Morphological and Anatomical Characterization of Mature Culms of *Guadua angustifolia* Kunth as a Raw Material for Transformation Processes

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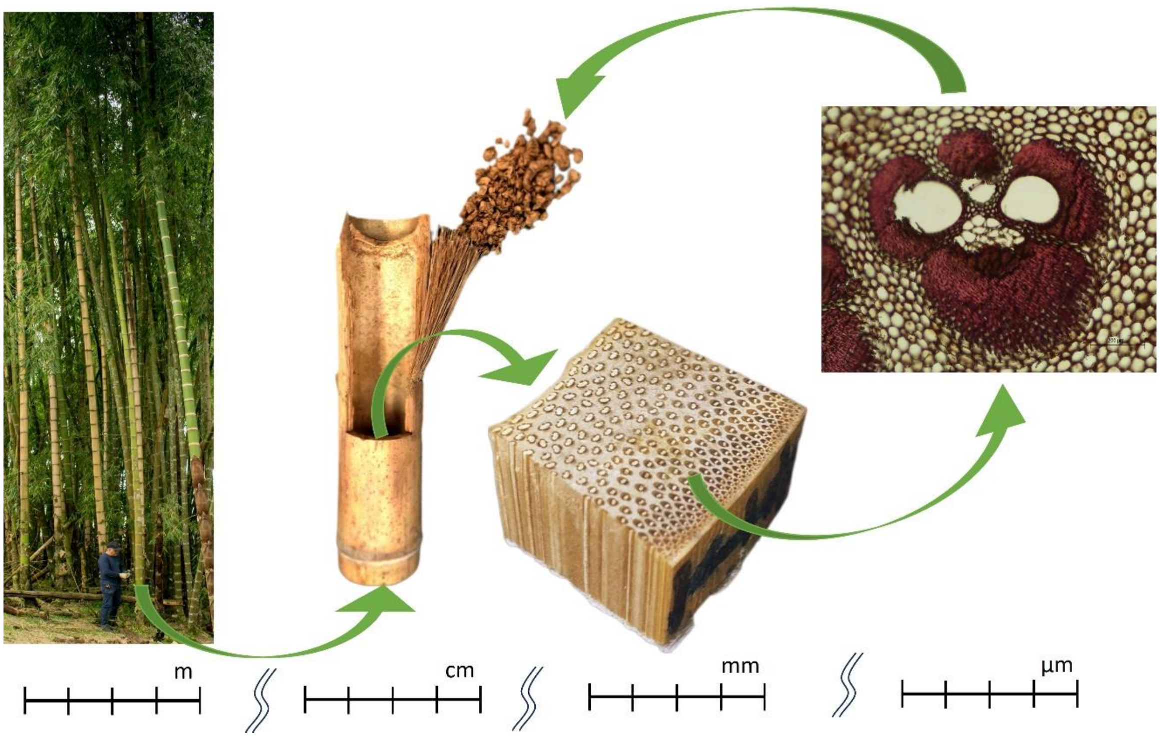
GRAPHICAL ABSTRACT



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The global bamboo market reached USD 70 billion in 2022, and moso bamboo (*Phyllostachys edulis*) is the species with the greatest international impact. Guadua (*Guadua angustifolia* Kunth) is a promising species, that has been traditionally used for the construction and manufacture of handcrafts in several countries in Central and South America. In this research, progress was made in the morphological and anatomical characterization of mature guadua culms. On average the culms are 21 m in total length and there are 53.2 kg of dry biomass. The culm morphology was characterized throughout its length; the average diameter, wall thickness, and density at breast height are 14.8 cm, 21.5 mm, and 0.595 g/cm^3, respectively. The fibers of the vascular bundles of internode number 7 have an average length of 2146 µm, a diameter of 20.4 µm, and a diameter of 8.7 µm wall thickness. The morphological and anatomical characteristics of guadua offer productive and comparative advantages and could be competitive in the bamboo market. The research, technological development, and innovation processes related to this material should be encouraged to promote the guadua industry.

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Keywords: Bamboo morphology and anatomy; Guadua angustifolia Kunth; Guadua; Biomass; Fibers

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INTRODUCTION

Bamboo has been recognized worldwide as a raw material with the potential to generate economic and social development in regions where a large part of the population still has many unmet basic needs. Therefore, various strategies have been promoted and evaluated to encourage industrial development around this raw material (Binfield *et al*. 2022). It is estimated that there are 50 million hectares of bamboo worldwide, and in 2021 more than USD 70 billion of bamboo products were sold (INBAR 2023). China is the world's leading country in producing, marketing, and consumption of bamboo products. That country has more than 4 million hectares of bamboo, mainly moso bamboo (*Phyllostachys edulis*), and is also the leader in research, technological development, and innovation around bamboo. The products at the market are varied and with different degrees of sophistication in their transformation. It should be noted that the products with the highest unit value reported on the platform of TRADEMAP.org are chopsticks (USD 5707/t), work baskets (USD 5650/t), furniture (USD 4145/t), and products made from bamboo paper such as plates, cups, and food containers (USD 3207/t).

The high growth rates of bamboo make it an important alternative for CO_2 fixation, which is the main gas responsible for the greenhouse gas emissions problem (Camargo *et al*. 2011). Bamboo's biomass is a raw material with diverse morphological, anatomical, physical, chemical, and mechanical characteristics that allow the development of many applications ranging from biomass energy valorization to the manufacture of carbon nanotubes for the development of light and strong materials with possible applications in the medical and aerospace sectors, among other applications (Akinlabi *et al*. 2017).

