Anatomical and Microstructural Features of Rattan (*Calamus caesius*)

Linpeng Yu,[a,b,c] Fukuan Dai,[b,c] Kangjian Zhang,[a] Zehui Jiang,[b,c] Genlin Tian,[b,c,]* and Youhong Wang [a,]*

Calamus caesius, one of the most valuable high-quality rattans, has emerged as an economical material for use in commercial products. This study systematically investigated the anatomical and microstructural characteristics of *Calamus caesius* in terms of the frequency, radial diameter, tangential diameter, and form factor of the vascular bundles in both inner and outer regions, as well as the frequency, proportion, length, diameter, and length-diameter ratio of the vessel elements, and the size, double wall thickness, lumen diameter, and ultrastructure of the fibers. The results revealed that the sizes of both vascular bundles and vessel elements in the inner regions were larger than the outer regions, while the fiber proportions and morphological features remained relatively constant. The fibers have a multi-layered structure, most of which exhibited a four-layered structure in their secondary walls. The properties of various tissue structures reflect rattan's desirable characteristics for use as high-quality commercial timber.

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Keywords: Calamus caesius; Anatomical properties; Vascular bundles; Fiber; Ultrastructure; Secondary wall

*Contact information: a: School of Forestry and Landscape Architecture, Anhui Agricultural University, Hefei, Anhui 230036, China; b: Institute of New Bamboo and Rattan Based Biomaterials, International Center for Bamboo and Rattan, Beijing 100102, China; c: Key Laboratory of National Forestry and Grassland Administration/Beijing for Bamboo & Rattan Science and Technology, Beijing 100102, China; * Corresponding authors: tiangenlin@icbr.ac.cn; wangyh@ahau.edu.cn*

INTRODUCTION

Rattan is a climbing palm that always gets tangled with other trees and grows together, but some of it also grows straight up (Jiang 2007). Rattan is a fascinating and versatile renewable natural resource in the tropical and subtropical rainforests of Africa and the Asia Pacific region (Vorontsova *et al.* 2016), which accounts for over 600 species in 13 genera worldwide (Dransfield 1979; Chen *et al.* 2018; Hanna 2018). The stem is the main part of rattan, which has excellent flexibility. This makes it an ideal material for use in fine and practical furniture and household items. The excellent flexibility is probably due to the large microfibril angle in the S2 sublayer of its fibers, and generally, a higher microfibril angle means lower stiffness and strength, but higher flexibility and extensibility (Abasolo *et al*. 2000). This attribute implies that the rattan considered in the present investigation might have advantages for applications requiring high flexibility or high toughness, rather than stiffness (Abasolo 2008). Moreover, certain *Calamus* species bear fruits that are suitable for human consumption. In addition, some rattan stems can also be used as a medicinal herb to treat diseases (Kuo *et al.* 2022). Current research on rattan has been undertaken in a wide range of areas, including cultivation and breeding (Li 2019; Xu *et al.* 2019), anatomical and mechanical properties (Ren *et al.* 2018), and chemical properties (Ji 2019). According to investigations conducted between 2017 to 2021, there has been a notable increase ranging from 10% to 20% in both the production volume and pricing of rattan. Moreover, there are gradually expanding demands for this natural resource in various industrial sectors (Lin 2023).

Calamus caesius, a member of the *Calamus* subfamily of the Arecaceae family, is the best smaller diameter (<18 mm) rattan (Blažková and Jeníček 2006). The stems of *Calamus caesius* are pliable, with long internodes with no obvious protrusions and uniformly smooth and yellowish-white outer epidermis (Fig. 1a). Without the need of replanting, it can be harvested repeatedly because it has a multiple-stem habit (Jiang *et al.* 2008). These impressive features make it versatile in numerous commercial applications. For example, rattan has long been utilized as a traditional material to manufacture various ranges of handcrafts in practical applications, such as baskets, mats, carpets, ropes, and even supplies for construction purposes. It is one of the most commercially valuable high-quality rattans in the world (Lin 2023). The superior mechanical properties observed in natural biota are often attributed to their finely tuned internal structures (Ren *et al.* 2022; Wei *et al.* 2022). Rattan also exhibits a sophisticated internal structure. However, to the authors' knowledge, the anatomy of *C. caesius* is still seldom reported, and the microstructure has not been explored. This study aimed to comprehensively investigate the anatomical structure and microstructures characteristics of *C. caesius*, with the expectation of establishing a fundamental basis for its efficient utilization in practical applications.

