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Charcoal anatomy of seven species from an Araucaria Forest area in Southern Brazil

Anatomía al carbón de siete especies de un área de Bosque de Araucaria, sur de Brasil

Rafaela Stange [a]*, Helena Cristina Vieira [a], Angela Luiza Schutz [b], Lia Wilma Stiegler [c], Polliana D'Angelo-Rios [d], Graciela Ines Bolzon-de-Muniz [e], Simone Ribeiro Morrone [e], Silvana Nisgoski [e]

* Corresponding author: [a] Federal University of Paraná, Post-Graduate Program of Forest Engineering, Curitiba, PR, Brazil, tel.: 49 999545114, rafaellastange@hotmail.com

[b] Federal University of Paraná, Degree in Industrial Woodworking Engineering, Curitiba, PR, Brazil.

[c] Federal University of Paraná, Degree in Forestry Engineering, Curitiba, PR, Brazil.

[d] University of Santa Catarina State, Department of Forest Engineering, Lages, Santa Catarina, Brazil.

[e] Federal University of Paraná, Department of Forest Engineering and Technology, Curitiba, PR, Brazil.

SUMMARY

To support the identification of species and control illegal charcoal production, this study aimed to anatomically characterize the carbonized wood of species native to the Araucaria forest areas, including *Nectandra megapotamica*, *Ocotea indecora*, *Ocotea diospyrifolia*, *Ocotea puberula*, *Balfourodendron riedelianum, Chrysophyllum marginatum* and *Zanthoxylum rhoifolium*. The samples were collected in the Planalto Catarinense region (high plains of the state of Santa Catarina). Three disks were removed from three trees of each species, selected randomly. All discs were sectioned to obtain samples close to the different parts of the trunk (near bark, intermediate and near pith). These sample were then carbonized for 392 minutes in a muffle furnace using a ramp regime with a maximum temperature of 450 ºC. Following carbonization, there was no significant degradation of the anatomical structure and cell arrangement, enabling the characterization and identification of species through comparison with wood description references. The position of samples in the trunk did not influence the anatomical characteristics, facilitating species identification. Wood from the Lauraceae family exhibited greater vessel diameter and lower vessel frequency compared to samples from the Rutaceae and Sapotaceae families. Discrimination of *Chrysophyllum marginatum* from the other evaluated species was possible based on vessel grouping, ray width and ray frequency. The results obtained contribute to a charcoal database of Atlantic Forest species, providing valuable information for practical applications in forestry supervision.

Keywords: carbonized wood, inspection, Lauraceae, Rutaceae, Sapotaceae.

RESUMEN

Con el objetivo de brindar información que apoye la identificación de especies y el control de la producción ilegal de carbón, este trabajo tuvo como objetivo caracterizar anatómicamente la madera carbonizada de las especies *Nectandra megapotamica*, *Ocotea indecora*, *Ocotea diospyrifolia*, *Ocotea puberula*, *Balfourodendron riedelianum*, *Chrysophyllum marginatum* y *Zanthoxylum rhoifolium*, nativas de las áreas del Bosque de Araucaria. Las muestras fueron colectadas en la región del Planalto Catarinense (altiplano del estado de Santa Catarina). Se extrajeron tres discos de tres árboles de cada especie, seleccionados al azar. Todos los discos se seccionaron para obtener muestras cercanas a las diferentes posiciones del tronco (cerca de la corteza, intermedia y cerca de la médula), las cuales se carbonizaron durante 392 minutos en un horno de mufla en régimen de rampa y temperatura máxima de 450 ºC. Después de la carbonización, no hubo una degradación significativa de la estructura anatómica y la disposición de las células, lo que permitió caracterizar e identificar las especies en comparación con las referencias de descripción de la madera. La posición de las muestras en el tronco no influyó en las características anatómicas, lo que permitió la identificación de especies. La madera de la familia Lauraceae presentó mayor diámetro de vasos y menor frecuencia de vasos en comparación con las muestras de las familias Rutaceae y Sapotaceae. La agrupación de vasos, el ancho y la frecuencia de los rayos podrían discriminar a *Chrysophyllum marginatum* de las otras especies evaluadas. Los resultados obtenidos contribuyen a una base de datos de carbón vegetal de especies del Bosque de Araucaria que se puede consultar para aplicaciones prácticas en la supervisión forestal.

Palabras clave: madera carbonizada, inspección, Lauraceae, Rutaceae, Sapotaceae.

INTRODUCTION

Brazil is the world's leading producer of charcoal, primarily utilized in the steelmaking industry (Ibá 2019). Approximately 82 % of the produced charcoal is used domestically, with an annual consumption reaching around 4.6 million metric tons. According to 2017 data from the Food and Agriculture Organization (FAO), nearly 82 % of Brazilian charcoal is derived from wood harvested from planted forests.

In 2019, the total area of planted forests increased by 2.4 % compared to 2018, reaching 9 million hectares. Of this land area, 77 % is allocated to Eucalyptus plantations (6.97 million hectares), while 18 % is dedicated to Pinus species (1.64 million hectares). Specifically in the state of Santa Catarina, the corresponding areas for planted forest are 0.21 million hectares of Eucalyptus and 0.43 million hectares of Pinus (Ibá 2019).

Nevertheless, native wood species are still being utilized, and the illegal extraction of trees exacerbates deforestation (Souza *et al.* 2015). Controlling charcoal production in Brazil is crucial due to damages in native forests, but, in practice, it presents difficulties in material identification (Gonçalves & Scheel-Ybert 2012).

Additionally, Gonçalves & Scheel-Ybert (2012) note that the significant percentage of charcoal consumption from native biomes reflect the practices of many industries that aim to maximize profits by paying the lowest possible value for charcoal, without concern for its origin.

Species of the Lauraceae family are among the most common in Araucaria Forest areas, many with high silvicultural potential, a wide range of final uses, and high economic value. In Santa Catarina, a total of 39 species have been identified, of which 21 belong to the *Ocotea* genus (Gasper *et al.* 2013). Due to their diverse applications, high value of essential oils, and good wood quality, species of this family have been extensively exploited for making boats, luxury furniture and essential oils (Vattimo-Gil 1956), jeopardizing their preservation (Quinet & Andreata 2002). Because of this excessive exploitation, *Ocotea porosa* (Nees & Mart) Barroso and *Ocotea odorifera* (Vellozo) Rohwer are on the official list of endangered plant species in Brazil, along with other species of the same family (Mma 2014).

In the Lauraceae family, most genera have similar wood anatomical characteristics, making identification at the genus and/or species level complex (Castiglioni 1962, Richter 1981, Richter 1987), and thus require a large reference database.