The bamboo culm is hollow and divided into segments delimited by horizontal structures known as nodes. The space delimited by the nodes is known as the internode, and in the wall of the internode, there are different types of cells and tissues oriented in the axial direction and with diverse physiological functions that determine the properties and applications of this material.

Guadua (*Guadua angustifolia* Kunth) is one of more than 1400 species of bamboo in the world and is present in tropical regions of Central and South America mainly near to rivers. It is the most important native bamboo species in Colombia and growth in Andine region where there are presently around 34000 hectares of natural guadua forest. Of this amount, 76% are in the Colombian coffee belt (Caldas, Risaralda, Quindío, Tolima, and northern Valle del Cauca departments). Guadua is traditionally used for handcrafts and construction (Londoño 1990). In this same region, 1739200 hectares have been identified with high and moderate suitability for the establishment of guadua plantations (Ministerio de Agricultura y Desarrollo Rural 2020).

Colombia established a protocol for the formulation of Management Plans and Sustainable Use of the Natural Guadua Forest. This plan defines the guidelines and actions to be taken and the number of culms that can be extracted from a natural guadua forest without putting at risk the ecosystems in which guadua grows. This protocol states that after a particular analysis of the guadua forest, and from a statistical sampling of the population, the structure of the population of guadua culms and the permissible extraction rate are determined. In general terms, it is possible to extract between 20 to 30% of the mature and over-mature culms of the guadua forest in 18-month cycles (Camargo *et al*. 2008).

There are different types of products that can be made from guadua that provide new and innovative applications for this material, and each of them is related to the traditional uses of the species, its availability, and particular characteristics. This research aims to contribute to the consolidation of primary and secondary information related to the morphological and anatomical characteristics of guadua, a vegetal species representative of Colombian biodiversity. It also aims to increase its knowledge and identify comparative advantages and promising applications to promote the development of research processes, technological development, and innovation to reinforce the guadua industry and increase its environmental, economic, and social impact.

EXPERIMENTAL

Materials

Samples of mature culms of *Guadua angustifolia* Kunth were obtained from the Botanical Garden facilities in the Technological University of Pereira-UTP located at 4°47'24.9''N 75°41'17.5''W where a natural guadua forest is present at an altitude of 1450 m.a.s.l. with an annual rainfall average of 2209.7 mm (Maya *et al*. 2017). Seven culms were selected, harvested, sized, and transported to the laboratories of the Sustainable Wood Processes Engineering Program of the UTP where natural drying was carried out for 1 month and the characterization tests were performed.

Methods

For the morphological characterization of the culms of guadua, measurements of total culm length, node diameter, node length, and wall thickness were recorded. Moisture content and density were determined at internodes numbers 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, and 63; depending on the characteristics of the total length of culms. The density of the samples was evaluated according to ASTM D2395 (2016) method B - Volume per immersion in water. The dry weight of each internode was determined by mass balance calculations.

The anatomical characterization of the samples included determining the fibrous percentage area and the dimensions of fiber cells. To determine the percentage of fibers, a LEICA EZ4 W (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) stereoscope was used to acquire images of the cross-section of the entire internode wall in each of the selected internodes; and using the IMAGEJ (Wayne Rasband and contributors, National Institutes of Health, V 1.54g, USA) software, through the analysis of the images the percentage of the area corresponding to the fiber sheaths in the total area of each internode wall was established. Additionally, samples of chips from internode 7 were dehydrated, embedded in paraffin, and cut in a rotary microtome LEICA RM2125 (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland). The cuts between 5 and 30 µm were washed and stained for observation in a LEICA DM500 (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) microscope. Finally, for the measurement of the fibers, chips from internode 7 were softened and bleached, and their cells and tissues were disintegrated according to the procedure proposed by Kiaei and Samariha (2011). Images of the different cell types were captured with the LEICA DM500 (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland) microscope at magnifications between 4x and 40x. The images were analyzed using LAS EZ software (Leica Application Suite, Version 3.4.0 (Build:272), using a slide with 10 µm divisions for calibration. A sample of the aqueous suspension of the disintegrated fibers was placed on a slide and photographs of at least 50 fibers were sized, recording the length, diameter, and wall thickness of the fibers. Scanning electron microscope (SEM) images of guadua fibers previously obtained by a soda chemical process (Ruiz *et al*. 2019) were obtained in a NEOSCOPE JCM-5000 Microscope available at the Laboratory of Engineering and Design in TECNOPARQUE SENA Node Cali, Colombia.

RESULTS AND DISCUSSION

Description of the *Guadua angustifolia* Kunth Forest

During the preliminary inventory carried out in the natural guadua forest in the UTP Botanical Garden and from the external morphological characteristics, the growth stages were established in the forest. A density of 5100 culms/ha was estimated with more than 60% of the culms in a mature or over-mature state and 11.8% of the culms dying. These results agree with the range established for a natural guadua forest (Camargo *et al*. 2007).

Morphological Characterization of the Culms of *Guadua angustifolia* Kunth

Mature culms of guadua (4 to 6 years age) were identified from their external morphological characteristics; presence of branches and leaves, absence of stem leaves, light green color, presence of fungi and lichens (Camargo *et al*. 2008), and harvest with a chainsaw and manually extracted from the forest during February (predominantly dry season in Colombia). The culms with 70 to 90% moisture content were sized into pieces between 3 and 4 m to preserve the internodes of interest and then weighed. The fresh branches and leaves of each culm weighed between 10.1 and 12.3 kg, and the fresh culms weighed between 161 and 182 kg. At the warehouse of the Sustainable Wood Processes Engineering Program at UTP, the culms were washed with pressurized water to remove fungi and lichens. The internodes were marked in ascending order starting from internode 2 and then with the help of a flexible measuring tape and a Vernier caliper, the characteristic dimensions of all the internodes were determined.