EXPERIMENTAL

Materials

The *Calamus caesius* used in this work was dried material that was ready for manufacturing processes purchased from Indonesia in Southeast Asia (Deng Jinzhi Rattan Factory, Guangdong, China). Three healthy rattans were randomly selected (8.77 ± 0.40 m in length with a diameter of 12.19 ± 0.29 mm). Internodes without any imperfections that were located at the 2-m point from the ground were selected (Fig. 1a). The acquired internodes have an average length of 27.15 ± 1.46 cm and diameter of 13.32 ± 1.45 mm. The rattan stems were air-dried and stored for use; moisture content of the sample is about 11%.

Methods

Obtaining frequency and tissue proportion

Clear visible cross-sections of vascular bundles and parenchymal cells were obtained using a precision cutting saw (Buehler IsoMet4000; Buehler, Lake Bluff, IL, USA). A high-resolution scanner (Epson perfection V850 pro, Seiko Epson Corporation, Shenzhen, China) was used to capture images of the cross-sectional areas of the rattan at a resolution of 9600 ppi in 16-bit grayscale mode (Fig. 1b). ImageJ software (National Center for Biotechnology Information, version 1.53e, Bethesda, MD, USA) was used to obtain the frequency of vascular bundles and metaxylem vessels from the obtained cross-sectional images. Vascular bundles or metaxylem vessels were counted within 1 mm^2 area (Fig. 1c and d), more than 30 data points from both the outer and inner parts were measured. In addition, the tissue proportions of fibers, vessels, sieve tubes, and parenchyma cells were measured from both the inner and outer parts (Wang *et al*. 2014, 2016). The 10 × 10 point counting method was used, those points falling on a given cell type were counted and the number expressed as a proportion of the total points counted for all tissue types. The tissue types are xylem, primary phloem, fibers, and parenchyma tissue. Each group was counted ten times.

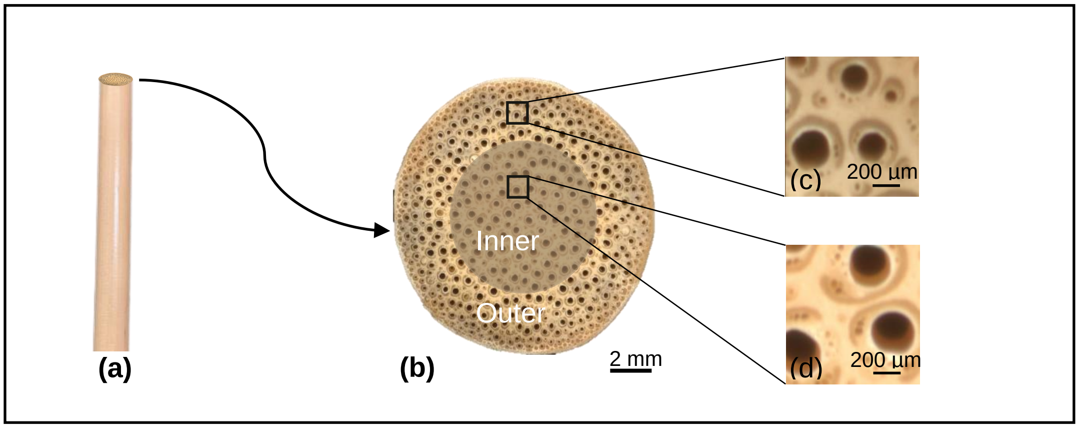

Fig. 1. a: The internode at 2 m from the ground of *Calamus caesius*; b: Cross-sectional scan image (the darker colour for the inner section has been added digitally); c & d: The number of vascular bundles and metaxylem vessels per square mm in the outer and inner regions.

Obtaining microstructures features by SEM

The samples (diameter of 13.15 mm) of approximately 1 cm in length were selected and placed in a beaker filled with water and microwaved for 15 min (G80W23CSP-Z, Galanz, Foshan, China). Subsequently, a sliding microtome (Leica SM2010R, Leica, Wetzlar, Germany) was used to polish the sample. Prior to imaging, the samples were coated with a layer of gold using an E-1010 sputter coater (Quorum Technologies, Emsworth, UK) for 90 s. The polished cross-section was observed by environmental SEM (GeminiSEM 360, Carl Zeiss, Oberkochen, Germany) at an acceleration voltage of 3 kV.

