MESOSTRUCTURAL CHARACTERIZATION OF PHYLLOSTACHYS AUREA BAMBOO USING OPTICAL MICROSCOPY AND DIGITAL IMAGE PROCESSING

Mario Seixas, Pontifical Catholic University of Rio de Janeiro (PUC-Rio), Department of Civil and Environmental Engineering, and Bambutec Design, Brazil, marioseixas@puc-rio.br
Sidnei Paciornik, Pontifical Catholic University of Rio de Janeiro (PUC-Rio), Department of Chemical Engineering and Materials, Brazil, sidnei@puc-rio.br
Daniel Cardoso, Pontifical Catholic University of Rio de Janeiro (PUC-Rio), Department of Civil and Environmental Engineering, Brazil, dctcardoso@puc-rio.br
Luís Eustáquio Moreira, Federal University of Minas Gerais, Department of Structural Engineering, Brazil, luis@dees.ufmg.br

ABSTRACT
This study investigates the *Phyllostachys aurea* bamboo mesostructure through optical microscopy and digital image processing. Specimens were produced using a bamboo full-culm with cross-sections sliced from 1, 4, and 6 meters distant from the ground. Digital images captured through light microscopy with the magnification of 200x were digitally processed for the segmentation of sclerenchyma, vessels, parenchyma, and epidermis phases, allowing for the extraction of the material's anatomical features. Binary images obtained from each phase were produced and, then, measured, showing portions of the constituent phases of *P. aurea* cross-sections at the bottom, middle, and top parts of the culm, respectively.

KEYWORDS
bamboo, full-culm, bio-based material, hierarchical graded structure, composites, optical microscopy, digital image processing

INTRODUCTION
Bamboo is a giant arborescent grass from the *Poaceae* family and *Bambusoideae* subfamily. Bamboo is constituted by a lignocellulosic ramifying system of vegetative axes formed by roots, rhizomes, culms, branches, and leaves. In addition to the environmental benefits related to bamboo plantations, like preservation of water sources, reforestation, and consumption of carbon dioxide from the atmosphere, the use of bamboo culms and strips have been applied as a sustainable building material in architectural design and civil engineering. Although the high-quality characteristics attributed to the physical and mechanical properties of bamboo, such as lightness, strength, flexibility, and workability, these qualities have not been completely explored, due to the lack of information about bamboo as an engineering material.

Bamboo is a bio-based functionally graded composite material (FGCM) at macro, meso, micro, and nanoscales (Ghavami et al., 2003), with an optimized hollow and cylindrical geometry. The culm wall is composed of vascular bundles surrounded by axially aligned fiber strands, immersed in a parenchymatous matrix of lignin and hemicellulose. The constituent phases of bamboo as a vegetable composite material are well-known and were extensively studied (Liese, 1998). The fiber distribution along the culm wall increases from the inner to the outer portion of the bamboo cross-section and also from the base to the top part of the culm. It was observed that Young's modulus and the tensile strength parallel to the fibers are determined linearly to the fiber volumetric fraction along the culm wall, through microscope investigations of laminae obtained from bamboo specimens (Zhou et al., 2012). A comparative study between *Moso*, *Guadua*, and *Tre Gai* bamboos showed that the modulus of elasticity and the modulus of rupture presents a linear variation considering the increment of fiber density from the inner to the outer portion in the culm wall (Dixon et al., 2015). The ratio between fiber density, matrix, and voids into the culm wall was studied for *Dendrocalamus giganteus* bamboo using optical microscopy and x-ray microtomography by (Krause et al., 2016). It was observed for this species that voids inside the cross-sectional area increase towards the outer portion of the culm wall, associated with a higher presence of sclerenchyma and interfaces with the parenchymatous matrix. Voids can be seen in the multi-phase structure of bamboo and are observed in vessels, lumen (sclerenchyma voids), and parenchyma pores.

