm center (Fig. 3a). At the upper edge of the diaphragm, many small vascular bundles turn horizontally and twist repeatedly (Fig. 3b, yellow dotted box). Further observation of the small bundles at high voxel resolution reveals that the fibrous sheath surrounding the vessel is well-developed (Fig. 3c). Between the nodal ridge and the upper edge of the diaphragm, there are more fibers around the vessel than on the diaphragm.

Table 2: Statistical results from vascular bundle frequency of bamboo diaphragm

Bamboo species	Vascular bundle frequency (No. mm^2)		
	The inner	The middle	The outer
I. latifolius	2.37 ± 0.10c	3.73 ± 0.16b	5.93 ± 0.29a
S. chinensis	21.70 ± 1.07c	26.51 ± 1.01b	38.76 ± 1.97a

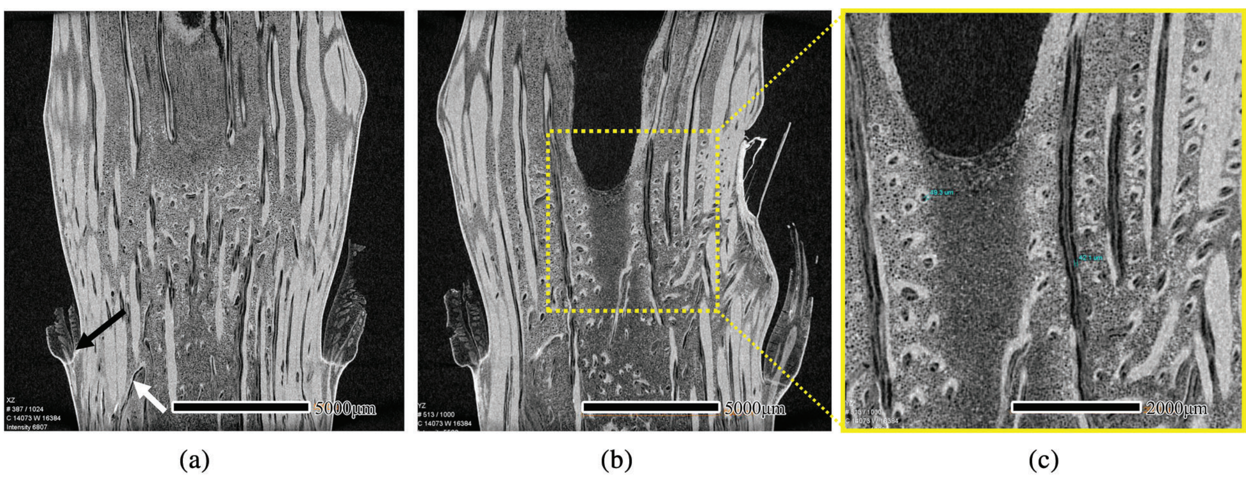

Figure 3: μCT scanning images of an axial section of *I. latifolius*. XZ (a) and YZ (b) of μCT images, scale bars = 5000 μm. Small vascular bundle at high voxel resolution (c), scale bars = 2000 μm

The 3D structure of *I. latifolius* nodes was reconstructed by continuous transverse and axial slices (Fig. 4). The different tissues are distinguished by color. The bright areas represent vascular bundles, whereas the dark areas represent parenchyma cells. Most vertically arranged vascular bundles near the peripheral of the nodal regions pass directly through the node, while those near the inner of the nodal regions pass into the diaphragm in the form of "Γ", and turn horizontally and twist repeatedly within the diaphragm (Fig. 4d). The finding is consistent with previous studies [3,10]. In the diaphragm, vascular bundle systems are a net-like structure composed of horizontal and axial vascular bundles. This network structure not only contributes to the mechanical properties, such as flex resistance and split resistance [3,7,23], but also facilitates the tangential and axial transport of moisture and nutrients [5,8,11].

3.2 Structure of S. Chinensis Nodes

The scanning area and transverse section of *S. chinensis* nodes are presented in Fig. 5. *S. chinensis* culm belongs to a multiple-branch type (Fig. 5a). By the transverse section from the bottom to the top of the nodal region, Figs. 5b–5e illustrates the development of branches into secondary axes. In Fig. 5b, the transverse section was taken close to the lower part of the sheath scar. The vascular bundle frequency of the vascular bundle of *S. chinensis* nodes decreases from the outer to the inner area of the culm (Tab. 2), which is similar to the results of the nodes of *I. latifolius*. A higher proportion of fiber surrounding vessels was observed in the outer of the culm than that in the inner (Figs. 5b–5d). In the diaphragm, vascular bundle frequency was significantly higher in *S. chinensis* nodes than in *I. latifolius* nodes (Tab. 2). In sections closer to the nodal ridge, bamboo culm was divided into two parts by the primary