According to the Brazilian Institute of Environment and Renewable Natural Resources (Ibama 2010), irrespective of social condition and biome, deforestation and charcoal production without a license from SISNAMA (National Environmental System) and / or a DOF (Forest Origin Document) are considered crimes, resulting in material apprehension and criminal prosecution.

Charcoal is the final product of the carbonization process or the result of incomplete combustion. In most situations, the anatomic structure of the wood is preserved after the process, enabling botanical identification. This depends on the anatomical characteristics of the species, dimensions of charcoal fragments, and the conservation state of the material, which can complicate the identification process and, consequently, inspection by authorities (Marguerie & Hunot 2007, Muñiz *et al.* 2012). Considering these factors, it is necessary to build a reference database to support the identification process (Afonso *et al.* 2015).

Dimensional changes due to wood carbonization do not interfere with the qualitative aspects of charcoal's anatomical characteristics, enabling the identification of wood species from carbonized material based on wood description (Muñiz *et al.* 2013). Studies by Prior and Alvin (1983), Prior and Gasson (1993), Kim and Hanna (2006), Gonçalves *et al.* (2012), Muñiz *et al.* (2013), Nisgoski *et al.* (2014), Afonso *et al.* (2015) and Stange *et al.* (2018), among others, have demonstrated that the wood's anatomical structure is normally preserved after carbonization. Other important studies, such as Gonçalves & Scheel-Ybert (2016), Pinto *et al.* (2017) and Scheel-Ybert & Gonçalves (2017), anatomically describe charcoal derived from a diverse range of species and contribute to increased wood and / or charcoal databases, making species identification possible.

The objective of this study is to anatomically characterize charcoal produced from seven species with significant occurrence in an Araucaria Forest area in Santa Catarina. Additionally, the study aimed to evaluate changes in radial position and generate technical data to contribute to the development of a database for charcoal identification in the field, facilitating the control of illegal logging.

METHODS

Wood samples were collected in the Planalto Catarinense region of southern Brazil, in the state of Santa Catarina, which includes the municipalities of Campos Novos, Brunópolis, Curitibanos, Frei Rogério, São José do Cerrito and Vargem. The study area is associated with the São Roque hydroelectric plant. Due to the construction of the hydroelectric plant, the region experienced flooding, leading to the suppression of vegetation. For this study, wood discs were cut at breast height (BH) from three randomly selected trees of each species. Botanical material from each tree was deposited in the Lages Herbarium of Santa Catarina State University (LUSC). A disc was obtained from each tree at breast height for further evaluations (table 1). All samples were registered with the Brazilian Council for Management of Genetic Heritage (CGEN / SISGEN) under the identification number AF3EDDC.

Samples from each disc were carefully cut to preserve the orientation of growth rings. These samples were obtained from three distinct trunk regions: near the bark, intermediate, and near the pith. A total of nine samples with

Table 1. Important data of the studied species.

Datos importantes de las especies estudiadas.

Species	DBH (cm)	Geographic coordinates	Altitude (m a.s.l.)	Wooden disk	Register number
Nectandra megapotamica (Spreng.) Mez. [L]	23.1	Lat: -27.493872 Long: -50.803839 WGS84	677	40-1 40-2 40-3	LUSC 6,264 LUSC 6,265 LUSC 6,266
Ocotea diospyrifolia (Meisn.) Mez. [L]	38.8	Lat: -27.497069 Long: -50.810492 WGS84	703	42-1 42-2 42-3	LUSC 6,270 LUSC 6,271 *
Ocotea indecora (Schott) Mez. [L]	34.1	Lat: -27.493061 Long: -50.807292 WGS84	680	41-1 41-2 41-3	LUSC 6,267 LUSC 6,268 LUSC 6,269
Ocotea puberula (Rich.) Nees. [L]	33.7	Lat: -27.482531 Long: -50.811214 WGS84	695	43-1 43-2 43-3	LUSC 6,273 * LUSC 6,275
Balfourodendron riedelianum (Engl.) Engl. [R]	37.6	Lat: -27.492872 Long: -50.812292 WGS84	773	7-1 7-2 7-3	LUSC 6,185 LUSC 6,186 LUSC 6,187
Zanthoxylum rhoifolium Lam. [R]	21.0	Lat: -27.483303 Long: -50.807792 WGS84	731.5	59-1 59-2 59-3	LUSC 6,321 LUSC 6,322 LUSC 6,323
Chrysophyllum marginatum (Hook. & Arn.) Radlk. [S]	19.3	Lat: -27.4938 Long: -50.810561 WGS84	793	15-1 15-2 15-3	LUSC 6,201 LUSC 6,202 LUSC 6,203

L: Lauraceae; R: Rutaceae; S: Sapotaceae family; DBH: diameter at breast height (1.30 m); Lat: latitude; Long: longitude; *: no register in the Lages Herbarium of Santa Catarina State University.

dimensions of 2 x 2 x 2 cm (length, width and thickness) were carbonized. Radial sampling was employed to capture the majority of anatomical variations that could affect wood identification. These samples were extracted from the heartwood, transition zone, and sapwood, with variations dependent on the diameter of the tree.

Once the material reached a moisture content of 12 ± 1 %, the samples were encased in aluminum foil and subjected to carbonization in a muffle furnace. The process involved a heating rate of 1.66 °C min^{-1}, reaching a final temperature of 450 °C, and maintained for two hours, following the procedure outlined by Muñiz *et al.* (2012)**.**

The anatomical characterization of the charcoal samples adhered to the guidelines set by the International Association of Wood Anatomists (Iawa 1989). The process was carried out using a Discovery V12 stereomicroscope from Zeiss, equipped with Axio Vision Rel. 4.7 software. Thirty measurements were recorded for each sample (tangential diameter of vessels, height and width of rays in micrometers, frequency of vessels and rays). Detailed images were captured using a Hitachi TM-1000 tabletop scanning electron microscope (SEM) directly from the material, without the application of coating.

To analyze the data, a 7 x 3 factorial design was employed, taking into account the various species (*Nectandra megapotamica*, *Ocotea diospyrifolia*, *Ocotea indecora*, *Ocotea puberula*, *Balfourodendron riedelianum*, *Zanthoxylum rhoifolium, Chrysophyllum marginatum*) and radial trunk positions (near bark, intermediate, near pith).

The Kolmogorov-Smirnov test was utilized to assess the normal distribution of the data, and Bartlett test was conducted to confirm variance homogeneity. Data that did not exhibit normal distribution underwent Box-Cox transformation. Once all assumptions were met, analysis of variance (ANOVA) was carried out using the Scott-Knott test at 95 % probability, employing the Sisvar 5.6 Build 77 software (Ferreira 2011).

RESULTS

The anatomical characteristics present in the transverse plane of the different species studied are illustrated in figure 1. It is noteworthy to mention certain observations, such as wall thickness and the behavior exhibited by vessel cells following the carbonization process.