The bamboo structure is constituted by vascular bundles reinforced by cellulosic fibers, embedded in a polymeric matrix of lignin and hemicellulose. The lignocellulosic hierarchical graded structure of bamboo plays an important role in its engineering applications (Youssefian & Rahbar, 2015). The vascular bundles are constituted by xylem and phloem vessels, distributed into the bamboo structure as follows: one protoxylem vessel, two metaxylem vessels, and multiple phloem vessels. The protoxylem is responsible for nourishing the culm in its first growth stage. Metaxylem is responsible for transporting water and minerals through capillarity from roots towards the top part of the culm. On the other hand, phloem is responsible for transporting photoassimilate nutrients by leaves, in the opposite direction to the flow of sap transported through xylem vessels (Liese, 1998). Sclerenchyma is a supporting tissue that surrounds the vascular bundles reinforcing them, being constituted by cellulosic fiber sheaths. Some parameters that influence the mechanical properties of sclerenchyma are fiber diameter, size, the structure of crystalline and non-crystalline regions of fibrils, the angle between fibrils (Li et al., 1995), the degree of polymerization of the bamboo wall, and the orientation of the cellulosic chains (Chunhui, 2019). The amount of fiber sheaths increases with the aging of bamboo, as reported by (Liese & Weiner, 1996). These authors studied the aging of *Phyllostachys viridiglaucescens* into the fiber and parenchyma domains. It was observed that bamboo cells are completely lignified after the first growing cycle, which takes approximately three months. During the first year, sclerenchyma cells (fibrils) increase from 1 to 3 lamellae. During the adult stage of the culm, there is an increment in the thickness and the number of fibers' lamellae, and between 9 to 12 years of age, there is an increment of 5 to 6 lamellae, as observed through scanning electronic microscopy (SEM). The mechanical properties of bamboo culms vary according to the different ages of the culms and are highly influenced by fibers. Ghavami & Marinho observed that bamboo species studied at PUC-Rio reached the highest Young's modulus and the peak of their tensile strength at around three years old (Ghavami & Marinho, 2005). Bamboo species present four categories of vascular bundles, as follows: vascular bundles' type I known as Open Type, vascular bundles' type II known as Tight-waist, vascular bundles' type III known as Broken-waist, and finally, vascular bundles' type IV known as Double-broken (Grosser & Liese, 1973). Vascular bundles' type I can be observed in leptomorph bamboos with creeping habit and vascular bundles' types II, III, and IV can be observed in pachymorph bamboos with clumping habit (Hidalgo-López, 2003). Liese performed the anatomical characterization of the vascular bundles of *Phyllostachys edulis* through cross-sections obtained from 6 different heights along the culm, showing their anatomical variations from the bottom to the top part and also from the inner to the outer layer for the analyzed cross-sections (Liese, 1998).

Hidalgo-López proposed the subdivision of the bamboo culm cross-section into three layers, where the outer layer is stiffer and stronger due to the higher volumetric fraction of fibers and the inner layer exhibits the lower mechanical properties (Hidalgo-López, 2003). Gomes performed the anatomical characterization of different bamboo species through images captured in an optical microscope using digital image processing at PUC-Rio. In this work, the cross-sections were subdivided into 6 layers and the vascular bundles were analyzed according to their anatomy and distribution along the bamboo cross-sections (Gomes, 2001). The subdivision of the bamboo cross-section and segmentation was also used to perform the characterization of Moso bamboo (*Phyllostachys pubescens*) to determine the influence of hornification on its physical and flexural properties, as studied by (Cid et al., 2020) and to determine the influence of heat on shrinkage and water absorption of *Dendrocalamus giganteus* bamboo (Azadeh & Ghavami, 2018). In these works, the bamboo cross-section was subdivided into layers and, then, its mechanical properties were evaluated at the mesostructure level. The present paper investigates *Phyllostachys aurea* bamboo species, endemic to the subtropical regions of China, being introduced in Brazil by the Portuguese conquerors with good adaptation to the Brazilian tropical climate (Salgado, 2014). The application of *P. aurea* in engineering structures has been studied at PUC-Rio at micro and mesoscales (Cruz, 2003), and also in full-scale structures, such as active bending arched systems (Seixas et al., 2021a) and lightweight spatial structures (Seixas et al., 2021b). *P. aurea* consists of a strategic bamboo species in the bio-based materials value chain in Brazil, needing more studies about its physical and mechanical behavior.