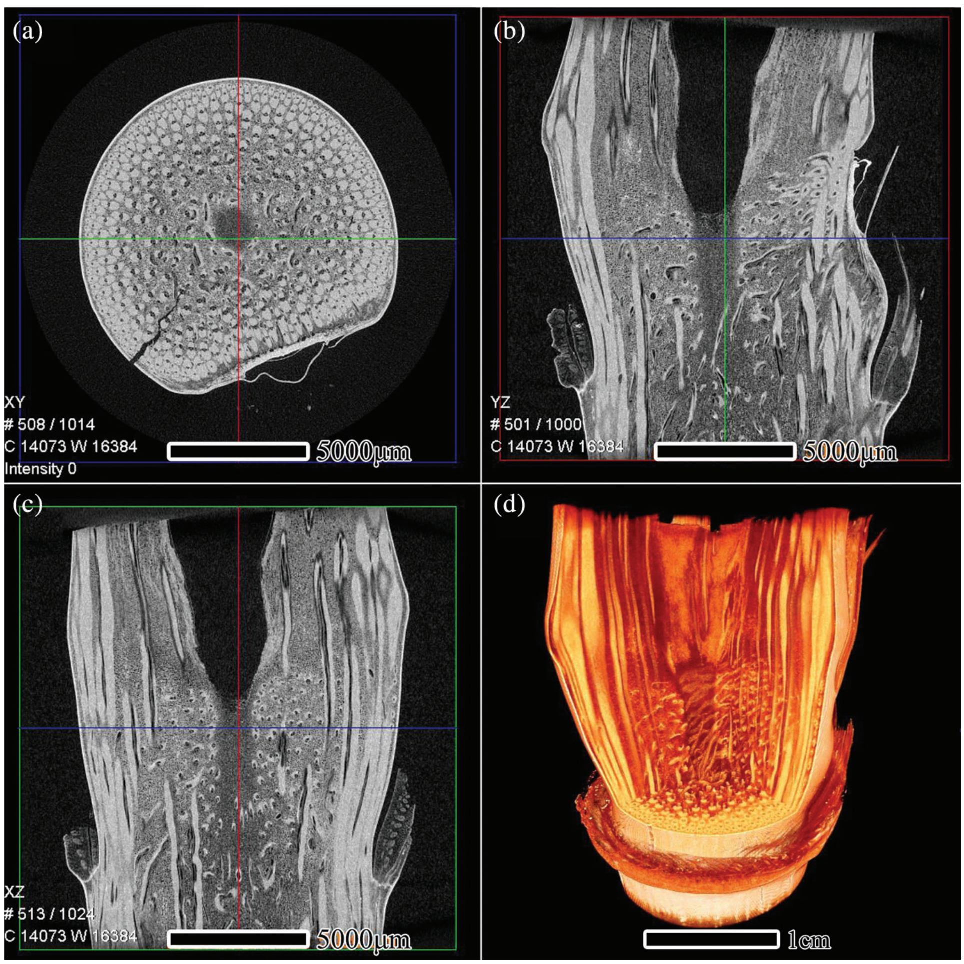

Figure 4: μCT reconstruction of the nodal region of *I. latifolius*, XY (a), YZ (b), and XZ (c) of μCT images, 3D reconstructed image (d)

axis and secondary axes. Horizontal tubular vascular bundles and fibrous cells were observed in the junction between the primary axis and secondary axes (Fig. 5e, white arrow). After distinctively separated into new axes in the top part of the nodal ridge, the size of the vascular bundle in the primary axis and secondary axes with a medullary cavity is larger than that without a medullary cavity (Fig. 5f).

The axial section of *S. chinensis* nodes is shown in Figs. 6a and 6b. The sheath leaf drops off the culm leaving the sheath scar at the sheath node. Many horizontal vascular bundles were observed in the diaphragm. This anatomical structure of the diaphragm may result from its biological functions, such as the transverse transport of moisture and nutrients within the bamboo node [6]. Some of the vascular bundles in the primary axis move transversally and bend into the secondary axes, and fiber cells were visible at the boundary between the primary axis and secondary axes. This structure helps strengthen the connection strength between the primary axis and secondary axes. Another study also postulated that stressed regions occurred mostly in the lower regions of secondary axes and in the interconnecting zones among other branches during compression [9]. Horizontal vascular bundles were observed on the bamboo wall without a medullary cavity in the primary axis or secondary axes; all cells in the bamboo wall recovered the axial alignment when there was a medullary cavity in the primary axis or secondary axes. This is consistent with results of Peng et al. for nodes of *Pleioblastus gozadakensis* [5].