Obtaining the dimensions of fibers and metaxylem vessels using maceration

As shown in Fig. 1b, the specimen was split into two parts, inner and outer, and used for the maceration. To analyze the characteristics of the metaxylem vessel elements and fibers, matchstick-sized samples were prepared by cutting them with a razor blade and placed in glass vials containing a 50:50 solution of 30% hydrogen peroxide and glacial acetic acid. The vials were then heated at 60 °C for about 8 h to soften the material. After it became soft and white, the samples were rinsed with deionized water and stored in glass test tubes filled with water. A small droplet of the cell suspension was placed on a microscope slide for observation under a microscope (Leica Microsystem, Biberach, Germany). Thirty intact fibers and metaxylem vessel elements were randomly selected and the diameter and length of the metaxylem vessel elements from each sample were measured.

Obtaining the wall layer structure of fiber cells by TEM

The outer and inner of the rattan were processed into matchstick-sized samples. The specimens were embedded in Spurr's epoxy resin and subjected to transverse ultra-thin sectioning (70 to 90 nm), using an ultramicrotome (Leica EM UC7) and a diamond knife. The sections were then treated with 1% w/v $KMnO_4$ for a duration of 2 min at an ambient temperature of 25 °C. Subsequently, the cell wall layering properties were analyzed *via* transmission electron microscopy (TEM, JEM-1200EX; Japan Electronics Technology Corporation, Tokyo, Japan), operating at an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

Microstructures

As a vascular plant, rattan is mainly comprised of vascular bundles and ground tissue (Fig. 2). In *C. caesius,* the ground tissue consists of parenchyma cells. The vascular bundles are scattered and embedded in the parenchymatous tissues. It is evident that rattan is a natural composite material, in which vascular bundles act as a reinforcing phase while parenchyma cells act as a matrix phase (Gupta 2020).

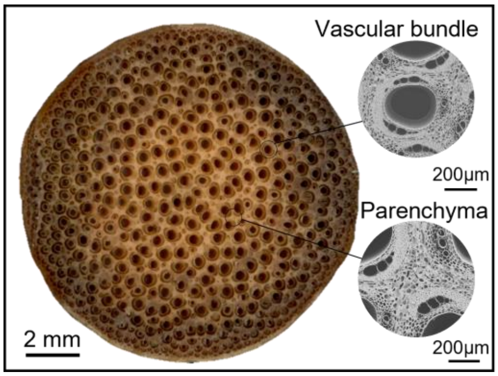

Fig. 2. Cross-sectional scan image of the softened sample

Figure 3 shows SEM images of cross-sectional morphology of vascular bundles and fibers in the inner- and outer regions of *C. caesius.*