METHODOLOGY

In this work, the mesostructural characterization of *Phyllostachys aurea* bamboo was performed through optical microscopy and digital image processing techniques. The study aimed to determine the portions of sclerenchyma, parenchyma, vascular bundles, and epidermis phases distributed along the bamboo cross-section at the bottom, middle, and top parts of the culm. Digital images captured through light microscopy with the magnification of 200x were digitally processed using Fiji software with the Weka plugin. 24-bits RGB images were transformed into 8-bits images for segmentation of phases and extraction of the material anatomical features. Binary images obtained from each phase were produced and, then, measured, showing portions of the constituent phases of *P. aurea* bamboo.

MATERIALS AND METHODS

Phyllostachys aurea bamboo culms were selected and harvested from an experimental cultivation farm with 16 years old, located in Paty do Alferes at Rio de Janeiro State, Brazil (22º23’59.80” S and 43º26’53.50” W), situated 680 m above the sea level with an average temperature of 19.9º C along the year and an average annual rainfall of 1,575 mm. Mature culms were felled with ages between 4-5 years old, external diameters measuring 25-50 mm, and wall thicknesses measuring 2.5-6.0 mm. The culms were harvested in July 2020 during the dry season and air-dried in the shade for a period of 1 year. The harvesting procedure followed the recommendations of the ISO Standard 22157-1 (International Standard, 2004). A portion approximately 1-m long from the base of the felled culms was discarded. The culms were washed with water to remove dirt and dust from their external surface. The harvesting of the culms and their preparation is seen in figure 1. Three 10-mm long segments were cut from an untreated single bamboo culm at distances of 1, 4, and 6 m from the ground level obtained from a culm rising 9 m on average, as presented in figure 2. Major and minor external diameters and wall thicknesses of each specimen were measured, according to the Brazilian Standard NBR 16828 (ABNT, 2020), as presented in figure 2a. The specimens were sanded using 400, 600, and 1,200-grain sandpaper. Then, specimens were polished with a diamond paste using 6, 3, and 1 μm grains in an Arotec Aropol polisher from the Laboratory of Metallography and Thermal Treatments (LMTT) from the Department of Chemical Engineering and Materials of PUC-Rio.


a) Felling of the culms with a handsaw

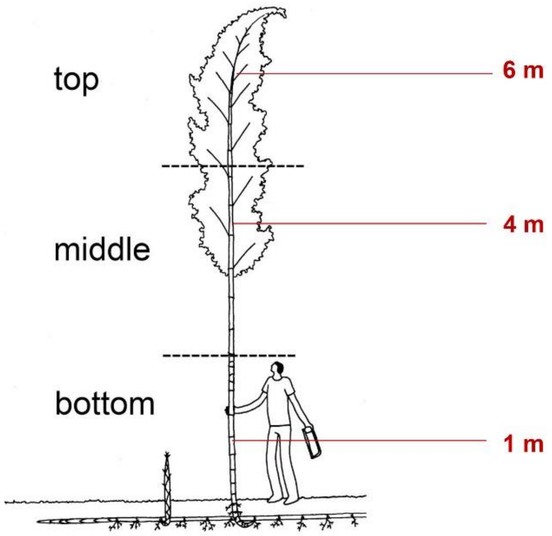

b) Washing of the specimens


c) Green specimens

Figure 1: Harvesting procedure and preparation of *Phyllostachys aurea* bamboo culms

The images of *P. aurea* cross-sections were captured at the Digital Microscopy Laboratory from the Department of Chemical Engineering and Materials at PUC-Rio, using a Zeiss light microscope AxioImager M2m model with the magnification of 200x. The capturing of images was first performed using juxtaposed mosaics and then, superimposed and aligned by correlation, known as the stitching technique (Paciornik & Maurício, 2004).

