

Article

Structural Variation Patterns in Xylem Vessels and Parenchyma Cells and Their Association with Tree Evolution

Linghui He , Yunlin Fu ^{*}, Zhigao Liu  and Penglian Wei

Key Laboratory of National Forestry and Grassland Administration on Cultivation of Fast-Growing Timber in Central South China, College of Forestry, Guangxi University, Nanning 530004, China

^{*} Correspondence: fuyunlin@gxu.edu.cn

Abstract: Xylem vessels and parenchyma cells perform functions such as water transport and nutrient storage in trees. However, they are highly variable in different trees. Therefore, this study aimed to explore the structural change patterns in vessels and parenchyma cells in the sapwood, transition wood, and heartwood of tree species with different degrees of evolution. The structural characteristics of the two types of cells in the sapwood, transitional wood, and heartwood were measured in six species with different levels of evolution, namely, *Michelia macclurei* Dandy, *Cinnamomum camphora* (L.) presl, *Erythrophileum fordii* Oliv, *Melaleuca leucadendron* L., *Parashorea chinensis* Wang Hsie and *Tectona grandis* L.F. The results showed that the more evolved species had larger earlywood vessel lumen diameters, thicker walls, and wider hydraulic diameters, as well as smaller latewood vessel densities, reflecting better water transport effectiveness and higher safety. From the sapwood to the transition wood and heartwood, the earlywood vessel lumen diameter of the more primitive species tended to be stable and then decrease, while that of the more evolved species gradually decreased. The latewood vessel density of the more primitive species tended to be stable and then increase, while that of the more evolved species tended to be stable. Additionally, the starch grains of the more primitive species were mainly distributed in the axial or ray parenchyma cells of the sapwood, while those of the more evolved species were abundantly distributed in the axial and ray parenchyma cells of the sapwood, and the distribution of the starch grains was reduced in the transition wood and heartwood. From the sapwood to the heartwood, the ray parenchyma cell fraction tended to be stable in the more primitive species, and tended to be stable before decreasing in the more evolved species. The pit density in the horizontal wall of the ray parenchyma cells tended to be stable and decrease in the more primitive species, while in the more evolved species it tended to be stable or decrease before stabilizing. Overall, trees' vessels have gradually undergone the optimal selection of vessels during evolution, and the structural variation in the parenchyma cells contributes to their nutrient storage and transport.

Keywords: xylem anatomy; comparison of trees; sapwood; transition wood; heartwood



Citation: He, L.; Fu, Y.; Liu, Z.; Wei, P. Structural Variation Patterns in Xylem Vessels and Parenchyma Cells and Their Association with Tree Evolution. *Forests* **2023**, *14*, 950. <https://doi.org/10.3390/f14050950>

Academic Editor: Angela Lo Monaco

Received: 27 February 2023

Revised: 12 April 2023

Accepted: 2 May 2023

Published: 4 May 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The main role of a xylem vessel is transporting water [1], while parenchyma cells perform a variety of functions, such as nutrient storage [2], defense against pathogenic bacteria [3], mechanical support, and regulation of xylem conductivity [4,5]. The axial parenchyma is composed of many cells, and its main function is to store and distribute nutrients [6]. The ray parenchyma plays a role in nutrient storage and radial transport [7].

In the long-term evolution process of trees, the characteristics of vessel element length, lumen diameter, density, and wall thickness vary greatly. Previous studies have shown that the variation range in the vessel lumen diameter is between 10.00 μm and 435.12 μm [8], the density is between 0.99 and 191.08 number $\cdot \text{mm}^{-2}$, the potential hydraulic conductivity is between 4.50 and 1640.00 $\text{kg m}^{-1} \text{Mpa}^{-1} \text{s}^{-1}$, and the wall thickness is between 0.00 and 0.32 mm [9].

Tree species with different degrees of evolution have changed from having multiple perforations to a single perforation, which resulted in less resistance in the process of water transport, and thus, these trees have higher water transport efficiency [10]. However, the variation pattern in the earlywood vessel lumen diameter, latewood vessel lumen diameter, earlywood vessel density, latewood vessel density, vessel wall thickness, and vessel fraction in tree species with different degrees of evolution is still unknown. According to the Hagen–Poiseuille theory, with the increase in the vessel diameter, the corresponding hydraulic conductivity will increase in proportion to the fourth power [11]. However, the vessels in plants do not increase in diameter infinitely. Currently, studies had confirmed that there is a trade-off between the effectiveness and safety of xylem transportation [12]. Nevertheless, some scholars have studied 400 plants on a global scale and found that there was only a weak correlation between the trade-offs [13]. Moreover, in some plants, there is common optimization to establish the effectiveness and safety of the hydraulic system [14]. The relationship between the two is reflected in the structure of a tree itself, which is determined by the relationship between the vessel lumen diameter and the vessel density and between the vessel lumen diameter and the vessel wall thickness. Large vessel diameters and densities are prone to embolism. Thus, to ensure water transportation safety, there is usually a trade-off between the vessel size and density [15]. The thickness of the vessel wall affects the thickness of the pit membrane [16], and a thick pit membrane is more resistant to the cavitation that is induced by drought because it is more difficult for it to stretch [17]. Therefore, a thick vessel wall ensures water transportation safety. The difference between effectiveness and safety can be used to reflect the differences in the niches and evolution of species [13]. Consequently, studying the relationship between the vessel lumen diameter and vessel density and between the vessel lumen diameter and the vessel wall thickness of tree species with different degrees of evolution is helpful for understanding the variation pattern in the vessel structure due to tree evolution.

A study on the radial variation in the vessel size showed that the size of the vessels increased as the distance from the pith to the sapwood increased, which implies that the hydraulic conductivity of the sapwood is higher [11]. Growing trees can adjust their wood function by modulating tissue composition, cell size, and cell wall thickness, and by increasing their water transport capacity and mechanical strength [18]. However, with regard to the evolution of trees, the variation pattern in the vessel element length, density, wall thickness, and fraction of the sapwood, transition wood, and heartwood still needs to be explored.

A study of 2332 woody angiosperms worldwide showed that the total parenchyma, axial parenchyma, and ray parenchyma fractions changed from 6.88% to 64.20%, from 0% to 44.10%, and from 5.23% to 42.47%, respectively [8]. Thus, the variation range of the parenchyma cells in trees is large, and the axial and ray parenchyma tissues have diversity in their cell morphology, fractions, and abundance. However, there are limited studies on the functional significance of this variation and its role in plant ecological strategies [19]. The results of a study on 52 species of *Adenia* in the family Passifloraceae showed that the parenchyma tissue evolved synergistically with the vascular bundles and increased with evolution [20]. The variability in the parenchyma content over evolutionary time in closely related species has been analyzed [21]. These studies have shown a relationship between the parenchyma content and the phylogeny of the same family and related species, but the variation pattern in the parenchyma cells of tree species with different degrees of evolution still needs further research. During the evolution of trees, the characteristics of the ray parenchyma cells have changed, thus affecting the transport function [22]. Therefore, the variation pattern in the pits in ray parenchyma cells of tree species with different degrees of evolution needs to be clarified.

In a study of *Zelkova serrata* (Thunb.) Makino, it was found that from the sapwood to the heartwood, the ray area first fluctuated within a certain range and then decreased [23]. There was no significant difference radially in the ray parenchyma fraction of *Quercus suber* L. [24]. For *Hevea brasiliensis* Muell. Arg, it was found that from the sapwood to the heartwood, the ray parenchyma fraction decreased [25]. In terms of the perspective of phylogeny,

Zelkova serrata was the most primitive among the three, and *Hevea brasiliensis* was more evolved. Therefore, the parenchyma cells of tree species with different degrees of evolution also varied greatly among the sapwood, transition wood, and heartwood, but the functional significance of this variation remains unidentified. A study of the distribution of the active ray parenchyma cells and main bioactive substances during the heartwood formation in *Taiwania cryptomerioides* Hayata found that the ray parenchyma cells died within 1–2 annual rings of the transition wood. The stored starch granules also gradually disappeared, and the content of the extracts and bioactive substances gradually increased [26]. This shows that the starch granules in the parenchyma cells gradually disappeared from the sapwood, transition wood, and heartwood. Studying the distribution of starch granules in axial parenchyma cells and ray parenchyma cells of sapwood, transition wood, and heartwood could help us understand the changing patterns in parenchyma cells.

Consequently, this study aimed to explore the structural change patterns in vessels and parenchyma cells in the sapwood, transition wood, and heartwood of tree species with different degrees of evolution. Therefore, six species of trees with similar ages and different degrees of evolution were selected for a comparative study to quantify the characteristics of the vessel structure and parenchyma cells and answer the following questions: (1) “what is the pattern of the vessel structure variation in tree species with different degrees of evolution?” and (2) “what is the pattern of the variation in parenchyma cells of tree species with different degrees of evolution?” The findings may clarify the variation patterns in the structural features, such as the xylem vessels and parenchyma cells, during the evolution of trees, and reveal the relationship between wood structural variation and tree evolution.