The number of internodes in the guadua culms sampled was between 62 and 67 internodes with lengths between 13 cm and 41 cm and diameters between 14.9 cm and 3.7 cm in the basal and apical segments, respectively. The diameter, number of internodes and length of the culm are defined since the shoots sprout from the buds of the guadua rhizome, the diameter and length of the same internode are inversely proportional (Fig. 1). The internode length is lower at the base of culm and higher at top of the culm, and the apical internodes present variation in its length, maybe for ant attacks. The total length of the culms was between 18.1 m and 23.4 m. When compared with the dimensions reported by Londoño (1998) during 10 years of monitoring commercial guadua plantations (Table 1) it is possible to see the effect of the restoration processes in the facilities of the UTP Botanical Garden after more than 20 years.

Table 1. Morphological Characteristics of Commercial Plantations of *Guadua angustifolia* Kunth. (Londoño 1998)

Parameter	Initial	2 years	4 years	7 years	10 years	Control	UTP Botanical Garden 2024 *
Length (m)	0.3	1 to 1.5	5 to 10	10 to 15	15 to 18	ND	18.1 to 23.4
DBH (cm)	ND	0.5 to 1	7.45	8.44	9.45	12.5	14.8
Density (plants/ha)	650	ND	10938	14406	19031	6594	5100

* Results obtained in this work, Diameter to Breast Height-DBH

After 1 month of natural drying, rings of 5 cm in length were sectioned from the central part of each selected internodes. The wall thickness, moisture content, and density in triplicate at these rings were determined. There was an inverse correlation between the density of the culm wall and the wall thickness, *i.e.*, at the internode 3 (lower part) the density is 0.570 g/cm^3 and the wall thickness is 24 mm, meanwhile at internode 63 the (higher part) the density is 0.720 g/cm^3 and the wall thickness is 8 mm (Figs. 1 and 2).

From the average data on wall thickness, density, diameter, and internode length of mature culms of guadua, important production variables can be estimated to ensure a continuous operation of industrial processes. Considering that mature culms have an average length of 21.1 m and an average dry weight of 53.2 kg (Figs. 3 and 4), in a forest with a similar structure to the natural guadua forest in the UTP Botanical Garden and according to *Reference Terms for the formulation of Management Plans and Sustainable Use of Guaduales* (Camargo *et al*. 2008), around 800 mature culms could be extracted in 18-month cycles without causing irreversible damages in the forest. This would represent until 16900 m linear of guadua or 42600 kg of dry biomass to supply one or several transformation processes according to the specific characteristics of each culm segments. These results should be verified for each specific natural guadua forest to be harvested.

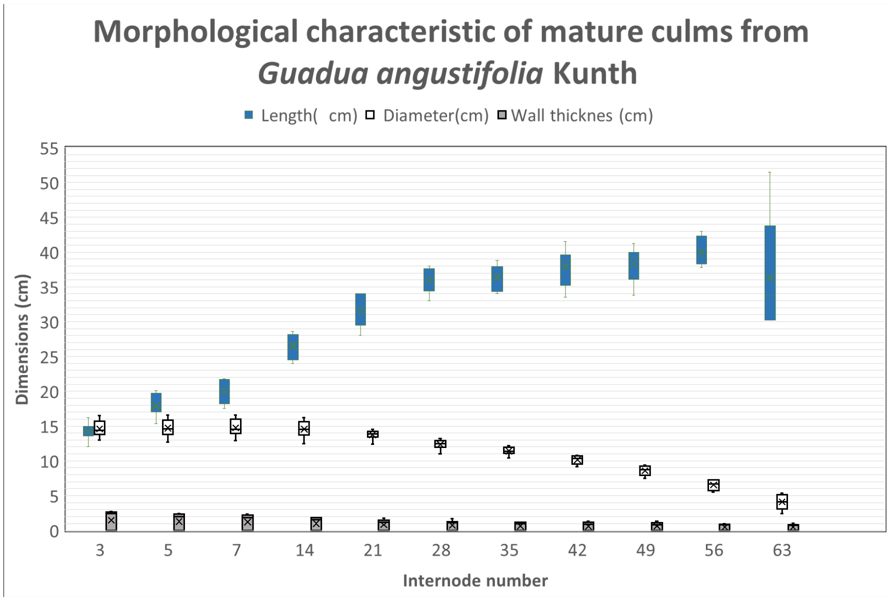


Fig. 1. Morphological characteristics of *Guadua angustifolia* Kunth culms. Length, diameter, and wall thickness variations in height of mature culms