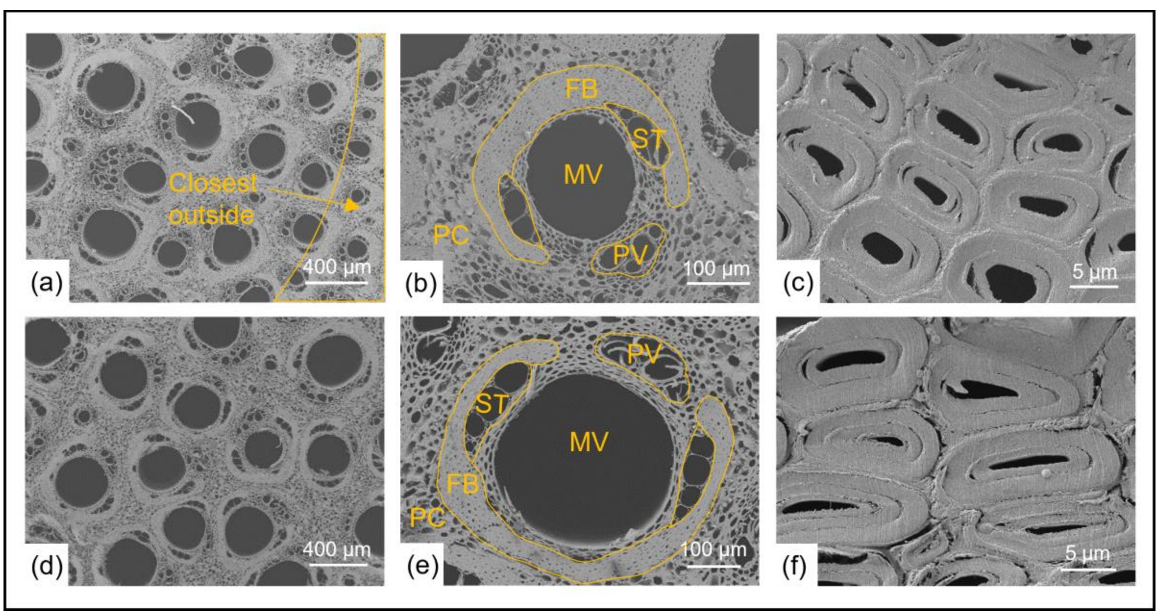

Fig. 3. SEM images of *C. caesius*. a and d: cross-section of the outer and inner; b and e: the vascular bundle of the outer and inner (FB: fiber; MV: metaxylem vessel; ST: sieve tube; PV: protoxylem; PC: parenchyma.); c and f: the section's fiber of the outer and inner

Vascular bundles are present in the inner regions are well developed that consists of parenchyma, vessels, sieve tubes, and fibers, which provide support structures, the metaxylem vessel element is located at the center of the vascular bundle (Fig. 3d & e.), and the fibrous tissue exhibits a horseshoe shape (Fig. 3d & e.). Two phloem fields are usually found on both sides of the metaxylem vessel element, and each is composed of 3 to 6 sieve tubes. The vascular bundles in the outer regions have smaller dimensions compared to the inner ones, but the density of vascular bundles was larger. These anatomical features are very similar to those of *Calamus simplicifolius*, which belongs to the same subfamily *Calamus* (Luo *et al*. 2012). From the cross-sectional distribution of vascular bundles, *C. caesius* can be inferred to be a functionally graded material, having vascular bundles that decrease in size but increase in frequency from inner to outer (Bhat *et al*. 1990; Akinbade *et al*. 2021).

The Bundle and Vessel Frequency and Tissue Proportion

The frequency results of the vascular bundle and metaxylem vessel of *C. caesius* are shown in Table 1. The mean number of the frequency of the inner vascular bundle and the metaxylem vessel is 3.6 pcs/mm^2 and 3.71 pcs/mm^2, respectively, while the outer vascular bundle and the metaxylem vessel is 5.91 pcs/mm^2 and 6.05 pcs/mm^2, respectively. These two observations both indicate that the outer has a higher frequency of vascular bundle and the metaxylem vessel than the inner, which aligns with the results reported by Wang *et al*. (2019). The inner frequency is similar to that of *Calamus spectatissimus* studied by Ren *et al.* (2018), but the frequency of both the vascular bundles and metaxylem vessels in either inner or outer parts of *C. caesius* is lower than that of *Plectocomia himalayan* (Zhang *et al.* 2021).

The mean tissue proportion of xylem, fiber, primary phloem, and parenchyma in inner and outer is 27.7% and 27.0%, 25.8% and 28.0%, 4.92% and 3.00%, and 41.5% and 42.0%, respectively (Table 1). Only the fiber proportion in the outer section increased 2.2% compared to the inner section. The tissue proportion of xylem, primary phloem, and parenchyma in the outer section was lower than those in the inner section, with primary phloem decreasing the most, by 1.92%, while the other two types only showed a slight decrease. In the same position, the fiber proportion in *C. caesius* was approximately 5% higher than *Calamus simplicifolius*, while the tissue proportion in *C. caesius* was approximately 14% lower than *Calamus simplicifolius* (Wang *et al.* 2019). The relationship between fiber proportion and the mechanical strength of a material is directly proportional to a certain extent (Yudodibroto 1985). This suggests that *C. caesius* may exhibit superior mechanical properties in comparison to *Calamus simplicifolius*.