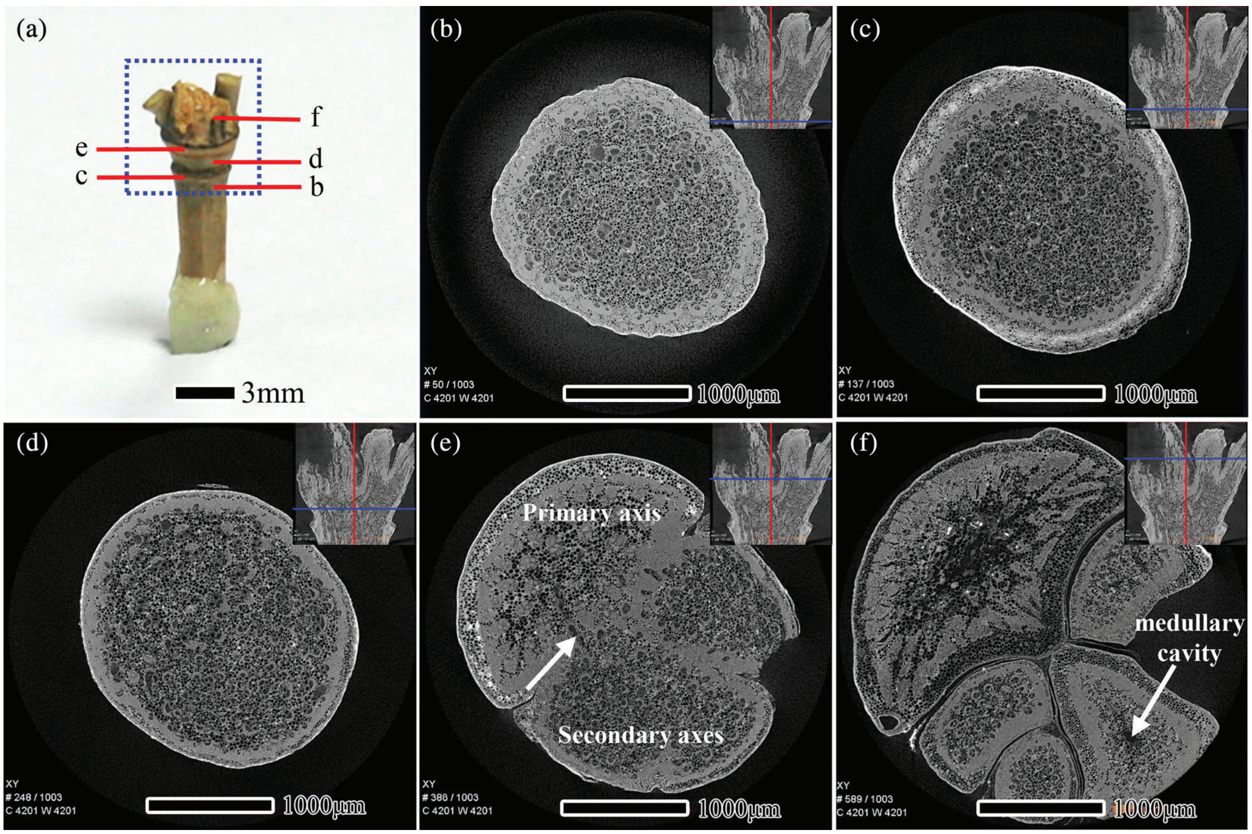

Figure 5: The scanning area and the transverse section μCT slices of *S. chinensis* nodes, scanning area of the sample (a), Scale bars = 3 mm. μCT slices located at the bottom region of the sheath node (b), sheath scar (c), diaphragm (d), nodal ridge (e), and the top region of the nodal ridge (f), Scale bars = 1000 μm

Figs. 6c and 6d present the 3D reconstruction of *S. chinensis* nodes by continuous transverse and axial μCT imaging, and show the internal anatomical structure of bamboo nodes. The anatomical structure of the bamboo node, especially in the arrangement of vascular bundles, was affected by branch type. This results in the arrangement of vascular bundles in the *S. chinensis* node being more complex than that in the *I. latifolius* node. Most of the vascular bundles in the secondary axes are derived from the relative position of the