2. Materials and Methods

2.1. Study Site and Species

The experimental site was located at the Xiashi Introduction Arboretum at the Experimental Center of Tropical Forestry at the Chinese Academy of Forestry, located in Pingxiang City, Guangxi Municipality (22°6′59″ N, 106°53′49″ E). It is located in a hilly area and has a subtropical monsoon climate with long summers and no winters, with abundant rainfall and the obvious characteristic of summer half-year and autumn half-year periods. The APG classification system is a new classification system for angiosperms proposed by the Angiosperm Phylogeny Group based on branching taxonomy and molecular systematics. It has been updated to the revised version of APG III in 2009. According to the APG III classification system, six species with different evolutionary degrees, namely, *Michelia macclurei*, *Cinnamomum camphora*, *Erythrophleum fordii*, *Melaleuca leucadendron*, *Parashorea chinensis*, and *Tectona grandis*, were selected (Table 1). In August 2021, a total of 18 individual trees with normal growth and no obvious defects were randomly selected for the experiment (3 from each species). The sampling took place 1.30 m above the ground level. Three wood cores were taken from each of the 18 individuals, where a growth cone (5.15 mm in diameter) was used to take them from the north bark, the pith, and the south bark, and they were sealed in plastic tubes immediately after marking the direction of the wood cores. Then, they were taken back to the laboratory and fixed them with Formalin-Aceto-Alcohol.

Table 1. Basic information of sample trees (mean ± SD).

Species	Family	APG III Serial Number	DBH/cm	Tree Height/m	Age/Year
<i>Michelia macclurei</i> Dandy	Magnoliaceae	17	24.27 ± 1.64	18.67 ± 2.05	51
<i>Cinnamomum camphora</i> (L.) presl	Lauraceae	28	25.73 ± 2.46	19.00 ± 2.83	50
<i>Erythrophleum fordii</i> Oliv.	Fabaceae	144	22.33 ± 1.34	15.00 ± 0.82	51
<i>Melaleuca leucadendron</i> L.	Myrtaceae Juss.	222	21.37 ± 2.22	26.67 ± 0.94	51
<i>Parashorea chinensis</i> Wang Hsie.	Dipterocarpaceae	256	24.13 ± 1.80	32.67 ± 0.94	51
<i>Tectona grandis</i> L.F.	Verbenaceae J. St.-Hil.	382	28.73 ± 0.26	20.33 ± 1.31	42

2.2. Material Preparation Method

The materials were prepared for observation under a light microscope (DM3000LED; Leica Microsystems, Wetzlar, Germany). Firstly, the wood slices were prepared by removing the bark from the sample cores and distinguishing the sapwood, transitional wood, and heartwood according to the color, and then the wood was taken from the middle of the sapwood, transition wood (the junction between the sapwood and heartwood), and heartwood. A sliding microtome (SM2010R; Leica Biosystems, Nussloch, Germany) was used to prepare the cross, radial, and tangential sections, and the section thickness was 20 μm . The sections were stained with 1% safranin for 15 min and with 1% alcian blue stain for 2 min, were washed in 70%, 90%, and 95% ethanol for 2 min, and were treated with a TO-type biological tablet transparent agent for 3 min. Next, they were laid out on slides and covered with neutral balsam to form a permanent seal. The inspection of the xylem structure was undertaken under objective lenses at 40 \times magnification. The microscopic images were captured using a digital camera (DFC295; Leica Microsystems, Wetzlar, Germany) that was attached to the light microscope. Secondly, for the wood dissociation, the specimens were split into matchsticks of 2–3 mm in diameter and about 5 cm in length, and 1–2 specimens were put into test tubes, submerged with a mixture of glacial acetic acid and hydrogen peroxide with a mass fraction of 30% (mixed in a 1:1 volume ratio), and heated in a constant temperature water bath at 100 $^{\circ}\text{C}$ for 2–6 h until the specimens turned white. After the separation was completed, the mixed solution in the tube was poured out, rinsed 2–3 times with distilled water, and placed on a slide. The inspection of the vessel element length was undertaken under objective lenses of 100 \times magnification. The microscopic images were captured using a digital camera attached to the light microscope.

The materials were then prepared for observation under the scanning electron microscope (SEM). A cross section, radial section, and tangential section of the sapwood, transitional wood, and heartwood were prepared using a sliding microtome with a thickness of 20 μm . After the sections were dried sufficiently, the samples were fixed on the sample holder with conductive adhesive tape. Gold spraying was completed after 2 min using a sputtering coater (Model 108; Cressington, Watford, UK). They were observed with the SEM (S-3400N; Hitachi, Tokyo, Japan) at a 15 kV accelerating voltage and 150–3000 \times magnification.

2.3. Observation of the Xylem Structure

The xylem vessels were observed using ImageJ software (Version fiji-win64, National Institutes of Health, Bethesda, MD, USA; <https://imagej.nih.gov/ij/download.html> (accessed on 1 May 2023)), and we analyzed and measured the vessel density of earlywood and latewood vessel wall thickness, fraction, element length, and lumen diameter. Then, we calculated the hydraulic diameter and potential hydraulic conductivity. All of the measurements were repeated three times, and the average value was calculated.

The earlywood/latewood vessel density ($\text{EV}_\text{D}/\text{LV}_\text{D}$, number $\cdot\text{mm}^{-2}$) was determined by counting the number of vessels in five randomly selected 500 μm square areas on each side of the sapwood, transitional wood, and heartwood.

Then, to determine the vessel wall thickness (T_{VW} , μm), the sum of the cell wall thicknesses of 30 connected vessels in a cross section was calculated randomly for the sapwood, transitional wood, and heartwood.

Next, the vessel fraction (VF, %) was determined using three pictures that were randomly selected from the sapwood, transition wood, and heartwood for measurement.

To determine the vessel element length (L_{VE} , μm), the length of 30 vessel elements in the sapwood, transition wood, and heartwood were randomly measured.

Subsequently, the earlywood/latewood vessel lumen diameter ($\text{ED}_\text{V}/\text{LD}_\text{V}$, μm) was determined for the sapwood, transition wood, and heartwood, and the cross-sectional area of 100 earlywood and latewood vessels was measured randomly [27]. The diameter of

each lumen was calculated based on the relationship between the cross-sectional area and diameter of the vessel. The formula is as follows:

$$D_V = 2\sqrt{\frac{A}{\pi}}, \quad (1)$$

where A is the area of the measurement area (mm^2), which was measured using ImageJ software.

For the vessel hydraulic diameter (D_H , μm), 100 earlywood vessel lumen diameters of sapwood were randomly measured using the following formula [28]:

$$D_H = \left(\frac{\sum_{i=1}^n D_i^4}{n} \right)^{\frac{1}{4}}, \quad (2)$$

where D_i is the earlywood vessel lumen diameter of the sapwood and n is the number of vessels.

Then, the potential hydraulic conductivity (K_p , $\text{kg m}^{-1} \text{Mpa}^{-1} \text{s}^{-1}$) is the hydraulic conductivity of a bundle of vessels of different diameters at a unit pressure gradient. Based on the average derived vessel densities and hydraulic diameters of the sapwood of each species, the potential hydraulic conductivities were calculated according to the Hagen–Poiseuille law [28]:

$$K_p = \frac{\pi \rho_w}{128 \eta} \times V_D \times D_H^4, \quad (3)$$

where ρ_w is the density of pure water at 20 °C (998.2 kg m^{-3} at 20 °C), η is the viscosity of water at 20 °C ($1.002 \times 10^{-9} \text{ Mpa} \cdot \text{s}$ at 20 °C), V_D is the vessel density of the sapwood, and D_H is the vessel hydraulic diameter of the sapwood.

For the observation of the xylem parenchyma cells, the axial parenchyma, ray parenchyma, and total parenchyma fractions, wood ray height, wood ray width, and pit density in the horizontal wall of the ray parenchyma cells were measured using ImageJ software, and the changes in the starch grain distribution were analyzed.

Firstly, for the axial parenchyma (APF, %), ray parenchyma (RPF, %), and total parenchyma fractions (TPF, %), three pictures were randomly selected for measurement of the sapwood, transition wood, and heartwood.

Secondly, for the ray height (HR, μm), the heights of 30 wood rays were measured randomly from the sapwood, transition wood, and heartwood.

Thirdly, for ray width (WR, μm), the widths of 30 wood rays were measured randomly from the sapwood, transition wood, and heartwood.

Fourthly, for the pit density in the horizontal wall of ray parenchyma cells (HRP_D , $10^{-3} \text{ number} \cdot \text{mm}^{-2}$), five rectangular areas that were 50 μm in length and 20 μm in width were randomly selected from the sapwood, transitional wood, and heartwood, and the number of pits was counted.

Lastly, the distribution of the starch grains was observed in five randomly selected microscope fields for the sapwood, transition wood, and heartwood.