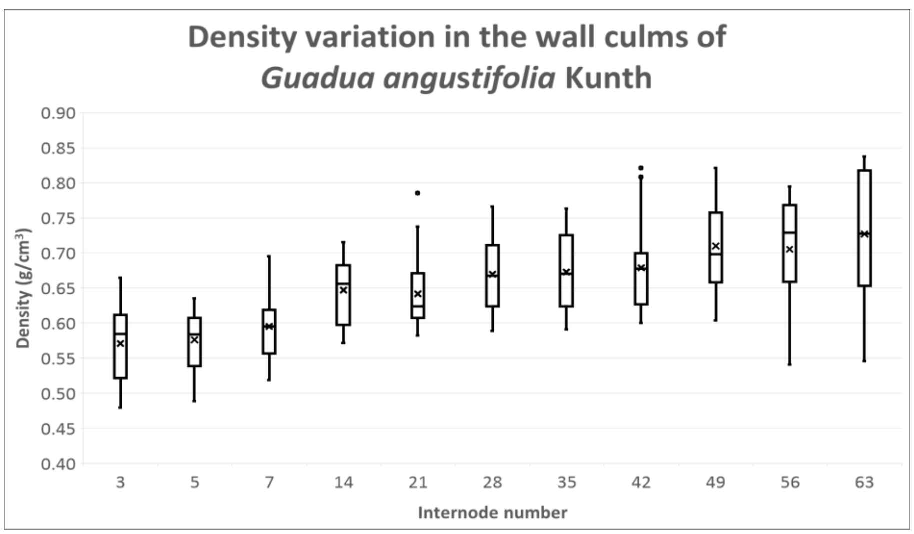


Fig. 2. Density variation in the wall of the internodes of mature culms from *Guadua angustifolia* Kunth

For the use of guadua in construction applications, culms of 10 to 12 cm in diameter are mainly used, so only culms from guadua plantations with more than 10 years of management could be harvested. The guadua culms analyzed in this work had an average Diameter to Breast Heigth-DBH of 14.8 cm with internode diameters greater than 10 cm in the middle and basal segment, so at least 80% of the harvested biomass would have potential use in this application. In culms with diameters greater than 12 cm, the application is restricted to posts or columns in specific projects. The uniformity of diameter and length of internodes is another aesthetic characteristic considered when selecting the culms that remain visible in design and construction projects; internodes 28 to 49 are those with the most uniform lengths and diameters between 10 and 14 cm.

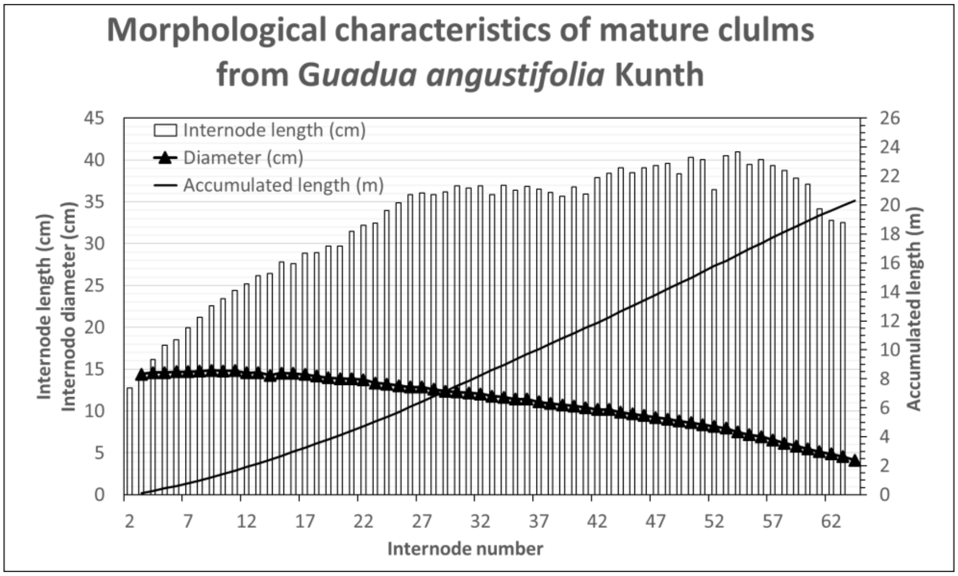


Fig. 3. Estimation of length production variables of mature culms of *Guadua angustifolia* Kunth

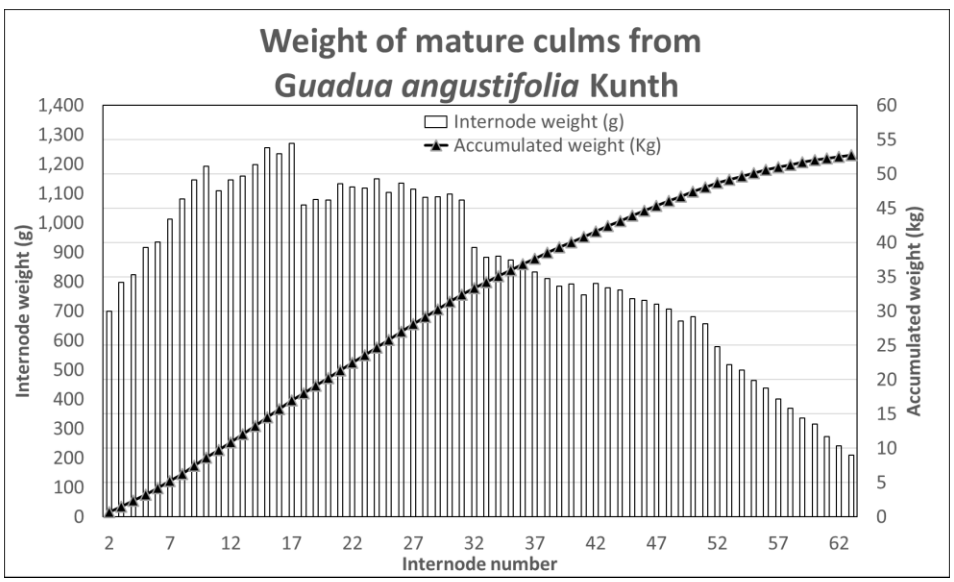


Fig. 4. Estimation of weight production variables of mature culms of *Guadua angustifolia* Kunth

Table 2. Comparative Morphological Characteristics of Mature Culms from *Guadua angustifolia* Kunth and Moso Bamboo