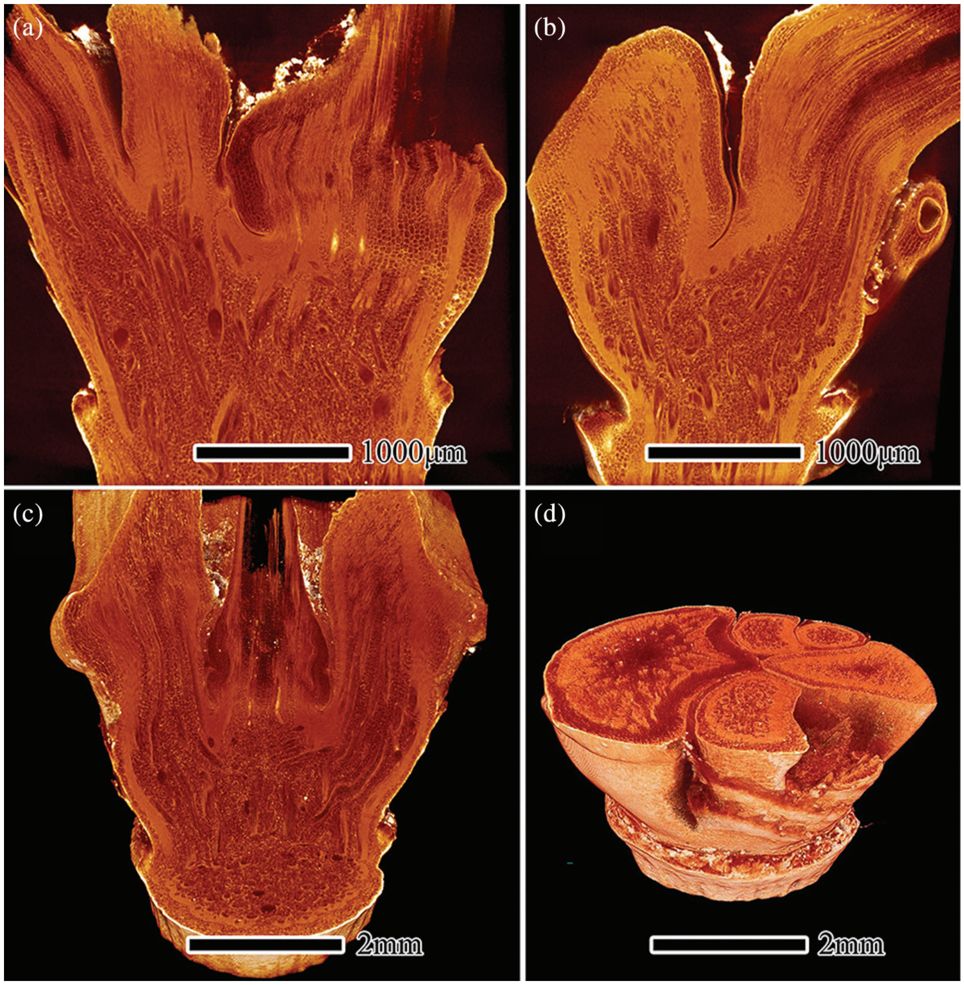

Figure 6: μCT image of axial section (a, b) and the 3D reconstruction (c, d) of *S. chinensis* nodes

diaphragm, and the other part came from the primary axis (Fig. 6c). The results are largely consistent with the finding of Palombini et al. on vascular bundle arrangement in new branches [9]. The nodes of *S. chinensis* comprise a primary axis and five secondary axes, and the secondary axes are arranged in a whorl (Fig. 6d).

4 Conclusions

The anatomical structure and 3D visual characterization of *I. latifolius* and *S. chinensis* nodes were investigated by high-resolution μCT.

1. The vascular bundle system in the nodal region of *I. latifolius* and *S. chinensis* is a net-like structure composed mainly of horizontal and axial vascular bundles. Furthermore, the fiber sheath surrounding metaxylem vessels tended to be shorter in the tangential direction. This structure of bamboo nodes facilitates the tangential and axial transport of moisture and nutrients.

2. The anatomical structure of bamboo nodes, especially in the arrangement of vascular bundles, was affected by branch type. In the nodal region of *I. latifolius*, most vertically arranged vascular bundles near the peripheral of the nodal regions pass directly through the node, while those near the inner of the nodal regions pass into the diaphragm in the form of "Γ". In the nodal region of *S. chinensis*, some of the vascular bundles in the primary axis move transversally and bend into the secondary axes, and fiber cells

were visible at the boundary between the primary axis and secondary axes. Vascular bundle frequency was significantly higher in *S. chinensis* nodes than in *I. latifolius* nodes.

3. μCT was indicated be a feasible and reliable method to characterize the anatomical structure of bamboo nodes nondestructively.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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