Table 1. The Frequency and Tissue Proportion of *C. caesius*

Position	Mean Frequency (pcs/mm^2)		Mean Tissue Proportion (%)			
	Vascular bundle	Metaxylem vessel	Xylem	Fiber	Primary phloem	Parenchyma
Inner	3.60 ± 1.00	3.71 ± 0.78	27.69 ± 5.76	25.85 ± 8.26	4.92 ± 3.33	41.54 ± 7.03
Outer	5.91 ± 1.35	6.05 ± 1.10	27.00 ± 4.55	28.00 ± 5.39	3.00 ± 1.81	42.00 ± 6.27

The data in the table represents mean ± SD; the same below.

The Morphological Characteristics of Vascular Bundles, Metaxylem Vessel Elements, and Fibers

The radial diameter, tangential diameter, and form factor of the vascular bundle in the inner and outer sections were determined as 0.59 mm and 0.37 mm, 0.60 mm and 0.32 mm, 1.01 and 0.88, respectively (Table 2). Form factor is the ratio of radial diameter and tangential diameter. The closer the ratio is to 1, the more circular the shape appears. It is evident that the shape of vascular bundles in the inner region appears more circular than in the outer region. The radial diameter and tangential diameter of vascular bundles in the outer region decrease sharply compared to those in the inner section. In general, the dimensions of the outer vascular bundles are only half the size of the inner ones.

The length, diameter, and length-diameter ratio of the metaxylem vessel elements in inner and outer sections are 2.97 mm and 2.69 mm, 0.33 mm and 0.18 mm, 8.83 and 14.51, respectively (Table 2), both the length and diameter are higher than those of *Plectocomia microstachys* and *Daemonorops margaritae* (Liu *et al.* 2010; Li *et al.* 2018). The length and diameter of the outer metaxylem vessel elements in *C. caesius* are lower compared to the inner ones, and the extent of diameter reduction is larger, resulting in the metaxylem vessel elements in the outer section appearing much more slender compared to those in the inner section. This is consistent with the data obtained from the measured length-diameter ratios for the inner and outer regions. Therefore, it can be concluded that the diameter of the metaxylem vessel element in the outer is about 55% of that in the inner when their lengths are similar. The frequency and diameter of metaxylem vessels are generally negatively correlated. The diameter of the metaxylem vessel affects the hydraulic efficiency, according to Hagen-Poiseuille's law (Tyree and Zimmermann 2002). Therefore, even a slight decrease or increase in vessel diameter can lead to a noticeable decrease or increase in hydraulic efficiency, indicating the hydraulic efficiency of the rattan in the inner is much higher than that in the outer.

Table 2. The Anatomical Characteristics of Three Tissues

Tissue	Anatomical Properties	Inner	Outer
Vascular Bundle	Radial Diameter (mm)	0.59 ± 0.05	0.37 ± 0.09
	Tangential Diameter(mm)	0.60 ± 0.05	0.32 ± 0.08
	Form Factor	1.01 ± 0.11	0.88 ± 0.14
Metaxylem Vessel Element	Length (mm)	2.97 ± 0.21	2.69 ± 0.23
	Diameter (mm)	0.33 ± 0.01	0.18 ± 0.02
	Length-Diameter Ratio	8.83 ± 0.62	14.51 ± 0.85
Fiber	Length (mm)	1.64 ± 0.42	1.78 ± 0.27
	Diameter (µm)	11.67 ± 0.26	11.87 ± 0.79
	Length-Diameter Ratio	141.79 ± 38.97	150.07 ± 13.61

The results of inner and outer fiber length, diameter, and length-diameter ratio are shown in Table 2. The length, diameter, and length-diameter ratio of the fiber in inner and outer sections are 1.64 mm and 1.78 mm, 11.67 µm and 11.87 µm, 141.79 and 150.07, respectively. The fiber length of *C. caesius* is similar to that of *Calamus guangxiensis*, but shorter than that of *Plectocomia kerrana* and *Calamus faberii* (Wang *et al*. 2011). The mean fiber diameter of *C. caesius* is smaller than the fiber diameter of all the three types of rattans mentioned above. The larger length-diameter ratio of the outer fibers is 150.07

compared to inner fibers, indicating that the fiber cells in the outer region are more elongated and slender.