2.4. Data Analysis (Model of ANOVA)

Firstly, the structural characteristics of the vessels and parenchyma cells of sapwood, transition wood, and heartwood among the different species and the same species were tested for homogeneity of variance. If $p > 0.05$, the homogeneity of variance was met, and a one-way analysis of variance (ANOVA) was performed; if $p < 0.05$, there was no homogeneity of variance, and a Kruskal–Wallis test was performed. If the difference was significant, multiple comparisons were made via the Duncan method. Additionally, a Pearson correlation analysis was used to study the relationship between the vessel lumen diameter and the vessel density and between the vessel lumen diameter and the thickness of the vessel wall of the tree species with different degrees of evolution. All of the data in this paper were processed and analyzed using the SPSS Statistics software (Version 19.0.0, International Business Machines

Corporation, Chicago, IL, USA; <http://www.spss.com/> (accessed on 1 May 2023)) and were plotted using the Origin software (Version 9.9.0.225, OriginLab, Northampton, MA, USA; <https://www.originlab.com/> (accessed on 1 May 2023)).

3. Results

3.1. Structural Variation Patterns in the Xylem Vessels

3.1.1. Variation in the Vessel Structure of Tree Species with Different Degrees of Evolution

Figure 1 shows the xylem anatomical images. The earlywood vessel lumen diameter (Figure 2a) and vessel wall thickness (Figure 2b) in the sapwood, transition wood, and heartwood of each tree species, as well as the vessel hydraulic diameter of the sapwood, tended to increase alongside tree evolution (Figure 3a). The earlywood vessel lumen diameters of *Michelia macclurei* were the smallest in the sapwood, transition wood, and heartwood, being $54.52 \pm 1.14 \mu\text{m}$, $51.99 \pm 1.99 \mu\text{m}$, and $47.73 \pm 0.83 \mu\text{m}$, respectively (Figure 2a). The earlywood vessel lumen diameters of *Tectona grandis* were significantly larger than those of all the other species, being $226.50 \pm 3.72 \mu\text{m}$, $212.84 \pm 4.52 \mu\text{m}$, and $210.77 \pm 4.90 \mu\text{m}$ (Figure 2a). Additionally, the vessel wall thicknesses in the sapwood, transition wood, and heartwood of *Michelia macclurei* were the smallest, being $5.39 \pm 0.32 \mu\text{m}$, $5.40 \pm 0.24 \mu\text{m}$, and $5.51 \pm 0.24 \mu\text{m}$, respectively (Figure 2b). The vessel wall thicknesses of *Tectona grandis* were the largest, being $20.70 \pm 1.75 \mu\text{m}$, $20.80 \pm 1.78 \mu\text{m}$, and $20.87 \pm 1.04 \mu\text{m}$, respectively (Figure 2b). Thus, the smallest vessel hydraulic diameter was $57.42 \pm 2.58 \mu\text{m}$ in *Michelia macclurei*, and the largest vessel hydraulic diameter was $240.68 \pm 3.15 \mu\text{m}$ in *Tectona grandis* (Figure 3a).

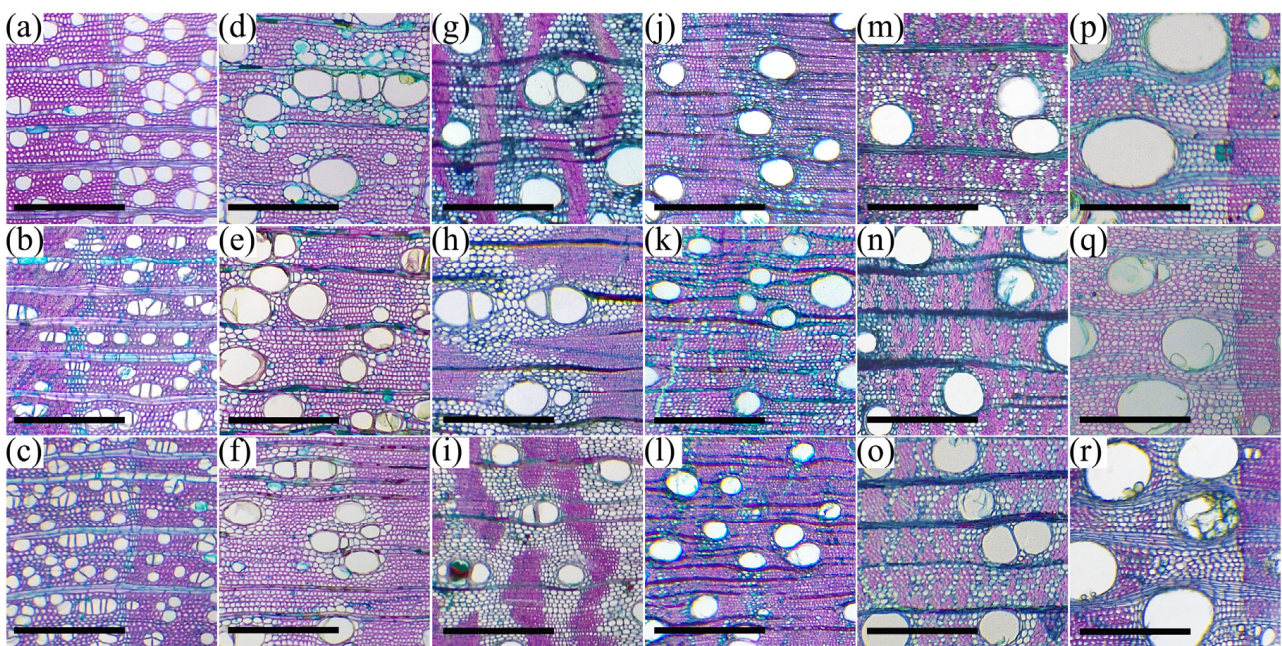


Figure 1. Anatomical images of the xylem of the sapwood (upper), transition wood (middle), and heartwood (lower) of trees with different evolutionary degrees. (a–c) *Michelia macclurei*. (d–f) *Cinnamomum camphora*. (g–i) *Erythrophloeum fordii*. (j–l) *Melaleuca leucadendron*. (m–o) *Parashorea chinensis*. (p–r) *Tectona grandis*. Scale bars = 500 μm .

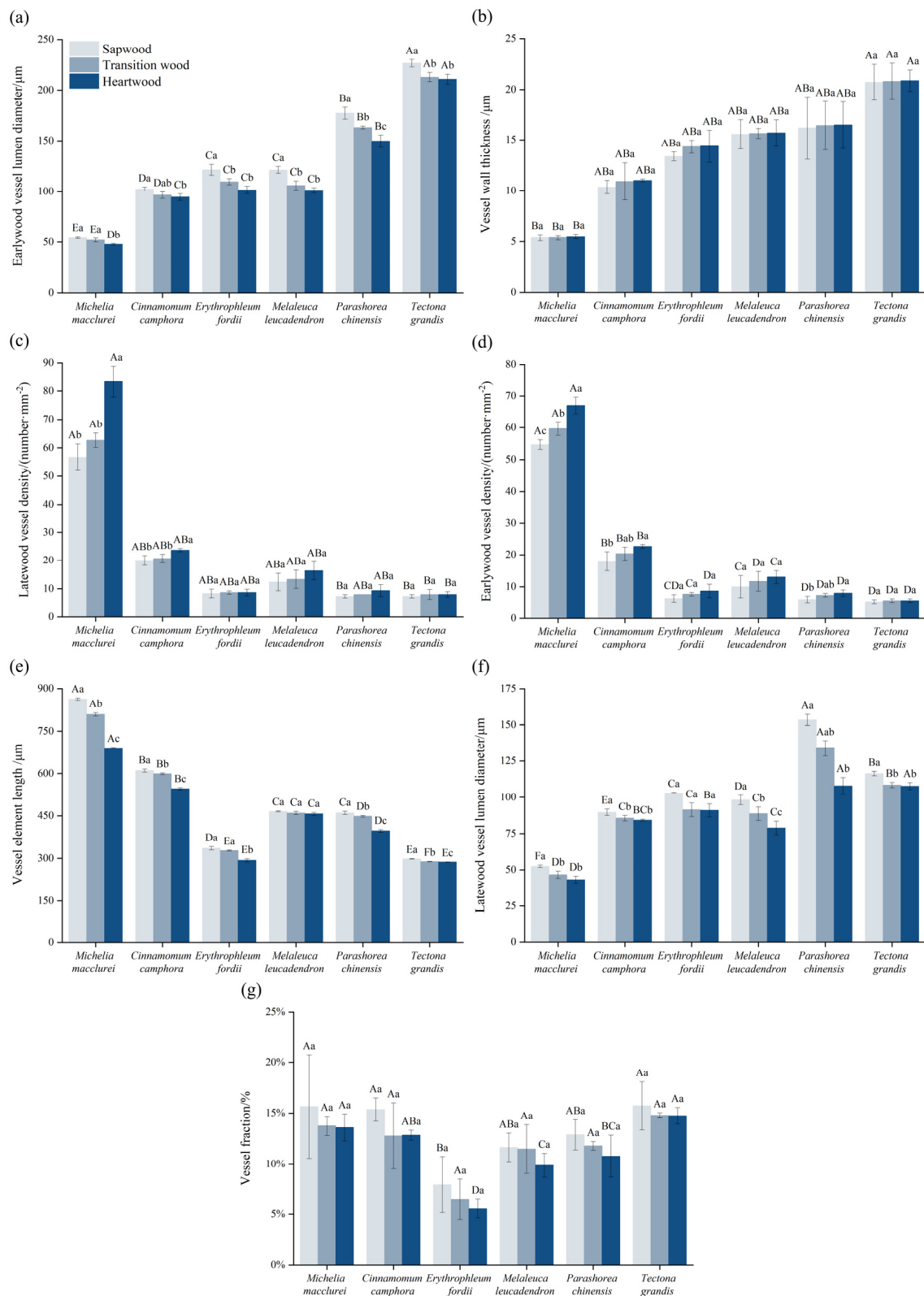


Figure 2. The difference in vessel structure in the sapwood, transition wood, and heartwood of the tree species with different evolutionary degrees (mean \pm SD). Capital letters represent significant differences among different tree species; lowercase letters represent significant differences among the sapwood, transition wood, and heartwood of the same tree species. (a) Earlywood vessel lumen diameter. (b) Vessel wall thickness. (c) Latewood vessel density. (d) Earlywood vessel density. (e) Vessel element length. (f) Latewood vessel lumen diameter. (g) Vessel fraction.