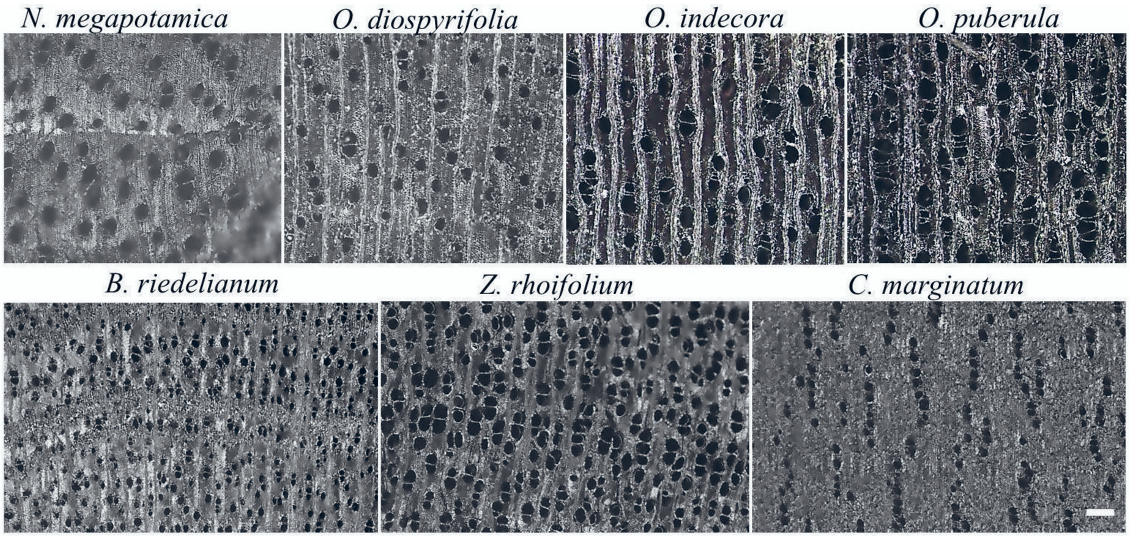

Figure 1. Macroscopic image of transversal sections of the studied species. Scale bar = 200 µm.

Imagen macroscópica de cortes transversales de las especies estudiadas. Barra de escala = 200 µm.

Qualitative anatomy of charcoal.

Scientific name: *Nectandra megapotamica* (Spreng.) Mez. (figure 2)

Vernacular name in Portuguese: Canela-loura, canela-imbuia

Family: Lauraceae

Growth rings: few, distinguished by differences in fiber cell wall (figure 2A). Vessels: diffuse-porous (figure 2A), solitary and in radial multiples of 2-5, simple perforate plate (figure 2B), intervessel pits alternate (figure 2E). Axial parenchyma: scanty paratracheal and vasicentric. Rays: heterogeneous, body ray cells procumbent with rows of square marginal cells (figure 2B), biseriate predominant, not storied (figure 2D); oil cells present (figure 2B, D, F), acicular crystals present in ray cells (figure 2C). Fibers: libriform, septate (figure 2E, F) and thin-walled.

Scientific name: *Ocotea diospyrifolia* (Meisn.) Mez. (figure 3)

Vernacular name in Portuguese: Canela

Family: Lauraceae

Growth rings: few, distinguished by differences in fiber cell walls (figure 3A). Vessels: diffuse-porous (figure 3A), solitary and in radial multiples of 2-5, simple perforate plate (figure 3D), intervessel pits alternate (figure 3E, F). Axial parenchyma: scanty paratracheal and vasicentric. Rays: homogeneous (figure 3B) and occasionally heterogeneous, body ray cells procumbent with rows of square marginal cells, multiseriate predominant, not storied (figure 3E); oil cells scarce, crystals present (figure 3C). Fibers: libriform, septate and thin-to thick-walled.

Scientific name: *Ocotea indecora* (Schott) Mez. (figure 4)

Vernacular name in Portuguese: Canela

Family: Lauraceae

Growth rings: few, distinguished by differences in fiber cell walls (figure 4A). Vessels: diffuse-porous (figure 4A), solitary and in radial multiples of 2-4, simple perforate plate (figure 4D), intervessel pits alternate (figure 4F). Axial parenchyma: scanty paratracheal and vasicentric. Rays: heterogeneous, body ray cells procumbent with rows of square marginal cells (figure 4B), multiseriate, not storied (figure 4E); oil cells (figure 4E) and crystals present (figure 4C). Fibers: libriform, septate and thin-walled.

Scientific name: *Ocotea puberula* (Rich.) Nees. (figure 5)

Vernacular name in Portuguese: Canela-sebo, canela-guaicá,

Family: Lauraceae

Growth rings: few, distinguished by differences in fiber cell walls. Vessels: diffuse-porous (figure 5A), most solitary, radial multiples of 2-4 present, simple perforate plate (figure 5D), intervessel pits alternate (figure 5F). Axial parenchyma: scanty paratracheal and vasicentric. Rays: heterogeneous, body ray cells procumbent with rows of square marginal cells (figure 5B), biseriate predominant, not storied (figure 5E); some oil cells present (figure 5E). Fibers: libriform, septate (figure 5F) and non-septate, thin-to thick-walled.

Scientific name: *Balfourodendron riedelianum* (Engl.) Engl. (figure 6)

Vernacular name in Portuguese: Pau marfim

Family: Rutaceae

Growth rings: distinguished by axial parenchyma (figure 6A). Vessels: diffuse-porous (figure 6B), solitary and in radial multiples of 2-3, some clusters present, simple