TOP	MIDDLE	BOTTOM
$D_1 = 26.4$	$D_1 = 39.0$	$D_1 = 43.0$
$D_2 = 27.9$	$D_2 = 40.3$	$D_2 = 46.7$
$t_1 = 2.7$	$t_1 = 3.9$	$t_1 = 5.2$
$t_2 = 3.3$	$t_2 = 4.1$	$t_2 = 6.0$

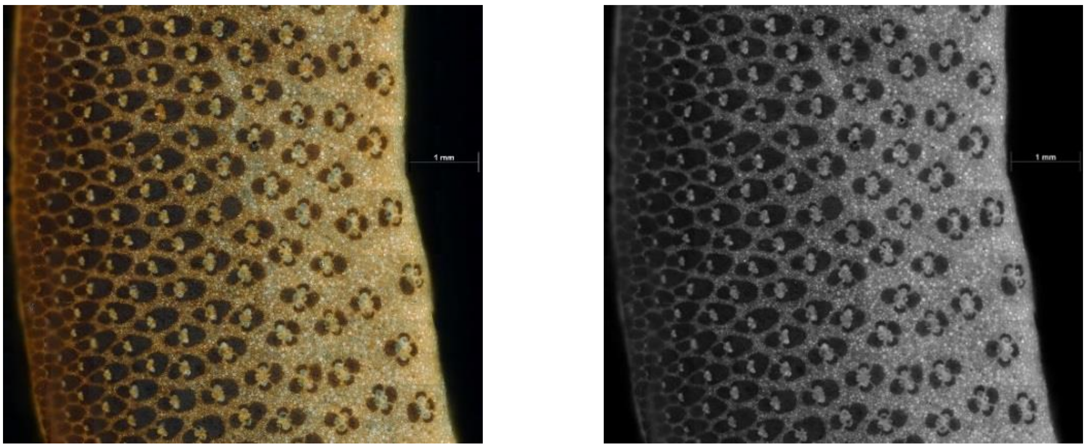


a) Selected cross-section specimens (unit: mm) b) Scheme for obtaining the specimens

Figure 2: Cross-section specimens sliced from a single *Phyllostachys aurea* bamboo culm

DIGITAL IMAGE PROCESSING

Multiple images were captured for each bamboo cross-section and, then, a single reference image was selected for each specimen, resulting in a total of three images for the segmentation procedure. The segmentation step was performed using the open-source Fiji software with the Weka plugin. The images captured through light microscopy were originally formed with 24-bits, i.e. RGB images with 8-bits of red, 8-bits of green, and 8-bits of blue, each one of these with 256 tones for each color, allowing for the representation of more than 16 million combined colors. Later, the 24-bits images were transformed into 8-bits images with 256 shades of gray, as shown in figure 3. The constituent phases of the bamboo cross-section were subdivided into classes according to the material anatomical phases, taken as the epidermis, sclerenchyma, vascular bundles and parenchyma, as presented in figure 4. An additional background class was added for the segmentation procedure (Fig. 4).



a) 24-bits image b) 8-bits image

Figure 3: Captured image from the bottom part of *Phyllostachys aurea* bamboo cross-section