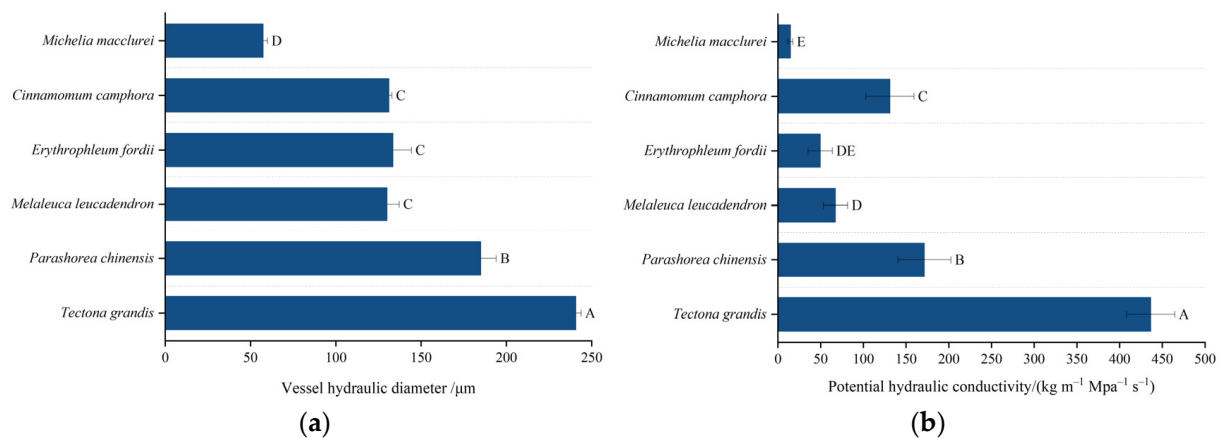


Figure 3. The difference in hydraulic characteristics of vessels among the tree species with different evolutionary degrees (mean \pm SD). Different letters represent significant differences among tree species at the 0.05 level. (a) Vessel hydraulic diameter. (b) Potential hydraulic conductivity.

The latewood vessel density in the sapwood, transition wood, and heartwood of each tree species had a decreasing trend alongside tree evolution (Figure 2c). The latewood vessel densities in the sapwood, transition wood, and heartwood of *Michelia macclurei* were the largest, and they were 56.67 ± 4.73 number \cdot mm $^{-2}$, 62.67 ± 2.52 number \cdot mm $^{-2}$, and 83.33 ± 5.51 number \cdot mm $^{-2}$, respectively. The latewood vessel densities in the sapwood of *Parashorea chinensis* and *Tectona grandis* were the smallest, being 7.33 ± 0.58 number \cdot mm $^{-2}$. Moreover, the latewood vessel densities in the transition wood and heartwood were the smallest in *Tectona grandis*, being 8.00 ± 1.73 number \cdot mm $^{-2}$ and 8.00 ± 1.00 number \cdot mm $^{-2}$, respectively. The earlywood vessel density (Figure 2d), vessel element length (Figure 2e), latewood vessel lumen diameter (Figure 2f), vessel fraction (Figure 2g), and potential hydraulic conductivity of each species had no obvious patterns in the species with different degrees of evolution (Figure 3b).

The more evolved species had larger earlywood vessel lumen diameters and thicker vessel walls. The earlywood vessel lumen diameter in the sapwood and transition wood of *Michelia macclurei* had a significant negative correlation with the vessel wall thickness (Figure 4a). In addition, the earlywood vessel lumen diameter of the transition wood was significantly positively correlated with the vessel wall thickness of the transition wood in *Cinnamomum camphora* (Figure 4b) and *Tectona grandis* (Figure 4f). The latewood vessel lumen diameter of the heartwood was significantly negatively correlated with the earlywood vessel density in *Melaleuca leucadendron* (Figure 4d). Furthermore, the latewood vessel lumen diameter of sapwood was negatively correlated with the earlywood vessel density of sapwood in *Parashorea chinensis* (Figure 4e). In contrast, *Erythrophleum fordii* did not demonstrate a relationship between the vessel lumen diameter and the vessel wall thickness or between the vessel lumen diameter and the vessel density (Figure 4c).

3.1.2. Variation in the Vessel Structure among the Sapwood, Transition Wood, and Heartwood of the Same Species

From the sapwood to the transition wood and the heartwood, the earlywood vessel lumen diameter of the more primitive species showed a trend of first being stable and then decreasing, and the earlywood vessel lumen diameter of the more evolved tree species showed a trend of gradually decreasing. Among the more primitive species, the latewood vessel density showed a trend of first being stable and then increasing, while the latewood vessel density of the more evolved species tended to be stable. Moreover, the earlywood vessel lumen diameter of the sapwood and the transition wood was significantly larger than that of the heartwood in *Michelia macclurei*, while the earlywood vessel lumen diameter of *Cinnamomum camphora* decreased gradually between the sapwood and the heartwood, and the earlywood vessel lumen diameter of the sapwood was significantly larger than

that of the transition wood and heartwood in *Erythrophleum fordii*, *Melaleuca leucadendron*, *Parashorea chinensis*, and *Tectona grandis*. Additionally, the latewood vessel density of the heartwood was significantly larger than that of the transition wood and sapwood in *Michelia macclurei* and *Cinnamomum camphora*, while there were no significant differences among the sapwood, transition wood, and heartwood of the slightly more evolved trees, such as *Erythrophleum fordii*, *Melaleuca leucadendron*, *Parashorea chinensis*, and *Tectona grandis*.