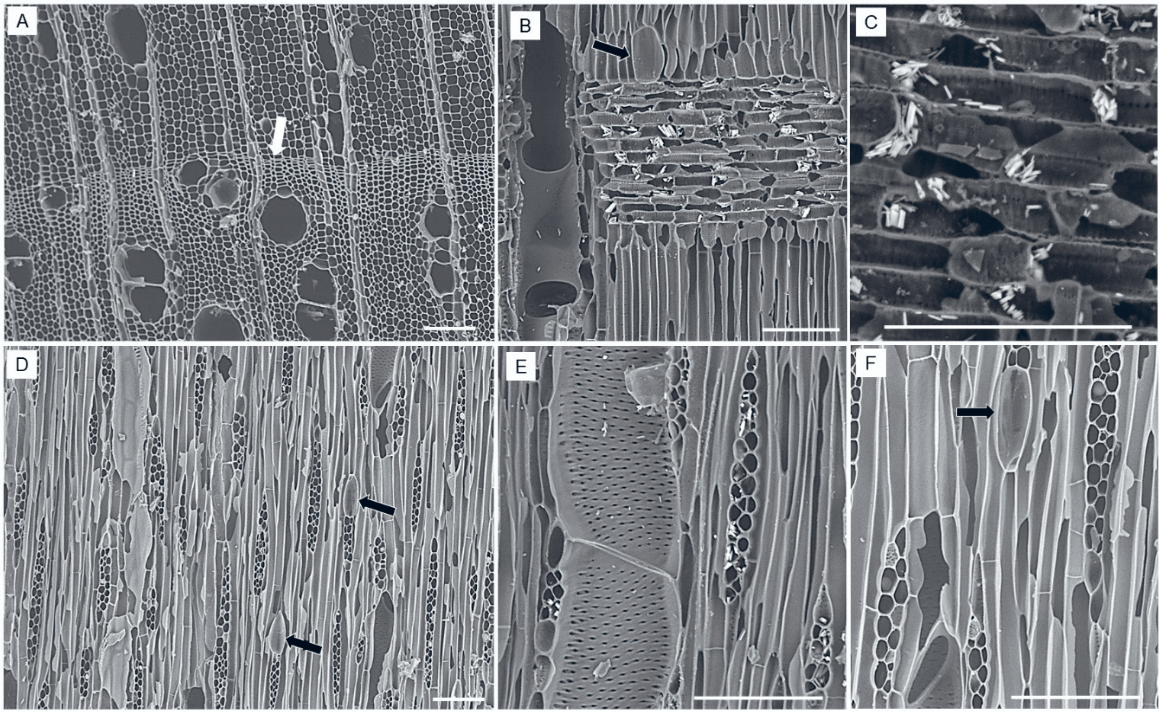

Figure 2. SEM images of *Nectandra megapotamica* charcoal. Transversal section (A), white arrow indicating growth ring; radial section (B, C), black arrow indicating oil cell; tangential section (D, E, F). Scale bar = 100 µm.

Imágenes SEM del carbón *Nectandra megapotamica*. Corte transversal (A), flecha blanca que indica anillo de crecimiento; sección radial (B, C), flecha negra que indica célula oleífera; sección tangencial (D, E, F). Barra de escala = 100 µm.

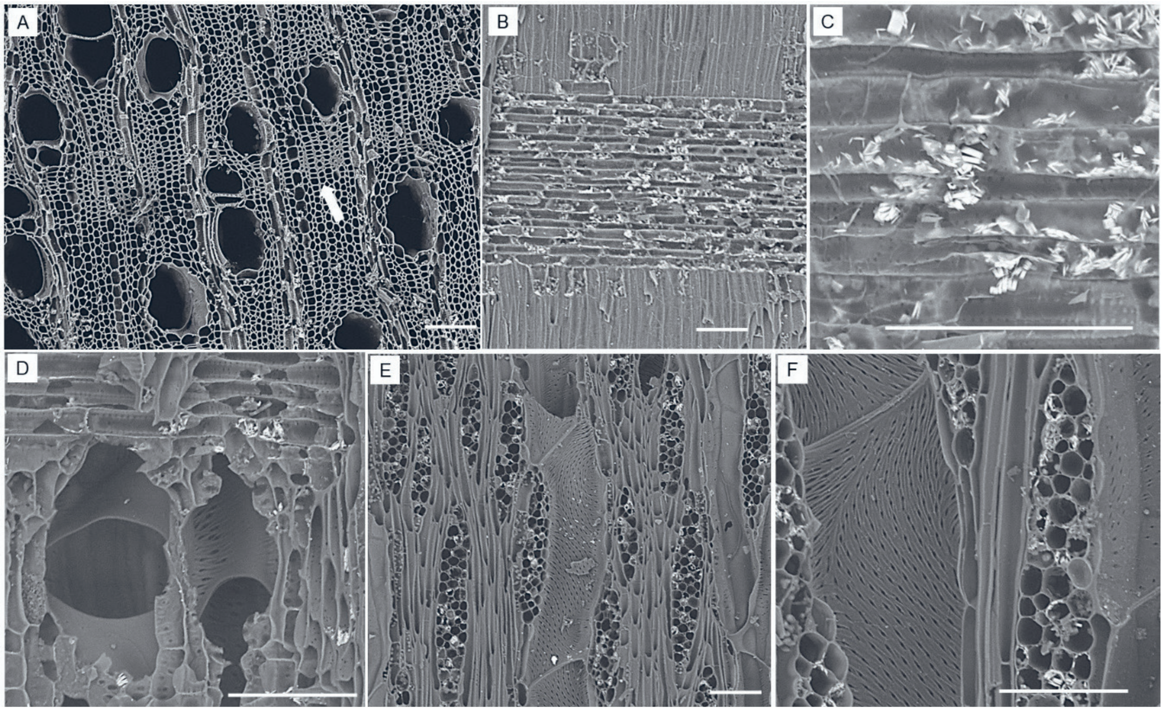

Figure 3. SEM images of *Ocotea diospyrifolia* charcoal. Transversal section (A), arrow indicating growth ring; radial section (B, C, D), tangential section (E, F). Scale bar = 100 µm.

Imágenes SEM del carbón vegetal *Ocotea diospyrifolia*. Sección transversal (A), flecha que indica el anillo de crecimiento; sección radial (B, C, D), sección tangencial (E, F). Barra de escala = 100 µm.