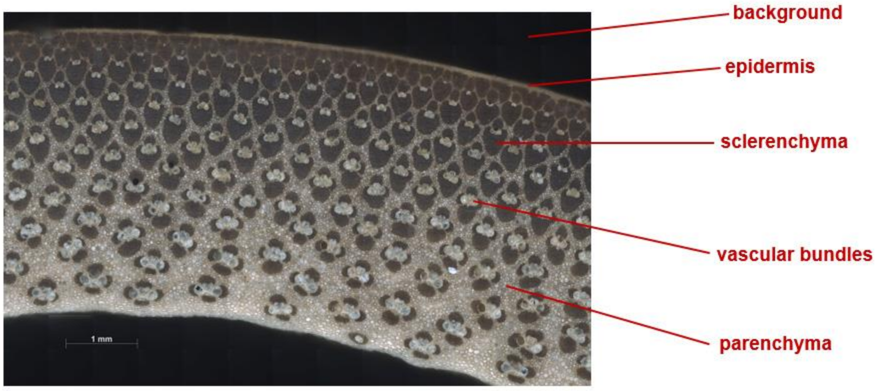
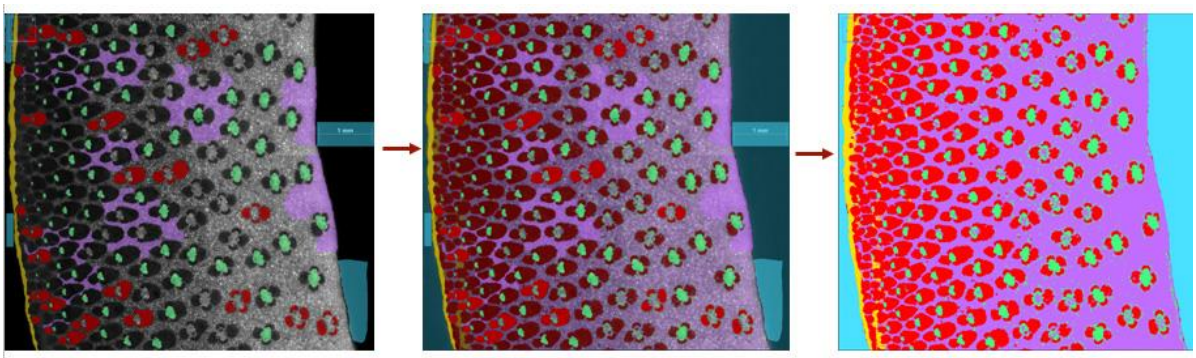


Figure 4: Constituent phases of the bamboo culm cross-section observed in the 24-bits image

Excerpts referring to the material phases were selected and assigned to each of the aforementioned classes for the training of the classifier and, after some tests, the output results of the classifier in RGB were generated. The training of the classifier can be seen in figure 5, as follows: figure 5a shows the selection of the material phases' excerpts into the cross-section, figure 5b shows the training of the classifier after selection, and figure 5c shows the classifier output in RGB, where yellow is attributed to the epidermis, red is attributed to sclerenchyma, pink is attributed to parenchyma, green is attributed to vascular bundles and blue is attributed to the background, respectively.



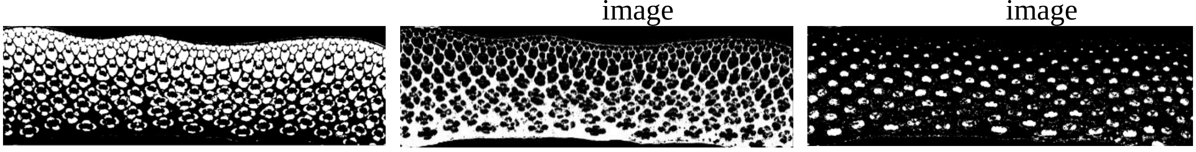
a) Image classification b) Training of the classifier c) Classifier output in RGB

Figure 5: Segmentation of the 8-bits image captured from the middle section of the bamboo culm using the Weka plugin