As shown in Table 3, the double wall thickness, lumen diameter, and wall-cavity ratio of the inner and outer fibers are 5.01 μm and 5.05 μm, 6.65 μm and 7.26 μm, 0.88 and 0.74, respectively. Double wall thickness is the thickness of the cell diameter minus the lumen diameter. The double wall thickness of the inner- and outer parts are very similar. The lumen diameter of the inner part is smaller than that of outer part, and the wall-cavity ratio of inner part is larger than that of outer part. As a comparison, all the lumen diameter, double wall thickness, and wall-cavity ratio of *C. caesius* are lower than those of *Daemonorops margaritae* (Ebanyenle and Oteng 2005; Wang *et al.* 2010). The physical and chemical properties of rattan are directly related to fiber characteristics, which have a noticeable impact on the rattan's utility. Specifically, fiber length and wall-cavity ratio are a crucial quality characteristic for the paper and pulp industry (Jiang *et al.* 2008). A wall-cavity ratio of less than 1 denotes a fiber with a thin fiber wall that felts easily, resulting in better paper quality and improved bonding. The wall-cavity ratio for both the inner- and outer *C. caesius* is less than 1, suggesting potential for use as raw material in pulp production (Viana *et al.* 2009).

Table 3. Wall Thickness, Lumen Diameter, and Wall-cavity Ratio of Fibers

Position	Double Wall Thickness (μm)	Lumen Diameter (μm)	Wall-Cavity Ratio
Inner	5.01 ± 1.31	6.65 ± 1.76	0.88 ± 0.24
Outer	5.05 ± 0.92	7.26 ± 1.74	0.74 ± 0.19

Ultrastructure Study

Figure 4 displays TEM images, showing the ultrastructure of fiber cells in *C. caesius*. The secondary walls of fibers typically contain 2 to 6 layers (Fig. 3c and f). Most fibers near either the outer or inner of rattan clearly exhibit a secondary wall with four layers: S1, S2, S3, and S4 (Fig. 4b and d). This pattern showcases a classic alternation between thick and thin layers (Gibson 2012), which was similar to the characteristics observed in *Calamus simplicifolius* and *Calamus manan* (Wen *et al*. 2021). The presence of multilayered cell walls is not limited to rattan fibers and appears to have undergone convergent evolution in multiple taxa. They have been reported in the root fibers of various woody monocotyledons as well as in the cell walls of several other plant species (Mueller and Beckman 1979). For instance, both the cell walls of the Douglas fir (*Pseudotsuga menziesii*) fibers and coconut (*Cocos nucifera*) stem fibers exhibit a multilayered structure (Parameswaran and Liese 1985).

The secondary wall of the fibers in bamboo *Dendrocalamus asper* also demonstrates a four-layered structure, the variation in the number of layers is much greater compared to that observed in *C. caesius* (Gritsch *et al.* 2004). The thick and thin layers have different microfibril angles, and the microfibril angle of the thick layer is normally smaller than that of the thin layer, while the alternation of thick and thin layers may create a balance between stiffness and flexibility for different functions of the plant cell wall (Osorio *et al*. 2018; Chen *et al*. 2020).