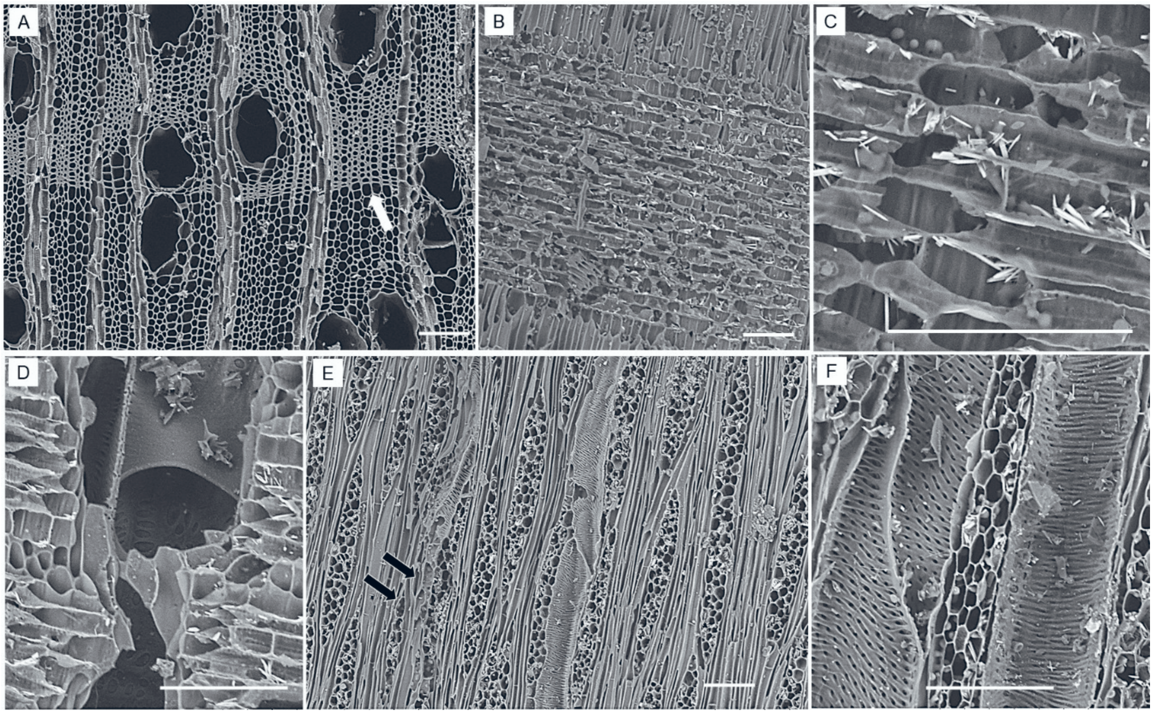

Figure 4. SEM images of *Ocotea indecora* charcoal. Transversal section (A), white arrow indicating growth ring; radial section (B, C, D); tangential section (E, F), black arrow indicating oil cells. Scale bar = 100 µm.

Imágenes SEM del carbón *Ocotea indecora*. Corte transversal (A), flecha blanca que indica anillo de crecimiento; sección radial (B, C, D); sección tangencial (E, F), flecha negra que indica célula oleífera. Barra de escala = 100 µm.

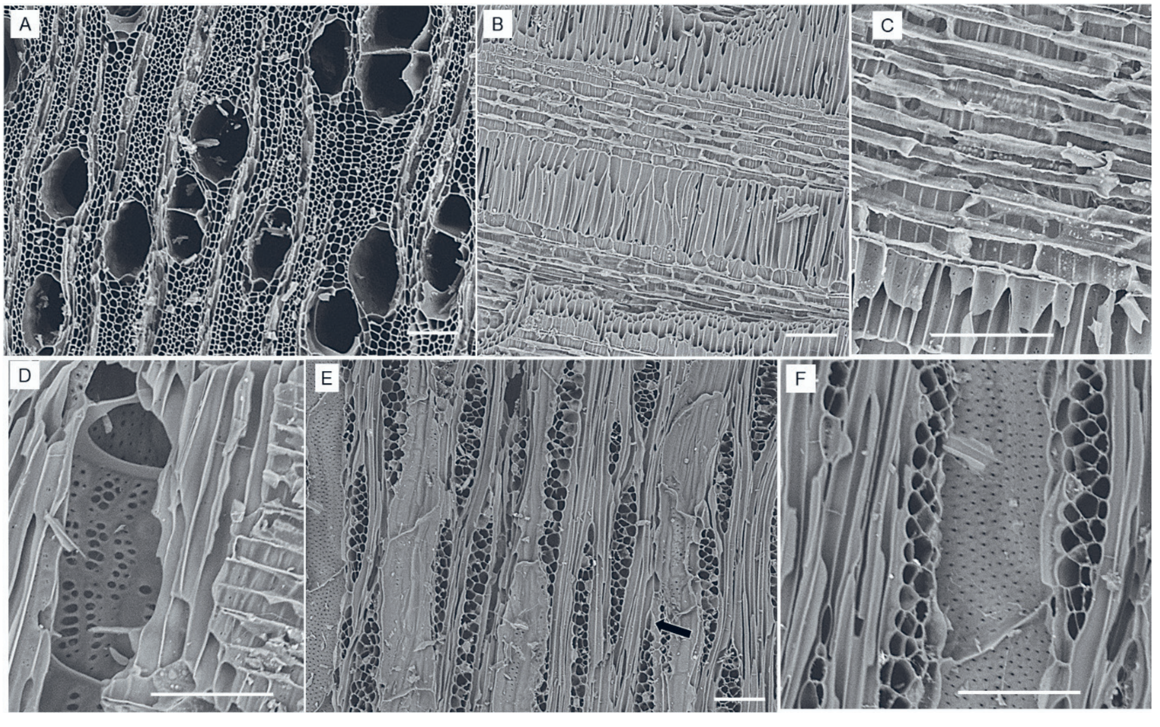

Figure 5. SEM images of *Ocotea puberula* charcoal. Transversal section (A); radial section (B, C, D), tangential section (E, F), black arrow indicates oil cell. Scale bar = 100 µm.

Imágenes SEM del carbón vegetal de *Ocotea puberula*. Sección transversal (A); sección radial (B, C, D), sección tangencial (E, F), la flecha negra indica célula oleífera. Barra de escala = 100 µm.