Parameter	*Guadua angustifolia* Kunth	Moso Bamboo
DBH (cm)	14.8	7.7-13.64*
Length (m)	20-23	10.67-15.92*
Biomass (kg)	50-53	8.01-25.62*
Density (kg/m^3)	570-720	601-640**
Wall Thickness (mm)	7-30	7-8**
Culm Density (culms/ha)	5000-8000***	3400-4700****

Reference: * (Jiang *et al.* 2022), **(Huang *et al.* 2015), ***(Camargo *et al.* 2007), ****(Xu *et al.* 2018)

The selection of segments with optimal internodes for construction applications is that of the most developed market; however, the extraction of vascular bundles with more than 30 cm in length can be carried out from internodes with a smaller diameter (internodes 45 to 60) and will allow for competition in applications of threads and fabrics made with coarse fibers of species such as fique, hemp, or jute. These same vascular bundles can compete as reinforcing material in the manufacture of composites that replace synthetic fibers such as fiberglass and other natural fibers.

Anatomical Characterization of the Culm Walls of *Guadua angustifolia* Kunth

In the internode wall of guadua, there are different tissues with cells oriented mainly in the axial direction and with various physiological functions that determine the properties of this material (Fig. 5-a). From the external surface and towards the internal surface of the culm wall one can find the following: the cortex (cuticle); a tissue of hard consistency with a thin waxy layer and that has the function of protecting the internal structures and regulating the exchange of fluids in the plant, respectively, and is where the highest concentration of inorganic substances (mainly silica) is found (Leksikowati and Rachmawati 2024). Moving towards the interior of the culm wall one can find the conductive system of the guadua; the so-called vascular bundles (which are known as fibers in some artisanal transformation processes). These vascular bundles are embedded in many parenchymal cells-Pq (Figs. 5-b y 5-c).

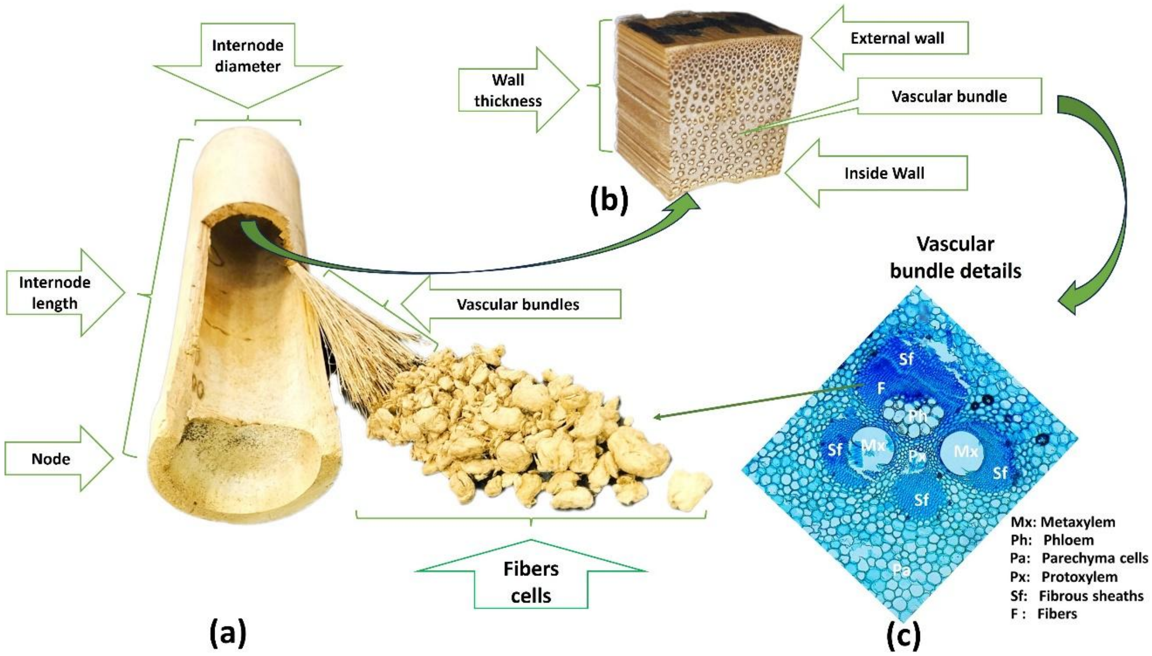


Fig. 5. Morphology and anatomy of the culm of *Guadua angustifolia* Kunth, (a) Internode characteristics; the vascular bundles and the internode have the same length, fiber cells separated by chemical process from the wall. (b) Detail of the cross-section of the culm wall; different density and dimensions of the vascular bundles in the external and inside wall. (c) Vascular bundle details; Metaxylem-Mx, Phloem-Ph, Fibrous sheaths-Fs, Fibers-F and Parenchyma cells-Pq

The proportions and dimensions of each type of cell and tissue vary between species, as well as in the same individual depending on the proportion of the wall being analyzed (periphery, transition, middle, and interior), the height of the internode, and the age of the culm. The vascular bundles (Fig. 5-c) are a complex system of transporting substances that allow the development of some of the important physiological activities of the plant. The transport of water and nutrients from the soil to the leaves is made through metaxylem-Mx and protoxylem-Px, while the transport and distribution of products produced from the leaves to other cells and tissues is made through phloem-Ph. Both are reinforced by the fibrous sheaths-Fs; group of lignified cells named fibers-F.