The segmentation of the images can be considered the critical step of digital image processing, making it possible to distinguish one phase from the others and the background. This distinction will allow for the program to interpret contiguous pixels and group them into regions, consisting of an empirical process adjustable for different images types. Segmentation is complex because it intends to inform the computer of a cognitive process carried out through the human vision (Paciornik, 2001). The output results provided by Weka after training the classifier were analyzed for each image and, then, improved and refined to distinguish the bamboo phases. Several outputs were produced until reaching satisfactory results. After the validation of the outputs, binary images were created for each material phase and the background (all of them depicted in white) using the thresholding selection, applying the intensity of pixel parameter based on the histogram of the images (Figs. 6-8). These images were adopted for the extraction of material anatomical features and quantification of each constituent phase within the selected cross-sections. The extraction of attributes of the constituent phases obtained through the binary images was performed by measurements of the area in the software, thus, particles were measured using ferets unit, then, automatically measured in square millimeters. As expected, an increment of the volumetric fraction of sclerenchyma from the inner towards the outer layer was observed, as well as from the bottom towards the top part of the culm. Inversely, it was observed an increment of parenchyma and epidermis from the top towards the bottom of the analyzed cross-sections.

The preliminary results regarding the extraction of quantitative attributes are shown in Table 1. Residual particles observed during segmentation of images were detected in the binary images and should be refined in further work.

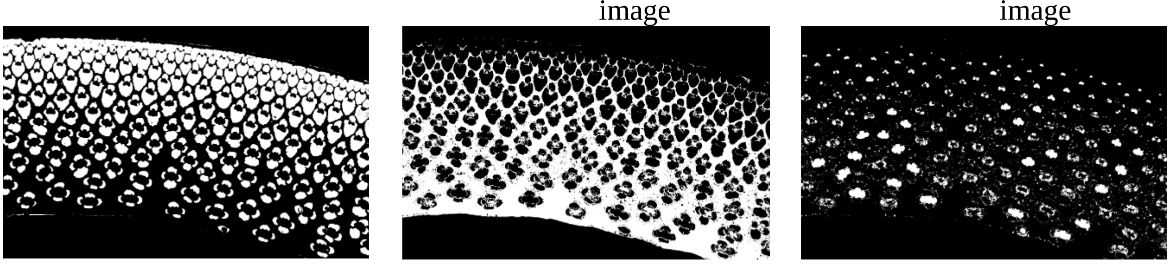
a) RGB image | b) Cross-section binary image | c) Epidermis binary image



d) Sclerenchyma binary image | e) Parenchyma binary image | f) Vascular bundles binary image

Figure 6: Segmentation images from the top portion cross-section

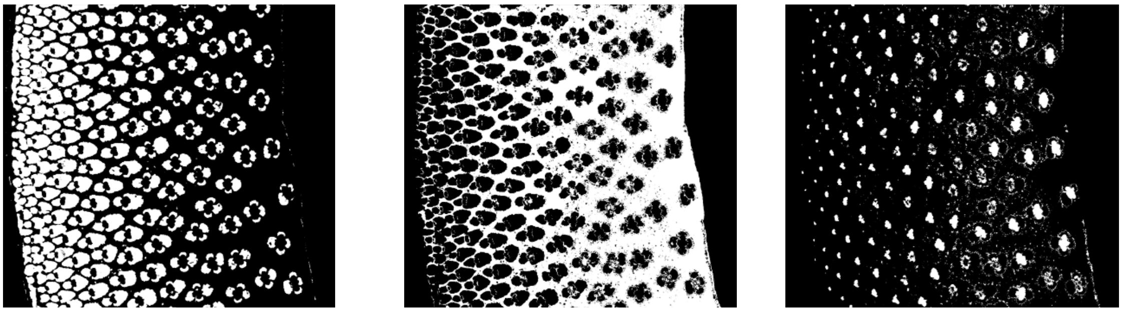
a) RGB image | b) Cross-section binary image | c) Epidermis binary image



d) Sclerenchyma binary image | e) Parenchyma binary image | f) Vascular bundles binary image