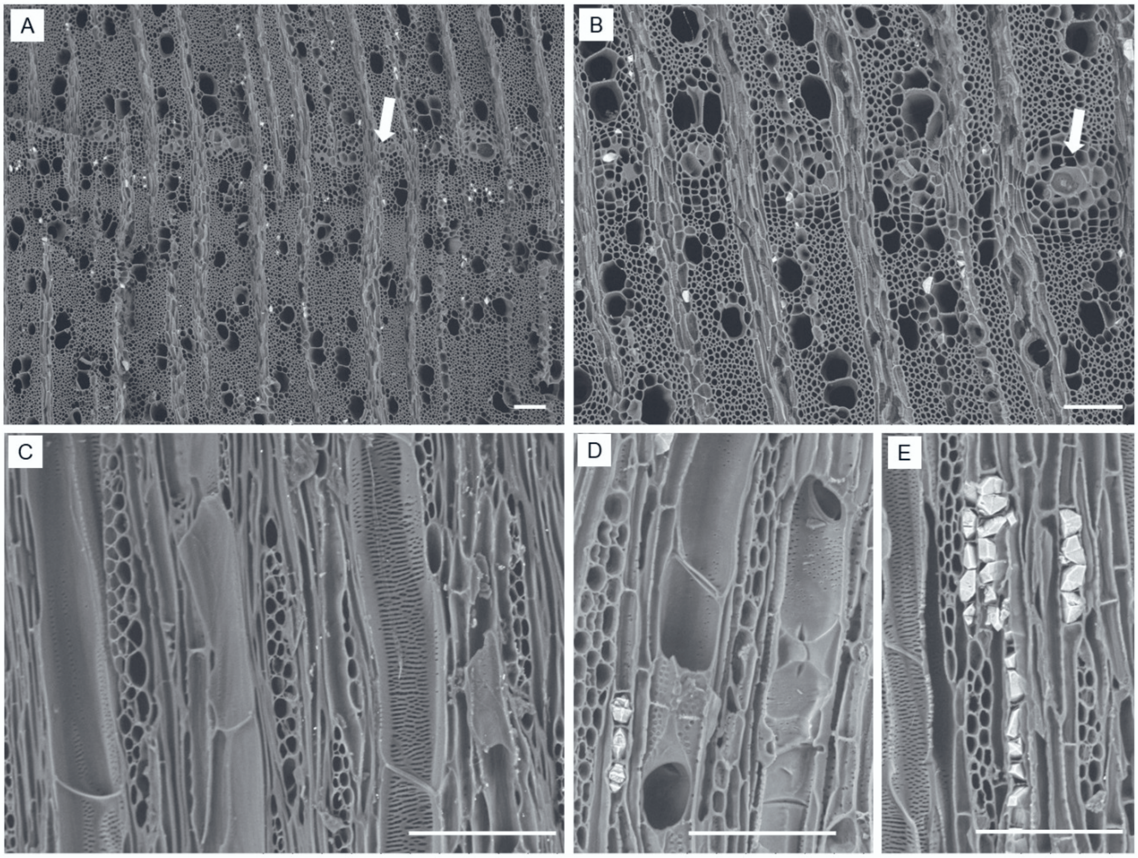

Figure 6. SEM images of *Balfourodendron riedelianum* charcoal. Transversal section (A, B), arrow indicating axial intercellular canal in marginal parenchyma; tangential section (C, D, E). Scale bar = 100 µm.

Imágenes SEM del carbón *Balfourodendron riedelianum*. Sección transversal (A, B), flecha que indica canal intercelular axial en parénquima marginal; sección tangencial (C, D, E). Barra de escala = 100 µm.

perforate plate (figure 6D), intervessel pits alternate (figure 6E). Axial parenchyma: marginal, scanty paratracheal, vasicentric and sometimes confluent. Prismatic crystals present (figure 6E) Rays: heterogeneous, body ray cells procumbent with rows of square marginal cells bi to triseriate, not storied (figure 6C, D, E). Fibers: libriform, non-septate and thin- to thick-walled. Axial intercellular canals (figure 6A, B) present in axial parenchyma.

Scientific name: *Zanthoxylum rhoifolium* Lam. (figure 7)
Vernacular name in Portuguese: Mamica-de-cadela
Família: Rutaceae

Growth rings: distinguished by axial parenchyma (figure 7A). Vessels: diffuse-porous (figure 7A, B), solitary, radial and tangential multiples of 2-5, simple perforate plate (figure 7D), intervessel pits alternate (figure 7F). Axial parenchyma: marginal, scanty paratracheal, vasicentric and sometimes confluent. Rays: heterogeneous (figure 7C), body ray cells procumbent with rows of square marginal cells bi to triseriate, not storied (figure 7E). Fibers: libriform, non-septate and thin-walled. Axial intercellular canals in short lines (figure 7A, B).

Scientific name: *Chrysophyllum marginatum* (Hook. & Arn.) Radlk. (figure 8)
Vernacular name in Portuguese: Guatambu-de-leite
Family: Sapotaceae

Growth rings: indistinct or few distinguished by differences in fiber cell walls. Vessels: diffuse-porous (figure 8A), in radial multiples of 2-4, in diagonal to radial pattern, simple perforate plate (figure 8C), intervessel pits alternate (figure 8F). Axial parenchyma: in narrow lines. Rays: heterogeneous, body ray cells procumbent with rows of square and upright marginal cells (figure 8C), with multiseriate portion as wide as uniseriate portion (figure 8D), not storied (figure 8D, E); silica bodies (figure 8B). Fibers: libriform, non-septate and thick-walled.

Charcoal quantitative anatomy. In the charcoal samples of the studied species, a significant difference in vessel diameter between species was observed among all regions studied (near bark, intermediate, near pith). Regarding the frequency of vessels, it was noted that only the species *Zanthoxylum rhoifolium* exhibited a significant difference between the studied regions (table 2).

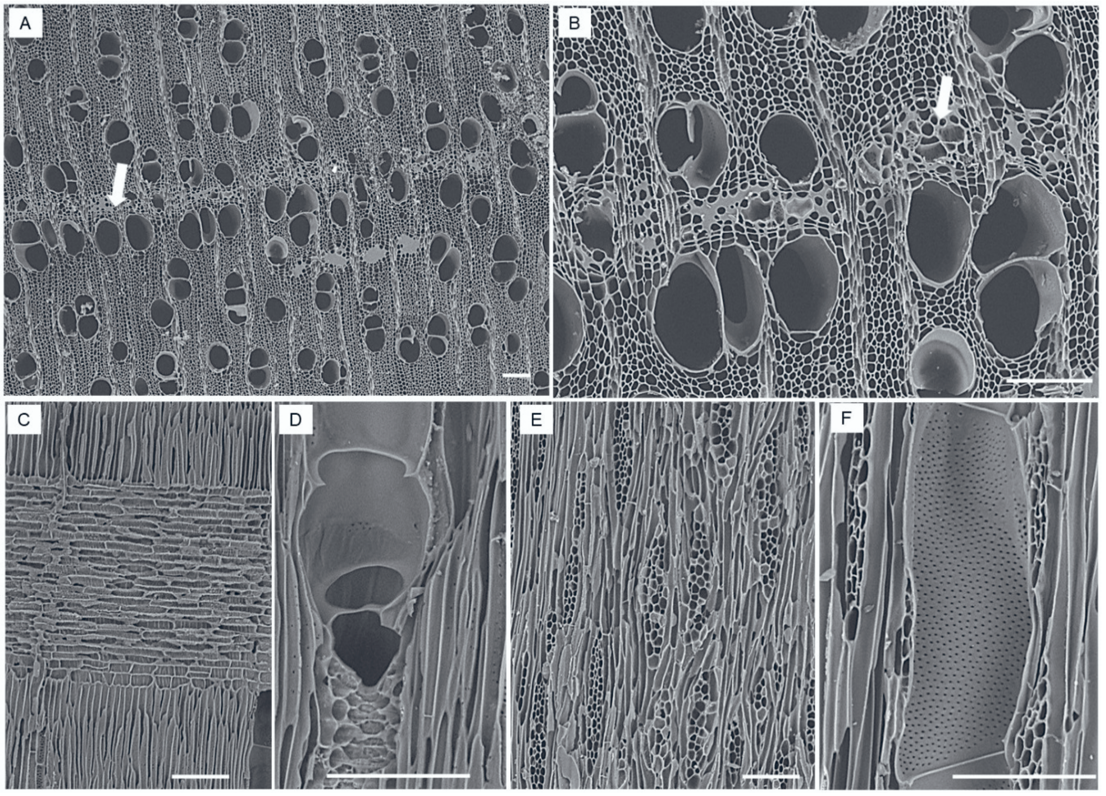

Figure 7. SEM images of *Zanthoxylum rhoifolium* charcoal. Transversal section (A, B) arrow indicates marginal parenchyma with intercellular canal; radial section (C, D) and tangential section (E, F). Scale bar = 100 µm.