The characteristics and proportions of the anatomical structures in the wall of bamboo determine their behavior during the utilization and transformation. The density, mechanical resistance, ease of impregnation and dehydration are strongly related to the anatomical characteristics of the selected material. In guadua culms, the ratio between vascular bundles-VB and parenchymal cells-Pq increases towards the outer surface of the culm wall (Fig. 6-a). At internode 7, in the inner part the ratio VB/Pq is lower (63 VB/mm^2 ,vascular bundles per mm^2), the vascular bundles are larger, with metaxylems between 160 and 190 µm. The fibrous sheaths surrounding the metaxylem vessels are independent of the fibrous sheaths surrounding the protoxylem and the phloem. There is still the presence of parenchyma cells in the middle, each vascular bundle is surrounded by a large number of parenchyma cells that allows observing each one independently and with the naked eye. At medium part of wall, the ratio VB/Pq increase (84 VB/mm^2), the metaxylems reduce their size 120 to 140 µm, while in external part the ratio VB/Pq is higher (218 VB/mm^2)

and the metaxilemas are less than 20 µm and the fibrous sheaths surrounding the phloem, the protoxylem, and the metaxylems fuse into a single tissue (Fig. 6-c).

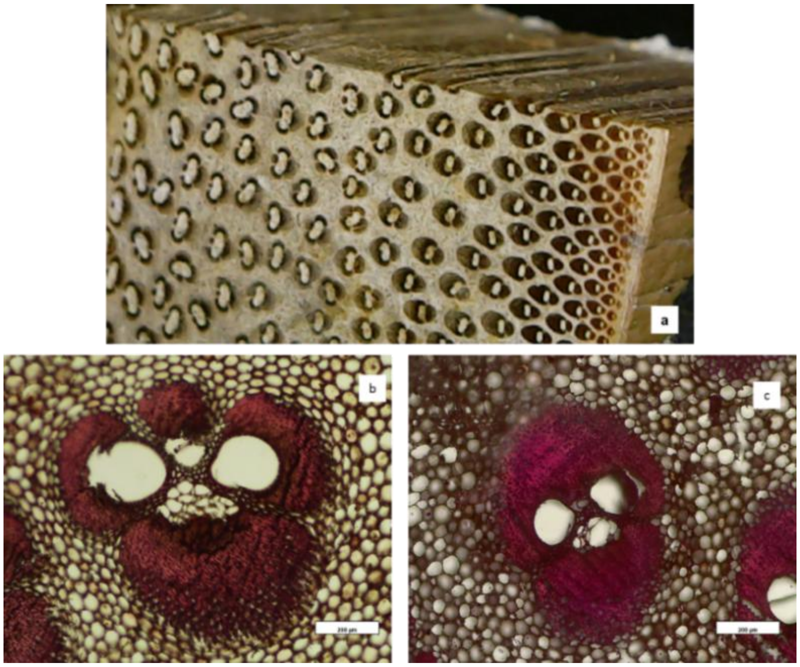


Fig. 6. Difference in density, dimensions and anatomical characteristics of vascular bundles in the culm wall of *Guadua angustifolia* Kunth: (a) Total wall, (b) Vascular bundle of inner region of the wall, and (c) Vascular bundle of transition region of the wall

The fibrous sheaths have a structural function and the amount and dimensions of fiber in fibrous sheaths are varied. The diameter of the lumen of the fibers is small and essentially allows the circulation of the metabolites required for the processes of lignification and thickening of its walls during the development of the plant. Through the analysis of images of the cross-section of the culm wall, it is possible to establish that the area of the fibrous sheaths represents between 30 and 36% of the cross-sectional area of the culm wall. It should be noted that the proportion of fibrous area increases in the internodes of the basal part (internodes 3 and 5) reaching up to 41% of the area of the wall in this area (Fig. 7). The average fibrous area in the culm wall at internode 7 is 33.4%, which would allow us to have an initial estimate of the maximum fiber yield that can be achieved from this raw material and the type of transformation. In previous research, a charcoal production yield of 22% has been achieved from guadua (Peña Gomez *et al*. 2021) and pulp for papermaking between 30 and 40% depending on the degree of delignification, of which this correlation must be validated in subsequent research (Ruiz *et al*. 2019).

Most of the products made with bamboo require intensive machining of segments to get pieces with homogeneous dimensions and improve the performance in transformation processes. The internal part of wall culm; with the lowest density of vascular bundles and more parenchyma cells, and the cuticle (external tissue); due to its composition (waxy layer) and structure should be removed to improve the gluing and surface finishing processes on all the products. This machining process generates a large amount of waste and therefore low yields; all these wastes contain cells and tissues that could be valorized as biomass resources in energy or biorefinery processes.

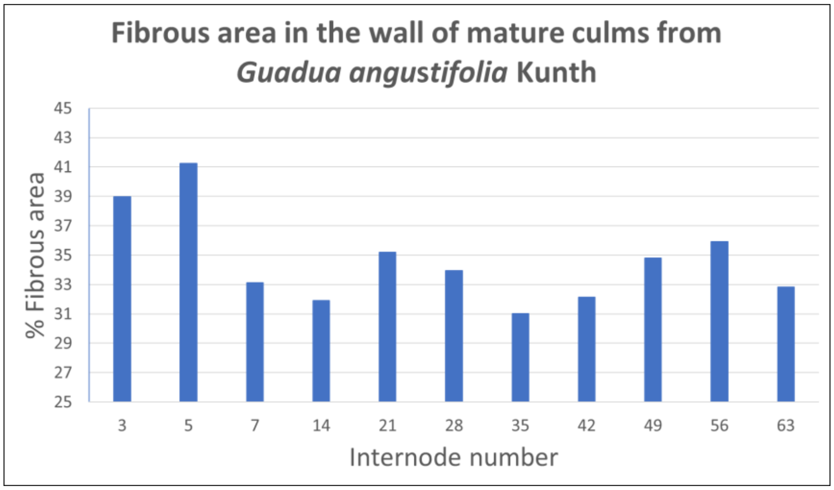


Fig. 7. Fibrous area in the internodes of the culm wall of *Guadua angustifolia* Kunth