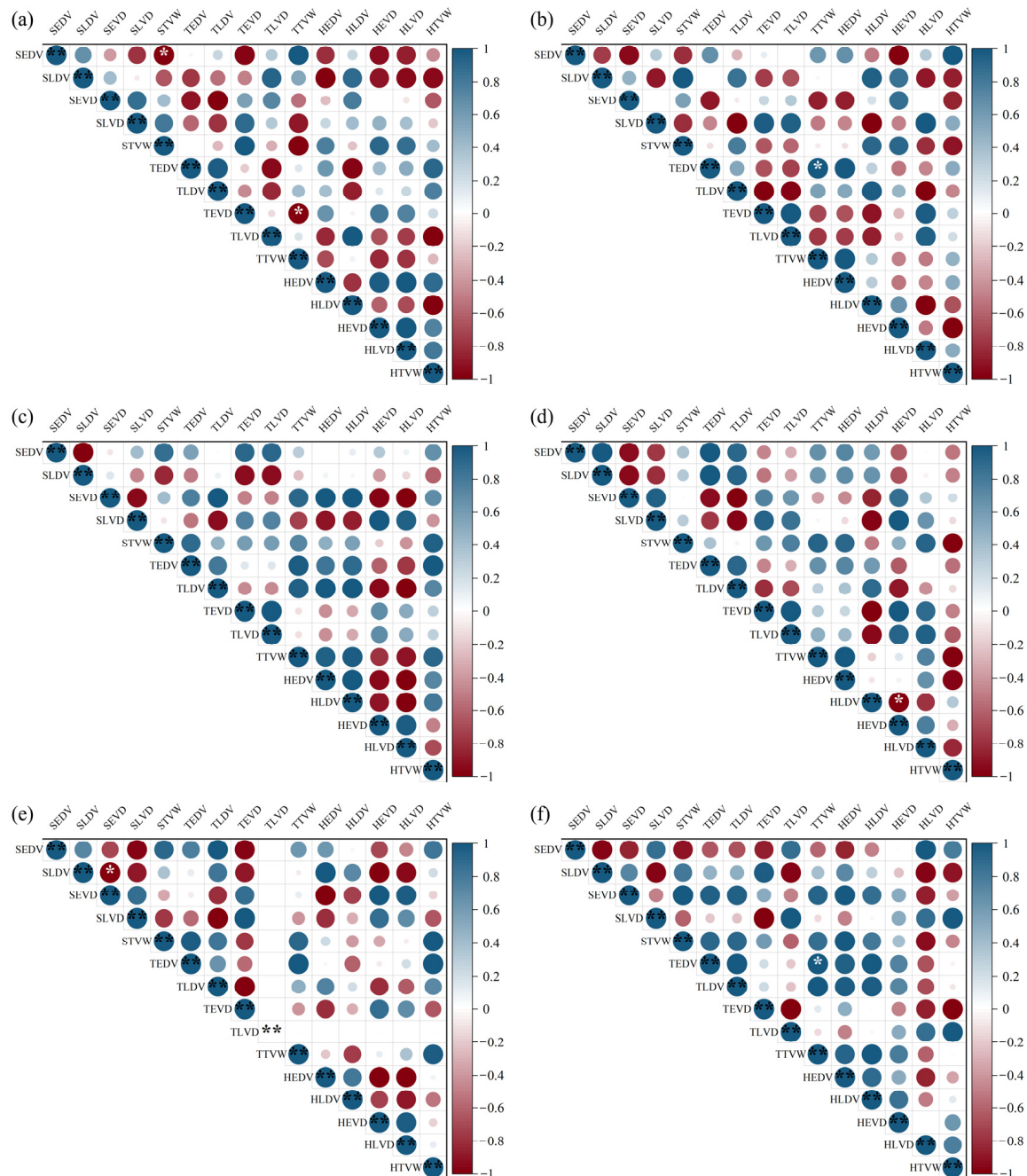


Figure 4. The relationship between the vessel lumen diameter and the vessel density and between the vessel lumen diameter and the vessel wall thickness in sapwood, transition wood, and heartwood of trees with different evolutionary degrees. (a) *Michelia macclurei*. (b) *Cinnamomum camphora*. (c) *Erythrophleum fordii*. (d) *Melaleuca leucadendron*. (e) *Parashorea chinensis*. (f) *Tectona grandis*. Prefixes S, T, and H represent sapwood, transition wood, and heartwood. “*” means a significant correlation at the 0.05 level, “**” means a significant correlation at the 0.01 level.

3.2. Structural Variation Patterns in the Parenchyma Cells

3.2.1. Variation in the Parenchyma Cell Structure of the Tree Species with Different Degrees of Evolution

No significant patterns were found in the ray height (Figure 5a), ray width (Figure 5b), axial parenchyma fraction (Figure 5c), ray parenchyma fraction (Figure 5d), total parenchyma fraction (Figure 5e), and pit density in the horizontal wall of the ray parenchyma cells in the sapwood, transition wood, and heartwood due to species evolution (Figure 5f).

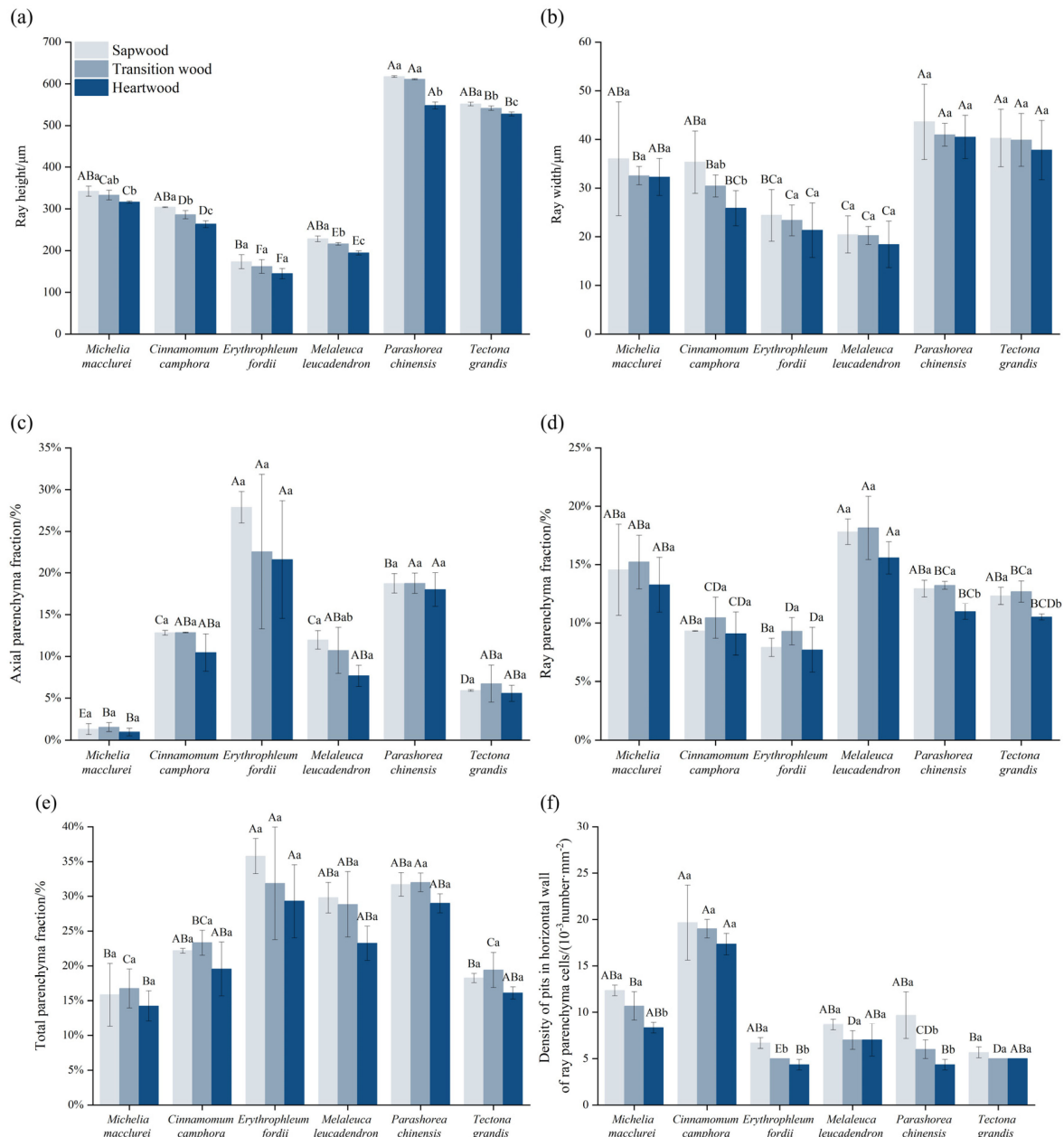


Figure 5. Structural differences in parenchyma cells in the sapwood, transition wood, and heartwood of the tree species with different evolutionary degrees (mean \pm SD). Capital letters represent significant differences among the different tree species; lowercase letters represent significant differences among the sapwood, transition wood, and heartwood of the same tree species. (a) Ray height. (b) Ray width. (c) Axial parenchyma fraction. (d) Ray parenchyma fraction. (e) Total parenchyma fraction. (f) Density of pits in the horizontal wall of ray parenchyma cells.

In the more primitive species, the starch grains were mainly distributed in the axial parenchyma cells or ray parenchyma cells of the sapwood, and in the more evolved species,

starch grains were abundantly distributed in both types of parenchyma cells in the sapwood (Figure 6). The starch grains in the sapwood of *Michelia macclurei* (Figure 6a) and *Melaleuca leucadendron* (Figure 6j) were distributed in small amounts in the corners of the ray parenchyma cells. The starch grains in the sapwood of *Cinnamomum camphora* (Figure 6d), *Erythrophleum fordii* (Figure 6g), and *Parashorea chinensis* (Figure 6m) were mainly distributed in the axial parenchyma cells, whereas the starch grains in the sapwood of *Tectona grandis* (Figure 6p) were distributed in large amounts in the axial and ray parenchyma cells. Additionally, the starch grains in the transition wood of *Michelia macclurei* (Figure 6b), *Melaleuca leucadendron* (Figure 6k), and *Tectona grandis* (Figure 6q) were distributed in the ray parenchyma cells in small amounts, and the starch grains in the transition wood of *Cinnamomum camphora* (Figure 6e) and *Erythrophleum fordii* (Figure 6h) were distributed in the axial and ray parenchyma cells in small amounts. Furthermore, the starch grains in the transition wood of *Parashorea chinensis* (Figure 6n) were distributed in the axial parenchyma cells in small amounts. The starch grains were not found in the heartwood of *Michelia macclurei* (Figure 6c), *Melaleuca leucadendron* (Figure 6l), and *Parashorea chinensis* (Figure 6o), and only a few starch grains were found in the ray parenchyma cells in the heartwood of *Cinnamomum camphora* (Figure 6f), *Erythrophleum fordii* (Figure 6i), and *Tectona grandis* (Figure 6r).