Imágenes SEM del carbón *Zanthoxylum rhoifolium*. La flecha de la sección transversal (A, B) indica parénquima marginal con canal intercelular; sección radial (C, D) y sección tangencial (E, F). Barra de escala = 100 µm.

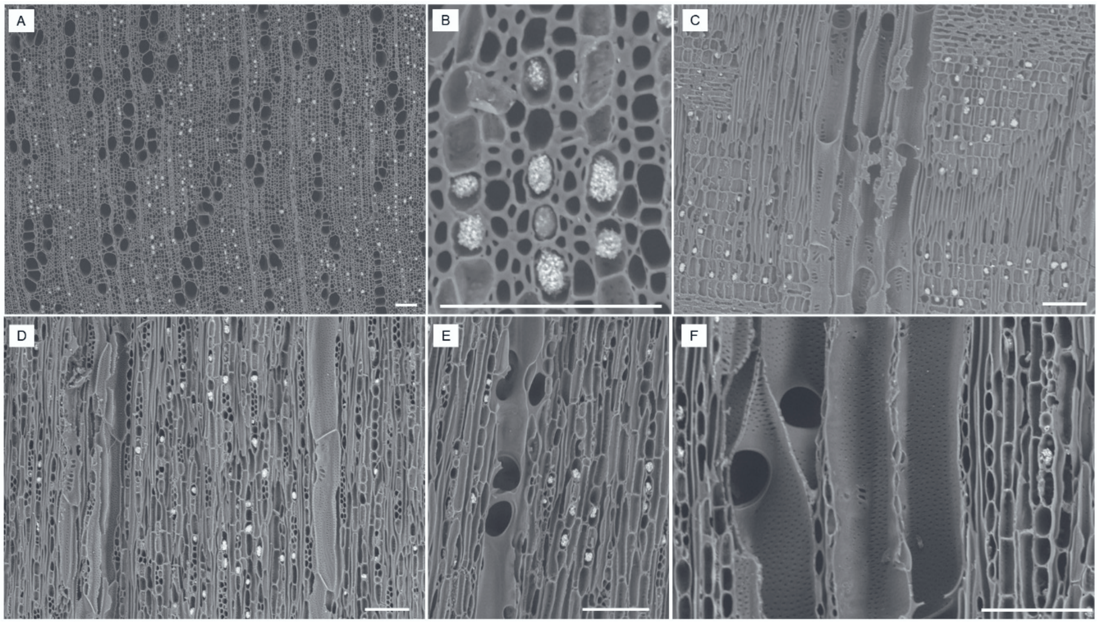

Figure 8. SEM images of *Chrysophyllum marginatum* charcoal. Transversal section (A, B), radial section (C) and tangential section (D, E, F). Scale bar = 100 µm.

Imágenes SEM de carbón *Chrysophyllum marginatum*. Sección transversal (A, B), sección radial (C) y sección tangencial (D, E, F). Barra de escala = 100 µm.

In terms of ray dimensions, it is evident that for the variable width of rays, all species exhibited variation, with a decrease in the width of rays from the region near the bark to near the pith. Concerning ray frequency, the majority of species demonstrated no significant difference between the radial regions studied, except for the species *Chrysophyllum marginatum* (table 3).

Table 2. Mean values of charcoal related to vessels in transversal sections.

Mean values of charcoal related to vessels in transversal sections.

Species	**Vessel diameter (µm)**			**Vessel frequency (n mm⁻²)**		
	B	**I**	**P**	**B**	**I**	**P**
Nectandra megapotamica L	99.61 Ab (20.21)	93.67 Ab (23.39)	108.34 Aa (49.96)	16.02 Da (25.62)	17.23 Da (31.01)	18.92 Da (33.27)
Ocotea indecora L	92.33 Aa (20.50)	97.41 Aa (26.77)	93.93 Ba (19.78)	10.69 Da (33.24)	11.83 Da (35.09)	9.77 Ea (29.76)
Ocotea diospyrifolia L	91.92 Aa (19.04)	85.14 Ba (20.93)	63.65 Db (60.98)	22.06 Ca (21.53)	18.52 Da (16.71)	20.74 Da (19.51)
Ocotea puberula L	94.49 Aa (20.48)	99.98 Aa (20.99)	75.36 Cb (20.38)	14.24 Da (26.84)	11.81 Da (33.82)	12.13 Ea (30.17)
Balfourodendron riedelianum R	46.51 Cb (24.00)	63.36 Ca (22.14)	46.47 Eb (25.56)	69.47 Ab (40.82)	82.03 Aa (19.03)	80.04 Aa (16.18)
Zanthoxylum rhoifolium R	71.01 Ba (21.03)	62.10 Cb (19.73)	53.04 Ec (21.97)	39.34 Ba (39.41)	25.99 Cb (29.62)	29.20 Cb (29.29)
Chrysophyllum marginatum S	53.77 Ca (28.44)	58.73 Ca (22.21)	53.74 Ea (21.31)	43.63 Ba (18.69)	46.03 Ba (21.53)	44.10 Ba (21.29)

B: near bark; I: intermediate; P: near pith; L: Lauraceae; R: Rutaceae; S: Sapotaceae. Mean values with the same letter do not differ statistically according to the Scott-Knott test at 95 % probability. Capital letters indicate species, and lower case letters indicate trunk position (near bark, intermediate, near pith). Values in parentheses represent the coefficients of variation.

Table 3. Mean values related to rays in charcoal tangential sections.

Mean values related to rays in charcoal tangential sections.