Through histological sections and maceration of chips from the wall of internode 7 of the culm of guadua, the presence of all types of cells that make up the different tissues can be verified. In Fig. 8-a vascular bundles can be observed in the middle zone of the culm wall, where between 6 and 15 parenchyma cells separate one vascular bundle from another, and the fibrous sheath that surrounds the protoxylem is separated by between 3 and 6 parenchyma cells from the rest of the vascular bundle. The metaxylems still maintain a significant diameter like that of the phloem and the sieve tubes inside the phloem have thin walls. The fibers that make up fibrous sheaths have variable diameters, wall thickness, and lumen diameter, and the fibers with the largest diameter and the smallest wall thickness are found mainly on the periphery of the fibrous sheaths (Fig. 8-b), possibly because they are parenchymal cells that begin their sclerification process.

The total length of the fibers is also variable and far exceeds the dimensions of the other types of cells found in the culm wall (Fig. 8-c).

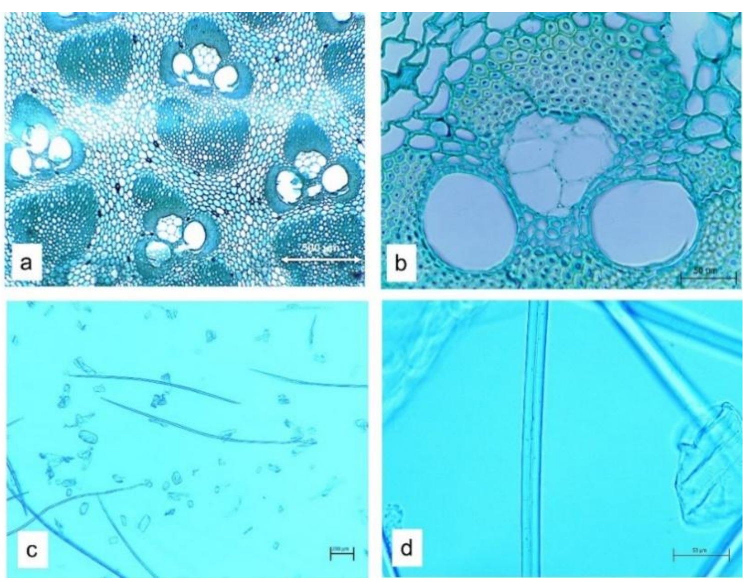
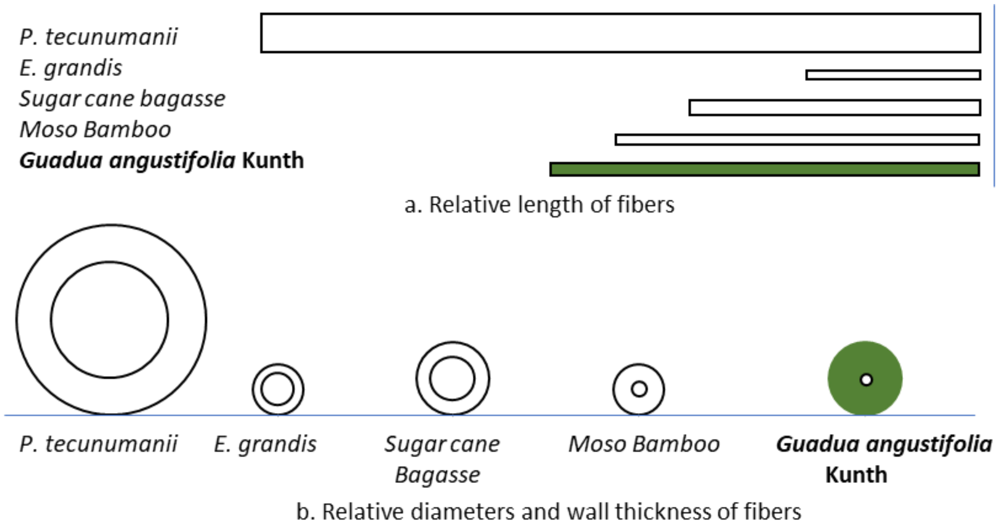


Fig. 8. Tissues and cells present in the culm of *Guadua angustifolia* Kunth: a. Vascular bundles in the transition zone of the culm wall, b. Detail of the fibrous sheaths in a vascular bundle, c. Relative size of the parenchyma cells and fibers, and d. Wall thickness and pitting in the fibers



	*Pinus tecunumanii**	*Eucalyptus grandis***	Sugarcane bagasse***	Moso Bamboo****	***Guadua angustifolia***
Length (µm)	3960	960	1590	2000	**2146.33**
Diameter (µm)	51.93	13.4	20	14	**20.46**
Wall thickness (µm)	9.48	2.4	4	5	**8.71**

Fig. 9. Comparison of the anatomical dimensions of *Guadua angustifolia* Kunth fibers with other commercial fibrous raw materials used in the pulp and paper industry: a. Lengths, b. Diameter and wall thickness. *Pinus tecunumanii**(Torres *et al.* 2005), *Eucalyptus grandis* **(Palermo *et al.* 2015), Sugar cane bagasse***(Samariha and Khakifirooz 2011), Moso bamboo**** (Gasparini Cid *et al.* 2020)

The morphological and anatomical characteristics of guadua make it a competitive raw material when evaluating its potential to participate in the bamboo products market segment. Its fibers are long and thick-walled, so they could contribute to the formulation of various types of paper. Moso bamboo is used in China as a raw material to produce pulp and paper, as well as the production of bamboo paper products that are important in the trade balance of bamboo. In Fig. 9, the average anatomical characteristics reported in the literature for the fibrous cells of moso bamboo and the main fibrous raw materials used industrially in Colombia for the manufacture of pulp, paper, and derived products (pine, eucalyptus, and sugarcane bagasse) are compared with the average dimensions of the guadua fibers that were obtained in this work.