Figure 7: Segmentation images from the middle portion cross-section

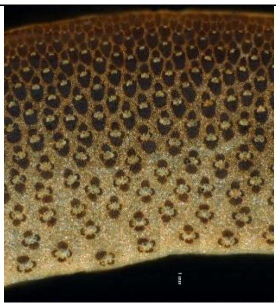
a) RGB image | b) Cross-section binary image | c) Epidermis binary image



d) Sclerenchyma binary image e) Parenchyma binary image f) Vascular bundles binary image

Figure 8: Segmentation images from the bottom portion cross-section

Table 1: Portions of the constituent phases observed along *P. aurea* bamboo cross-sections

Cross-section	Sclerenchyma	Vascular bundles	Parenchyma	Epidermis
 TOP PORTION	44%	13%	41%	2%
 MIDDLE PORTION	40%	10%	48%	2%
 BOTTOM PORTION	36%	9%	53%	2%

In a second stage, the images from the bottom, middle, and top cross-sections were subdivided manually into 3 layers taken as the inner, middle, and outer layers, allowing to depict vascular bundles surrounded by sclerenchyma for each layer. Representative vascular bundles and sclerenchyma particles were selected. The anatomical features observed in the cross-sections, such as the shape of sclerenchyma tissue and vascular bundles are presented in figure 9. Vascular bundles of *Phyllostachys aurea* bamboo are attributed as type I, known as Open Type vessels, due to its geometric arrangement. The changes observed on vascular bundles and sclerenchyma along the cross-section corresponding to each layer can be seen in figure 9.

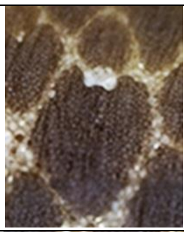
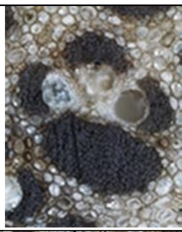
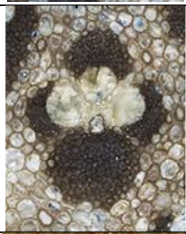
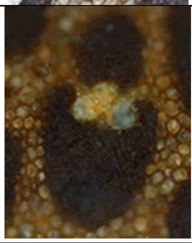
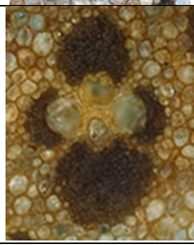
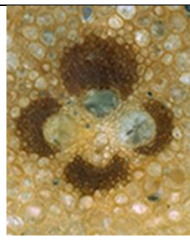
CROSS-SECTION	Outer 1/3	Middle 1/3	Inner 1/3
TOP PORTION			
MIDDLE PORTION			
BOTTOM PORTION			

Figure 9: Anatomical features of selected vascular bundles type I (Open type) surrounded by sclerenchyma observed along the layers into the cross-sections

CONCLUDING REMARKS

The mesostructural characterization of *Phyllostachys aurea* bamboo was performed using optical microscopy and digital image processing. The constituent phases were measured by area, showing portions of each phase in the analyzed cross-sections. Images showed the bamboo hierarchical graded structure, with a higher volumetric fraction of sclerenchyma (fibers) in the outer layer and a higher volumetric fraction of parenchyma (matrix) in the inner layer for all specimens. Additionally, an increase in the volumetric fraction of fibers was observed from the bottom toward the top part of the culm. On the other hand, it was observed inversely an increment of matrix from the top towards the bottom part on the analyzed cross-sections. Anatomical features on the shape of vascular bundles and sclerenchyma along the cross-sections were also identified. The study shows the potential of optical microscopy and digital image processing techniques for the characterization of bamboo, allowing for the extraction of attributes considering its functionally graded composite structure. The results collaborate with information regarding the physical properties of *Phyllostachys aurea* bamboo considering its application in engineering structures and advanced composite polymers.

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CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest associated with the work presented in this paper.

DATA AVAILABILITY
Data on which this paper is based is available from the authors upon reasonable request.