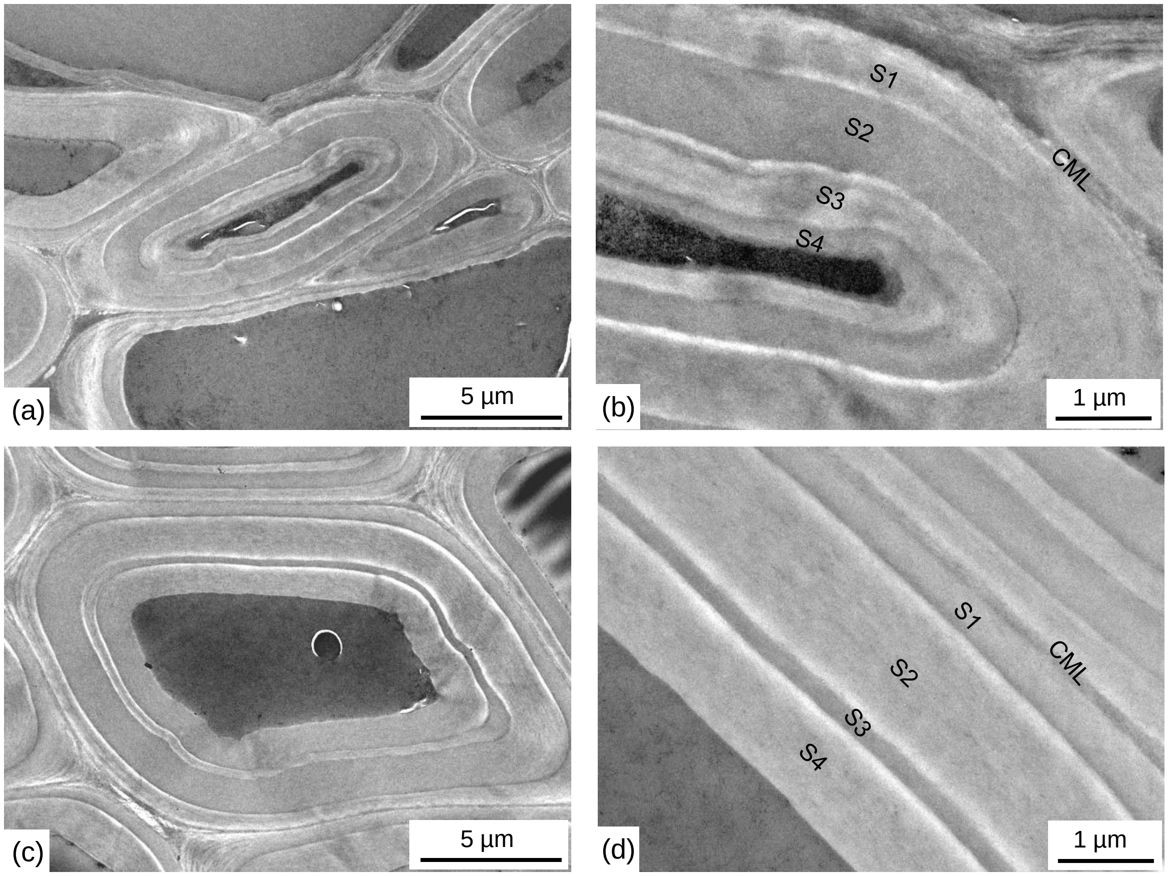

Fig. 4. TEM images of outer and inner fibers: a: the outer fibers; b: CML: compound middle lamella; S1, S2, S3, and S4 respectively represent the number of secondary wall layers; c: the inner fibers; d: CML: compound middle lamella; S1, S2, S3, and S4 represent the number of secondary wall layers

CONCLUSIONS

1. Anatomical study on *Calamus caesius* revealed that the frequency of the outer vascular bundles is greater than that of the inner ones, while the radial and tangential diameters and form factors of the outer bundles are smaller than those of the inner bundles. Furthermore, the frequency and length-diameter ratio of the outer metaxylem vessel elements are higher, but their length and width are lower than those of the inner elements.
2. The outer fibers have a higher tissue proportion, length, diameter, double wall thickness, lumen diameter, and length-diameter ratio, but a lower wall-cavity ratio than the inner fibers. Overall, the characteristics of the inner and outer fibers appear to be very similar to each other.
3. Most fiber cells in the inner and outer layers exhibit a four-layer structure of alternating thick and thin secondary walls. Consequently, the difference in material properties between the outer and inner of rattan is minor, making rattan suitable for direct use in the production of bindings, handicrafts, furniture, agricultural tools, and so on.

4. This study has revealed some interesting features of the microstructure of *Calamus caesius*. However, there are still many aspects that need further investigation, especially related to the mechanical properties of this material. For example, it would be valuable to measure the Young's modulus, tensile strength, flexural properties, and crush resistance of *Calamus caesius* fibers and compare them with other rattan or plant fibers. Moreover, it would be interesting to explore the wettability properties and compatibility of *Calamus caesius* surfaces with different adhesives, which could affect the performance and durability of biocomposites made from this material. Therefore, we suggest that future research should focus on these topics to better understand and utilize the potential of *Calamus caesius* as a sustainable and flexible biomaterial.

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Author Contributions Statement

L. Y. wrote the main manuscript, D. F. was responsible for data curation, K. Z. performed formal analysis, Z. J. supplied the resources and came up with the methodology, G. T. and W.Y were responsible for funding acquisition, methodology, and project administration. All authors reviewed the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statements

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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