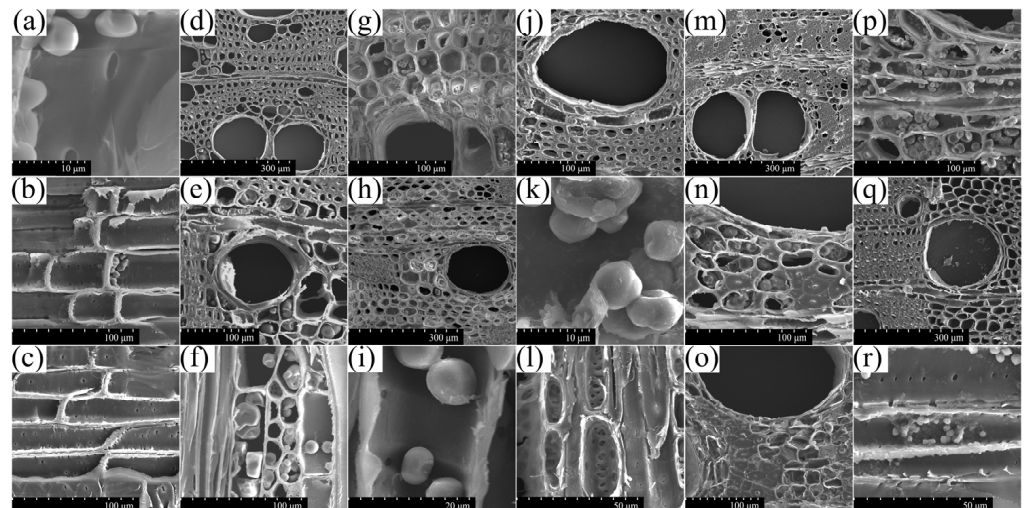


Figure 6. Images of the starch granules in the sapwood, transition wood, and heartwood of tree species with different evolutionary degrees. (a) Radial section of sapwood of *Michelia macclurei*. (b) Radial section of the transition wood of *Michelia macclurei*. (c) Radial section of the heartwood of *Michelia macclurei*. (d) Cross section of the sapwood of *Cinnamomum camphora*. (e) Cross section of the transition wood of *Cinnamomum camphora*. (f) Tangential section of the heartwood of *Cinnamomum camphora*. (g) Cross section of the sapwood of *Erythrophleum fordii*. (h) Cross section of the transition wood of *Erythrophleum fordii*. (i) Tangential section of the heartwood of *Erythrophleum fordii*. (j) Cross section of the sapwood of *Melaleuca leucadendron*. (k) Radial section of the transition wood of *Melaleuca leucadendron*. (l) Tangential section of the heartwood of *Melaleuca leucadendron*. (m) Cross section of the sapwood of *Parashorea chinensis*. (n) Cross section of the transition wood of *Parashorea chinensis*. (o) Cross section of the heartwood of *Parashorea chinensis*. (p) Cross section of the sapwood of *Tectona grandis*. (q) Cross section of the transition wood of *Tectona grandis*. (r) Radial section of the heartwood of *Tectona grandis*.

3.2.2. Variation in the Parenchyma Cell Structure among the Sapwood, Transition Wood, and Heartwood of the Same Species

From the sapwood to the transitional wood and heartwood, the ray parenchyma fraction tended to be stable in the more primitive species, while in the more evolved species it tended to be stable before decreasing. The pit density in the horizontal wall of the ray parenchyma cells tended to be stable and then decrease in the more primitive species, and tended to be stable or decrease before stabilizing in the more evolved species.

There were no significant differences in the ray parenchyma fractions among the sapwood, transitional wood, and heartwood of four species, namely, *Michelia macclurei*, *Cinnamomum camphora*, *Erythrophleum fordii*, and *Melaleuca leucadendron*. In addition, the ray parenchyma fraction in the sapwood and transitional wood was significantly larger than that in the heartwood of *Parashorea chinensis* and *Tectona grandis*. The pit density in the horizontal wall of the ray parenchyma cells in the transition wood was significantly greater than that in the heartwood of *Michelia macclurei*, while there were no significant differences in the densities of pits in the horizontal wall of the ray parenchyma in the transition wood and heartwood of *Cinnamomum camphora*, *Erythrophleum fordii*, *Melaleuca leucadendron*, *Parashorea chinensis*, and *Tectona grandis*. Additionally, the distribution of the starch grains between the sapwood and the heartwood was reduced in all species. Therefore, from the sapwood to the heartwood, the axial parenchyma fraction, total parenchyma fraction, ray height, ray width, and distribution of the starch particles did not have obvious patterns with regard to the evolution of the tree species.

4. Discussion

4.1. Structural Variation in the Xylem Vessels and Its Association with Tree Evolution

This study showed that more evolved species had larger xylem earlywood vessel lumen diameters, thicker walls, and wider hydraulic diameters, in addition to also having smaller latewood vessel densities. Large vessel lumen diameters [29] and thick walls are evolutionary trends in xylem vessels [30], and the general pattern exhibited by the vessel density is influenced by phylogeny [31]. When trees generate larger vessels, avoiding embolization in the transport of water due to an excessive vessel diameter is achieved by reducing vessel density [15]. Therefore, more evolved tree species reduce the vessel density when increasing the vessel lumen diameter, thus achieving the optimal selection and configuration of the vessels.

The results of this study showed that from the sapwood to the transition wood and heartwood, the earlywood vessel lumen diameter of the original tree species showed a trend of first being stable and then decreasing, and the earlywood vessel lumen diameter of the more evolved species tended to gradually decrease. This is consistent with previous findings that identified the earlywood vessel lumen diameter of the more evolved species *Catalpa bungei* C. A. Mey to increase with the increasing age of the forming layer [32]. An increased vessel lumen diameter reflects the need for high hydraulic efficiency in trees [18]. Trees need to maintain a certain level of water transport; however, an increase in the sapwood area results in metabolic costs for the new wood or affects the maintenance of parenchyma cell viability [33]. In contrast, generating larger vessels does not incur additional costs and increases the efficiency of water transport in the sapwood of the tree. This study showed that from the sapwood to the heartwood, the latewood vessel density of the more primitive species tended to be stable and then increase, while the latewood vessel density of the more evolved species tended to be stable. This indicates that the evolved species maintain a certain vessel density in the transition from sapwood to heartwood. Some scholars found that there was no significant difference between the latewood vessel density of the sapwood and heartwood in Legumes (Fabaceae) [34], which was consistent with the findings of this study. The latewood vessels can remain functional for many years, ensuring water transport security [35]. Thus, the more stable latewood vessel density reflects the need for hydraulic security in trees.

In the most primitive tree species, namely, *Michelia macclurei*, the earlywood vessel lumen diameter was significantly negatively correlated with the vessel wall thickness, while in the more evolved tree species, such as *Cinnamomum camphora* and *Tectona grandis*, the earlywood vessel lumen diameters were significantly positively correlated with the vessel wall thicknesses. This suggests that the more evolved species with larger earlywood vessel lumen diameters also have thicker vessel walls. A study on the relationship between the vessel wall thickness and vessel lumen diameter in 858 species of plants found that for plants with a vessel lumen diameter above 90 μm , the vessel wall thickness was moderately

positively correlated with the vessel lumen diameter [36], which is consistent with the results of this study. Thicker vessel walls increase the ability of the vessel to resist implosion, thereby increasing its embolic resistance [37]. Thus, a trade-off between the effectiveness and safety of the hydraulic transport was found in *Michelia macclurei*, but in the more evolved species, the trees improved both the efficiency and safety of water transport, which indicates a synergistic relationship between the two.

4.2. Structural Variation in the Xylem Parenchyma Cells and Its Association with Tree Evolution

This study showed that in the more primitive species, the starch grains were mainly distributed in the axial parenchyma cells or ray parenchyma cells of the sapwood, and in the more evolved species they were abundantly distributed in both types of parenchyma cells in the sapwood. Another study showed that the starch grains of *Pentaclethra macroloba* (Willd.) Kuntze, a Fabaceae species, were mainly distributed in the axial parenchyma cells, and the starch grains of *Ceiba pentandra* (L.) (Gaertn.) of the Malvaceae family were mainly distributed in the ray parenchyma cells. In contrast, in the *Brosimum* sp. of the family Moraceae, in *Acer pseudoplatanus* L. and *Aesculus hippocastanum* L. of the family Sapindaceae, and in *Fraxinus excelsior* L. of the family Oleaceae, there were a large number of starch grains in axial and parenchyma cells [38]. Oleaceae is more evolved than Malvaceae and Fabaceae, and Sapindaceae and Moraceae are more evolved than Fabaceae, which is in agreement with the results that were obtained in this study. More evolved tree species have a higher utilization of the axial and ray parenchyma cells' storage space, allowing for axial and parenchyma cells to perform multiple functions. In a study of 800 tree species in China, the axial parenchyma was found to be more correlated with theoretical hydraulic conductivity [39]. The paratracheal parenchyma plays an important role in embolism repair [8]. Carbohydrates that are stored as starch granules in the axial and ray parenchyma cells are broken down for spring leaf expansion, formative layer growth, embolism repair [40], and heartwood formation [41]. The reduced distribution of starch grains in the transition wood and heartwood of all species is because the sapwood contains stored nutrients, such as starch grains, that are synthesized and accumulated by the axial and ray parenchyma cells with different types of extractives after the conversion of sapwood to heartwood [42]. There is also a close relationship between the extractives and durability of the heartwood [43]. Therefore, as trees evolve, the starch grains that are distributed in large numbers in axial and ray parenchyma cells can better serve as an energy reserve for tree growth.