In addition to contributing to the strength of paper due to their length and wall thickness, guadua fibers can incorporate a positive environmental and social dimension in this sector if the Sustainable Management and Utilization Protocols for the Guadua forest are successfully implemented. These fibers could be used to manufacture paper for packaging, absorbent paper, textile fibers, and composite materials.

In previous tests, samples of guadua were subjected to chemical delignification processes with sodium hydroxide (Ruiz *et al*. 2019), obtaining fibers suitable for paper manufacturing. In Fig. 10, images of hand sheets made with fibers of different degrees of delignification are presented (Fig. 10-a). In the images acquired by SEM microscopy of the hand sheets, the abundant presence of parenchymal cells and vessels that were not degraded during the less intense chemical treatment and that produced the fibers with lower degrees of delignification can be confirmed, in Fig. 10-b y c shows parenchymal cells, vessels, and intact and cut fibers with intact walls (without the presence of detachment of microfibrils on the surface). In Fig. 10-c the detail of the metaxylem vessels with diameters much larger than the fibers can be observed. Fig. 10-d y e illustrates in dye, details of the surface of the fibers and the bundles of cellulose microfibrils that, when oriented axially, make up the primary wall of the fibers and the few pits that allow the circulation of the metabolites required in the lignification processes of the fiber walls.

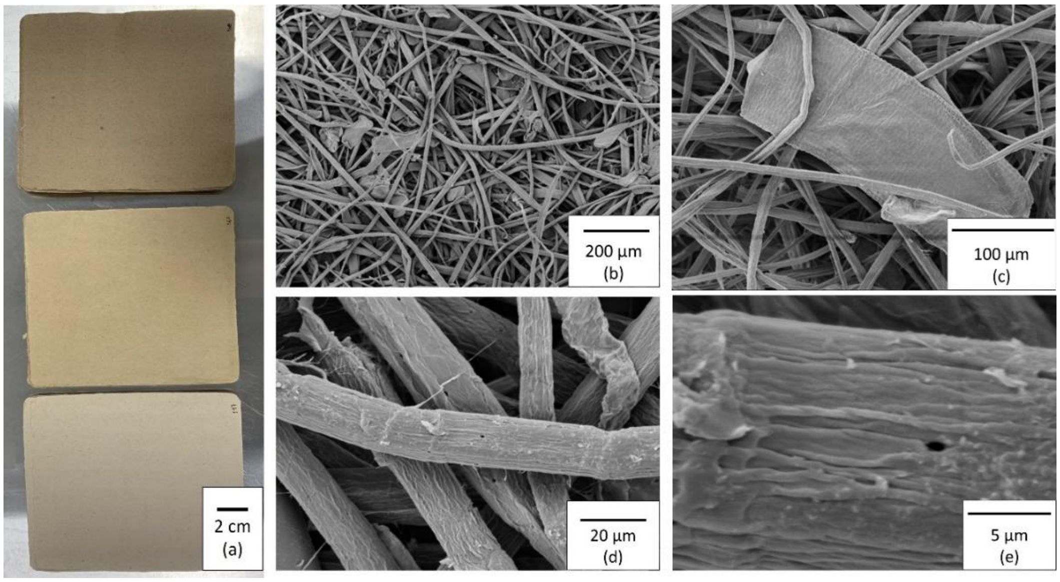


Fig. 10. Details of *Guadua angustifolia* Kunth fibers obtained by chemical methods: a. Different grade of delignification, b. Matrix of different kinds of cells, c. Details of metaxylem cells, d. Detail of the external fibers surface, and e. Pits and microfibrils on fibers surface

CONCLUSIONS

1. According to the results of the morphological characterization of *Guadua angustifolia* Kunth, it can be established that there are significant differences in the amount of biomass that produces the culms of this species (50 to 53 kg d.b.) and the biomass of moso bamboo (8 to 25 kg d.b.) highlighting the potential of guadua to participate in the bamboo products market. Under a schema of sustainable management of 1 ha of natural guadua each 18 months up to 16900 m of linear guadua or 42584 kg of dry biomass could be available to develop industrial transformations.
2. The length of the fibers of *Guadua angustifolia* Kunth (2150 µm) and developments around moso bamboo encourage continuing the research and development required for its incorporation into the offer of fibrous raw materials for the pulp and paper industry, a deeply study of pulping, refining and bleaching behavior is conducted.
3. The segregation of the culm segments; even the parts of the wall, according to their morphological and anatomical characteristics will make it possible to efficiently exploit the potential of this raw material in applications as the construction industry (internodes 28 to 49), the extraction of fibers for the pulp and paper industry (internodes 2 to 27), textiles (internodes 50 to 63), or the manufacture of products

based on these fibers (paper, composites, cellulose packages and other are in evaluation).

4. The recognition of the morphological and anatomical particularities of *Guadua angustifolia* Kunth and its productive implications in addition to chemical and mechanical properties previously studied should be the guidelines for the development of an ambitious research and technological development program oriented towards the sustainable use of this raw material and correct supply chain planning.

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