From the sapwood to the heartwood, the ray parenchyma fraction tended to stabilize in the more primitive species, and stabilize and decrease in the more evolved species. In [18], the radial variation in the xylem anatomical characteristics of five species, namely, *Neolitsea obtusifolia* Merrill (Lauraceae), *Azelia xylocarpa* Craib (Fabaceae), *Chukrasia tabularis* A. Juss. (Meliaceae), *Melia azedarach* L. (Meliaceae), and *Toona ciliata* M. Roem. (Meliaceae), was studied, and the results showed that the ray parenchyma fraction increased as the tree diameter increased in all the species except for *Neolitsea obtusifolia*. In the above study, the Lauraceae was more primitive, while both the Fabaceae and Meliaceae were more evolved; thus, these findings agreed with the results of this study. A study on the xylem anatomical characteristics of 15 species of subtropical woody plants in Japan found that the ray parenchyma fraction was positively correlated with the starch and non-structural carbohydrate (NSC) concentration [44]. Therefore, the larger ray parenchyma fraction of the sapwood and transitional wood of the more evolved species provides greater nutrient storage potential, which is conducive to providing the plants with greater environmental adaptability.

From the sapwood to the heartwood, the pit density in the horizontal wall of the ray parenchyma cells of the more primitive species tended to be stable and then decrease, whereas in the more evolved species it tended to be stable or decrease before stabilizing. In [45], the anatomical variation in the pit density in the horizontal wall of the ray parenchyma cells from the sapwood to heartwood of *Populus tomentosa* Carrière (Meliaceae)

demonstrated no significant difference, and Meliaceae is more evolved than Leguminosae, which is consistent with the results of this study. Moreover, a study of the water distribution in the sapwood, transitional wood, and heartwood of *Cryptomeria japonica* D. Don showed that many earlywood tracheids were again filled with free water in the dark-brown heartwood, and that water in the heartwood gradually diffused into the transitional wood and sapwood even though the cell walls of the transitional wood and latewood tracheid contained water [46]. A study on the movement of mineral nutrients from the heartwood to the sapwood in *Cryptomeria japonica* showed that when Cs were injected into the heartwood, they moved toward the boundary of the transition wood but failed to reach the transition wood and sapwood, and the anatomical characteristics of the transition wood prevented the movement of the mineral elements from the heartwood to the sapwood [47]. The ray parenchyma cells can transport fluids to neighboring ray parenchyma cells through the pits on the horizontal walls, which form a network structure [45]. Therefore, the stable pit density in the horizontal wall of the ray parenchyma cells between the transition wood and heartwood of the more evolved species may be helpful for nutrient transportation.

5. Conclusions

Evolutionary tree species have gradually carried out the optimal selection and configuration of their vessels. In this study, it was found that the vessel wall thickness, earlywood vessel lumen diameter, and vessel hydraulic diameter tended to increase alongside tree evolution, while the latewood vessel density tended to decrease alongside tree evolution. From the sapwood to the heartwood, the earlywood vessel lumen diameter of the more primitive species tended to be stable and then decrease, while in more evolved species it tended to gradually decrease; meanwhile, the latewood vessel density of the more primitive species tended to be stable and then increase, while in the more evolved species it tended to be stable. The more evolved species had larger vessel lumen diameters and thicker vessel walls with better water transport effectiveness and higher safety. The starch grains of the more primitive species were mainly distributed in the axial or ray parenchyma cells of the sapwood, while the more evolved species had more starch grains in the axial and ray parenchyma cells of the sapwood, which could be a better energy reserve for tree growth; the distribution of the starch grains was reduced in the transition wood and heartwood of all species. From the sapwood to the heartwood, the ray parenchyma fraction tended to be stable in the more primitive species and tended to be stable before decreasing in the more evolved species, which provides more nutrient storage potential for the trees. Additionally, the pit density in the horizontal wall of the ray parenchyma cells tended to be stable and then decrease in the more primitive species, and tended to be stable or decrease before stabilizing in the more evolved species. The smooth pit density in the horizontal wall of the ray parenchyma cells in the transitional wood and heartwood helps nutrient transport. These findings contribute to the understanding of the significance of the structural evolution of vessels and parenchyma cells and can serve as a basis for further studies on the evolutionary history of trees. In addition, the xylem consists of vessels, parenchyma cells, and fibers. However, only vessels and parenchyma cells were studied in this paper because both cells are closely related to heartwood formation, and parenchyma cells in transitional wood convert nutrients such as starch granules into extractives before death and diffuse these extractives to other parts through the pits of the vessels walls immediately connected to ray parenchyma cells. The fiber variation pattern can be further investigated in the future.

Author Contributions: Conceptualization, Y.F. and L.H.; methodology, Y.F.; software, L.H.; validation, Y.F., L.H. and Z.L.; formal analysis, Z.L.; investigation, Z.L.; resources, Y.F.; data curation, P.W.; writing—original draft preparation, L.H.; writing—review and editing, Y.F. and Z.L.; visualization, L.H.; supervision, P.W.; project administration, Y.F.; funding acquisition, Y.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 32171702.

Data Availability Statement: Data are contained within the article.

Acknowledgments: Our sincere gratitude extends to the Experimental Center of Tropical Forestry of the Chinese Academy of Forestry for providing the samples.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Slupianek, A.; Dolzblasz, A.; Sokolowska, K. Xylem parenchyma—Role and relevance in wood functioning in trees. *Plants* **2021**, *10*, 1247. [[CrossRef](#)] [[PubMed](#)]
- Tomasella, M.; Petrusa, E.; Petruzzellis, F.; Nardini, A.; Casolo, V. The possible role of non-structural carbohydrates in the regulation of tree hydraulics. *Int. J. Mol. Sci.* **2020**, *21*, 144. [[CrossRef](#)] [[PubMed](#)]
- Wheeler, E.A.; Baas, P.; Rodgers, S. Variations in dicot wood anatomy: A global analysis based on the insidewood database. *IAWA J.* **2007**, *28*, 229–258. [[CrossRef](#)]
- Burgert, I.; Eckstein, D. The tensile strength of isolated wood rays of beech (*Fagus sylvatica* L.) and its significance for the biomechanics of living trees. *Trees-Struct. Funct.* **2001**, *15*, 168–170. [[CrossRef](#)]
- Menard, D.; Pesquet, E. Cellular interactions during tracheary elements formation and function. *Curr. Opin. Plant. Biol.* **2015**, *23*, 109–115. [[CrossRef](#)]
- Obara, K.; Kuriyama, H.; Fukuda, H. Direct evidence of active and rapid nuclear degradation triggered by vacuole rupture during programmed cell death in zinnia. *Plant Physiol.* **2001**, *125*, 615–626. [[CrossRef](#)]
- Pfautsch, S.; Renard, J.; Tjoelker, M.G.; Salih, A. Phloem as capacitor: Radial transfer of water into xylem of tree stems occurs via symplastic transport in ray parenchyma. *Plant Physiol.* **2015**, *167*, 963–971. [[CrossRef](#)]
- Morris, H.; Gillingham, M.A.F.; Plavcova, L.; Gleason, S.M.; Olson, M.E.; Coomes, D.A.; Fichtler, E.; Klepsch, M.M.; Martinez-Cabrera, H.I.; McGlinn, D.J.; et al. Vessel diameter is related to amount and spatial arrangement of axial parenchyma in woody angiosperms. *Plant Cell Environ.* **2018**, *41*, 245–260. [[CrossRef](#)]
- Fichtler, E.; Worbes, M. Wood anatomical variables in tropical trees and their relation to site conditions and individual tree morphology. *IAWA J.* **2012**, *33*, 119–140. [[CrossRef](#)]
- Sperry, J.S.; Hacke, U.G.; Feild, T.S.; Sano, Y.; Sikkema, E.H. Hydraulic consequences of vessel evolution in angiosperms. *Int. J. Plant Sci.* **2007**, *168*, 1127–1139. [[CrossRef](#)]
- Sperry, J.S.; Hacke, U.G.; Pittermann, J. Size and function in conifer tracheids and angiosperm vessels. *Am. J. Bot.* **2006**, *93*, 1490–1500. [[CrossRef](#)] [[PubMed](#)]
- Pfautsch, S.; Harbusch, M.; Wesolowski, A.; Smith, R.; Macfarlane, C.; Tjoelker, M.G.; Reich, P.B.; Adams, M.A. Climate determines vascular traits in the ecologically diverse genus eucalyptus. *Ecol. Lett.* **2016**, *19*, 240–248. [[CrossRef](#)] [[PubMed](#)]
- Gleason, S.M.; Westoby, M.; Jansen, S.; Choat, B.; Hacke, U.G.; Pratt, R.B.; Bhaskar, R.; Brodribb, T.J.; Bucci, S.J.; Cao, K.F.; et al. Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytol.* **2016**, *209*, 123–136. [[CrossRef](#)]
- Liu, H.; Ye, Q.; Gleason, S.M.; He, P.C.; Yin, D.Y. Weak tradeoff between xylem hydraulic efficiency and safety: Climatic seasonality matters. *New Phytol.* **2021**, *229*, 1440–1452. [[CrossRef](#)] [[PubMed](#)]
- Sperry, J.S.; Meinzer, F.C.; McCulloh, K.A. Safety and efficiency conflicts in hydraulic architecture: Scaling from tissues to trees. *Plant Cell Environ.* **2008**, *31*, 632–645. [[CrossRef](#)] [[PubMed](#)]
- Wheeler, J.K.; Sperry, J.S.; Hacke, U.G.; Hoang, N. Inter-vessel pitting and cavitation in woody rosaceae and other vesselless plants: A basis for a safety versus efficiency trade-off in xylem transport. *Plant Cell Environ.* **2005**, *28*, 800–812. [[CrossRef](#)]
- Lens, F.; Sperry, J.S.; Christman, M.A.; Choat, B.; Rabaey, D.; Jansen, S. Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus acer. *New Phytol.* **2011**, *190*, 709–723. [[CrossRef](#)]
- Rungwattana, K.; Hietz, P. Radial variation of wood functional traits reflect size-related adaptations of tree mechanics and hydraulics. *Funct. Ecol.* **2018**, *32*, 260–272. [[CrossRef](#)]
- Carlquist, S. Living cells in wood 3. Overview; functional anatomy of the parenchyma network. *Bot. Rev.* **2018**, *84*, 242–294. [[CrossRef](#)]
- Hearn, D.J. Developmental patterns in anatomy are shared among separate evolutionary origins of stem succulent and storage root-bearing growth habits in *Adenia* (*Passifloraceae*). *Am. J. Bot.* **2009**, *96*, 1941–1956. [[CrossRef](#)]
- Martinez-Cabrera, H.I.; Zheng, J.M.; Estrada-Ruiz, E. Wood functional disparity lags behind taxonomic diversification in angiosperms. *Rev. Palaeobot. Palynol.* **2017**, *246*, 251–257. [[CrossRef](#)]
- Zhou, C.B.; Xia, H.U.; Song, Y.Y.; Gong, W.; Ting-Xing, H.U. Radial variation and its storage function of ray tissue. *J. Northwest For. Univ.* **2016**, *31*, 179–183.
- Tsuchiya, R.; Furukawa, I. Relationship between the radial variation of ray characteristics and the stages of radial stem increment in *zelkova serrata*. *J. Wood Sci.* **2010**, *56*, 495–501. [[CrossRef](#)]

24. Leal, S.; Sousa, V.B.; Pereira, H. Within and between-tree variation in the biometry of wood rays and fibres in cork oak (*Quercus suber* L.). *Wood Sci. Technol.* **2006**, *40*, 585–597. [\[CrossRef\]](#)
25. Naji, H.R.; Sahri, M.H.; Nobuchi, T.; Bakar, E.S. Radial variation of wood cell features under different stocking densities management of two new clones of rubberwood (*Hevea brasiliensis*). *J. Wood Sci.* **2013**, *59*, 460–468. [\[CrossRef\]](#)
26. Chen, S.Y.; Yen, P.L.; Chang, T.C.; Chang, S.T.; Huang, S.K.; Yeh, T.F. Distribution of living ray parenchyma cells and major bioactive compounds during the heartwood formation of *taiwania cryptomerioides* hayata. *J. Wood Chem. Technol.* **2018**, *38*, 84–95. [\[CrossRef\]](#)
27. Scholz, A.; Klepsch, M.; Karimi, Z.; Jansen, S. How to quantify conduits in wood? *Front. Plant Sci.* **2013**, *4*, 56. [\[CrossRef\]](#)
28. Sterck, F.J.; Zweifel, R.; Sass-Klaassen, U.; Chowdhury, Q. Persisting soil drought reduces leaf specific conductivity in scots pine (*Pinus sylvestris*) and pubescent oak (*Quercus pubescens*). *Tree Physiol.* **2008**, *28*, 529–536. [\[CrossRef\]](#)
29. Olson, M.E. From carlquist's ecological wood anatomy to carlquist's law: Why comparative anatomy is crucial for functional xylem biology. *Am. J. Bot.* **2020**, *107*, 1328–1341. [\[CrossRef\]](#)
30. Frost, F.H. Specialization in secondary xylem of dicotyledons. I. Origin of vessel. *Bot. Gaz.* **1930**, *89*, 67–94. [\[CrossRef\]](#)
31. Thomas, R.; Boura, A. Palm stem anatomy: Phylogenetic or climatic signal? *Bot. J. Linn. Soc.* **2015**, *178*, 467–488. [\[CrossRef\]](#)
32. Li, S.; Li, X.; Link, R.; Li, R.; Deng, L.P.; Schuldt, B.; Jiang, X.M.; Zhao, R.J.; Zheng, J.M.; Li, S.; et al. Influence of cambial age and axial height on the spatial patterns of xylem traits in *Catalpa bungei*, a ring-porous tree species native to China. *Forests* **2019**, *10*, 662. [\[CrossRef\]](#)
33. Brodersen, C.R.; McElrone, A.J. Maintenance of xylem network transport capacity: A review of embolism repair in vascular plants. *Front. Plant Sci.* **2013**, *4*, 108. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Jokanovic, D.; Vilotic, D.; Mitrovic, S.; Miljkovic, D.; Rebic, M.; Stankovic, D.; Nikolic, V. Correlations between the anatomical traits of *Gymnocladus canadensis* lam. In heartwood and sapwood of early- and latewood zones of growth rings. *Arch. Biol. Sci.* **2015**, *67*, 1399–1404. [\[CrossRef\]](#)
35. Kitin, P.; Funada, R. Earlywood vessels in ring-porous trees become functional for water transport after bud burst and before the maturation of the current-year leaves. *IAWA J.* **2016**, *37*, 315–331. [\[CrossRef\]](#)
36. Echeverria, A.; Petrone-Mendoza, E.; Segovia-Rivas, A.; Figueroa-Abundiz, V.A.; Olson, M.E. The vessel wall thickness-vessel diameter relationship across woody angiosperms. *Am. J. Bot.* **2022**, *109*, 856–873. [\[CrossRef\]](#)
37. Jacobsen, A.L.; Ewers, F.W.; Pratt, R.B.; Paddock, W.A.; Davis, S.D. Do xylem fibers affect vessel cavitation resistance? *Plant Physiol.* **2005**, *139*, 546–556. [\[CrossRef\]](#)
38. Plavcova, L.; Hoch, G.; Morris, H.; Ghiasi, S.; Jansen, S. The amount of parenchyma and living fibers affects storage of nonstructural carbohydrates in young stems and roots of temperate trees. *Am. J. Bot.* **2016**, *103*, 603–612. [\[CrossRef\]](#)
39. Zheng, J.M.; Martinez-Cabrera, H.I. Wood anatomical correlates with theoretical conductivity and wood density across China: Evolutionary evidence of the functional differentiation of axial and radial parenchyma. *Ann. Bot. Lond.* **2013**, *112*, 927–935. [\[CrossRef\]](#)
40. Spicer, R. Symplasmic networks in secondary vascular tissues: Parenchyma distribution and activity supporting long-distance transport. *J. Exp. Bot.* **2014**, *65*, 1829–1848. [\[CrossRef\]](#)
41. Nakaba, S.; Sano, Y.; Funada, R. Disappearance of microtubules, nuclei and starch during cell death of ray parenchyma in *abies sachalinensis*. *IAWA J.* **2013**, *34*, 135–146. [\[CrossRef\]](#)
42. Nagasaki, T.; Yasuda, S.; Imai, T. Immunohistochemical localization of agatharesinol, a heartwood norlignan, in *cryptomeria japonica*. *Phytochemistry* **2002**, *60*, 461–466. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Kokutse, A.D.; Stokes, A.; Bailleres, H.; Kokou, K.; Baudasse, C. Decay resistance of togolese teak (*Tectona grandis* L.f) heartwood and relationship with colour. *Trees* **2006**, *20*, 219–223. [\[CrossRef\]](#)
44. Kawai, K.; Minagi, K.; Nakamura, T.; Saiki, S.T.; Yazaki, K.; Ishida, A. Parenchyma underlies the interspecific variation of xylem hydraulics and carbon storage across 15 woody species on a subtropical island in Japan. *Tree Physiol.* **2022**, *42*, 337–350. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Yin, L.J.; Jiang, X.M.; Ma, L.Y.; Liu, S.J.; He, T.; Jiao, L.C.; Yin, Y.F.; Yao, L.H.; Guo, J. Anatomical adaptations of pits in two types of ray parenchyma cells in *populus tomentosa* during the xylem differentiation. *J. Plant Physiol.* **2022**, *278*, 153830. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Kuroda, K.; Yamashita, K.; Fujiwara, T. Cellular level observation of water loss and the refilling of tracheids in the xylem of *cryptomeria japonica* during heartwood formation. *Trees* **2009**, *23*, 1163–1172. [\[CrossRef\]](#)
47. Kuroda, K.; Yamane, K.; Itoh, Y. In planta analysis of the radial movement of minerals from inside to outside in the trunks of standing Japanese cedar (*Cryptomeria japonica* D. Don) trees at the cellular level. *Forests* **2021**, *12*, 251. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.