Species	**Ray height (µm)**			**Ray width (µm)**			**Ray frequency (n mm⁻¹)**		
	B	**I**	**P**	**B**	**I**	**P**	**B**	**I**	**P**
Nectandra megapotamica L	280.06 Ca (25.98)	246.38 Cb (28.05)	225.07 Bb (38.51)	34.71 Ca (28.52)	28.90 Bb (21.97)	27.29 Cb (21.20)	5.70 Ba (24.45)	5.64 Ca (19.57)	5.06 Ba (39.45)
Ocotea indecora L	379.75 Aa (28.92)	358.19 Aa (33.52)	290.25 Ab (37.06)	52.81 Aa (20.10)	52.69 Aa (19.47)	46.53 Ab (27.89)	6.01 Ba (17.90)	6.33 Ca (19.52)	5.42 Ba (19.93)
Ocotea diospyrifolia L	339.44 Ba (28.18)	309.19 Bb (29.01)	303.11 Ab (33.09)	53.00 Aa (23.48)	49.51 Aa (32.05)	42.49 Ab (35.63)	5.89 Ba (24.34)	5.46 Ca (26.63)	4.93 Ba (25.57)
Ocotea puberula L	365.92 Aa (36.97)	364.17 Aa (30.99)	288.45 Ab (36.81)	56.32 Aa (47.41)	43.02 Bb (28.05)	37.91 Bc (37.47)	5.94 Ba (23.16)	5.37 Ca (22.77)	5.54 Ba (26.51)
Balfourodendron riedelianum R	241.23 Db (25.44)	274.80 Ca (25.32)	248.63 Bb (32.12)	44.86 Ba (37.04)	40.11 Ba (39.78)	32.00 Cb (23.00)	7.10 Ba (34.11)	7.63 Ba (17.61)	6.11 Ba (20.15)
Zanthoxylum rhoifolium R	252.40 Da (27.52)	261.55 Ca (24.92)	237.85 Ba (26.90)	29.14 Da (34.16)	28.98 Ba (26.03)	28.84 Ca (24.50)	7.01 Ba (25.79)	5.80 Ca (32.61)	5.36 Ba (30.70)
Chrysophyllum marginatum S	281.71 Ca (31.39)	270.03 Ca (29.68)	261.82 Ba (32.66)	16.60 Ea (23.38)	14.79 Ca (27.36)	14.59 Da (22.47)	20.60 Ab (13.32)	21.93 Aa (10.54)	19.51 Ab (18.41)

B: near bark; I: intermediate; P: near pith; L: Lauraceae; R: Rutaceae; S: Sapotaceae. Mean values with the same letter are not statistically different according to the Scott-Knott test at 95 % probability. Capital letters indicate species, and lower case letters indicate trunk position (near bark, intermediate, near pith). Values in parentheses represent the coefficients of variation.

DISCUSSION

After carbonization, some alterations were observed in the samples. In those from the Lauraceae family, cracks were observed in rays, and contraction was verified in vessels, probably resulting from thinner fiber walls compared to the Sapotaceae and Rutaceae samples.

The presence of radial cracks and / or transversal and longitudinal splits in carbonized material is the result of rapid release of gas during the process (Pinto and Junior 2010, Théry-Parisot and Henry 2012). Splits are generally more related to the water content in wood than to temperature. However, if the wood is moist, an increase in carbonization temperature will result in more frequent splits (Théry-Parisot and Henry 2012).

In general, traits such as the shape and distribution of vessels, rays and fibers changed very little after carbonization, resulting in well-defined structures on the surface. When evaluating all seven species together and comparing transversal sections (figure 1), some aspects can be noted:

- In the Lauraceae family, *Ocotea indecora* had the widest rays, *Ocotea puberula* had the greatest number of multiple vessels, and *Nectandra megapotamica* had larger vessels compared to the other species. Although the differences were slight, they were sufficient for inclusion in a database for the identification of carbonized material.
- In *Chrysophyllum marginatum*, the presence of multiple radial vessels distinguished it from the other species.
- In the Rutaceae family, vessel diameter, arrangement and frequency can be distinguishing characteristics, but they must be applied with caution because there were some variations in function of radial position in the trunk (see table 2).

The mean values of tangential vessel diameter and vessel frequency (table 2) indicated significant differences between the species. In relation to trunk position, *i.e.* samples near bark, intermediate, and near pith, some variation was verified for vessel diameter position, without influence only in *Ocotea indecora* and *Chrysophyllum marginatum*. For vessel frequency, no effect was found for species from the Lauraceae and Sapotaceae family, and for the Rutaceae family, the near bark region was different from the other positions.

Species from the Lauraceae family exhibited a larger vessel diameter and lower frequency compared to the species from the Rutaceae and Sapotaceae families, consistent with expectations based on wood anatomical characteristics. Generally, a larger vessel diameter is associated with a lower frequency in wood (Lima *et al.* 2011), a trend confirmed in this study (table 2).

In charcoal, previous studies have also described this behavior, namely, variation in vessel diameter after carbonization. However, the relationship with changes in vessel frequency is influenced by the species (Muñiz *et al.* 2012, Gonçalves *et al.* 2012).

Differences in vessel diameter and frequency in charcoal, when compared to wood, were also verified in studies by Souza-Pinto & Scheel-Ybert (2021), who note that this behavior can occur due to the carbonization process, ecological factors, or intraspecific variability. Therefore, this variation does not interfere with species identification, as it falls within the expected values in wood anatomy (Gonçalves *et al.* 2012, Gonçalves & Scheel-Ybert 2016).

Another important point is the increase in vessel diameter concerning radial position, from the near pith to near bark regions in *Ocotea diospyrifolia* (30.76 %), *Zanthoxylum rhoifolium* (25.31 %) and *Ocotea puberula* (20.24%) (table 2). Stange *et al.* (2018) also described a gradual increase in vessel diameter from pith to bark in *Eugenia pyriformis.*

Pereira *et al.* (2016), in their evaluation of the effect of carbonization on the wood anatomy of six *Eucalyptus* clones, concluded that changes in vessel shape occurred. Specifically, there was elongation of vessels in the radial direction, particularly in sapwood, changing from circular in wood to oval in charcoal.

Some authors have reported that, during carbonization, there is higher contraction in the tangential direction compared to the radial contraction of the wood structure (Kim and Hanna 2006, Kwon *et al.* 2009). Kwon *et al.* (2009) attributed the elongation of vessels to the presence of rays, which can act by limiting the contraction in the radial direction.

For Lauraceae species, the near pith region presented different vessel diameter values than the other radial positions, indicating that this characteristic can be applied for species identification. In practice, this may be more efficient in young trees. For Rutaceae species, a distinction in vessel frequency was verified in the near bark position (table 2).

Mean ray dimensions and frequency (table 3) indicated a slight distinction of material, primariily in the Lauraceae family, with samples from the genus *Ocotea* having higher and wider rays than *Nectandra.* The radial position had an influence on ray dimensions in all species of the Lauraceae family and in *Balfourodendron riedelianum.* The species *Chrysophyllum marginatum* differed markedly from the other species in ray width and ray frequency. Ray frequency had a slight influence on trunk position only in *C. marginatum.*

Differences in ray dimensions in function of radial position in charcoal produced from native Atlantic Forest species have also been reported by Stange *et al.* (2018) and Stüpp *et al.* (2021).

When comparing wood and charcoal, a reduction in ray dimensions has been described, for example, by Muñiz *et al.* (2012) in *Enterolobium schomburgkii*, causing what is referred to as “retraction splits” (Théry-Parisot 2001). The varying changes in ray width described in the literature are contradictory. Some species exhibit an increase, while others show a decrease, indicating that the principal factor influencing these changes is the specific species under evaluation (Kim and Hanna 2006, Muñiz *et al.* 2012, Stange *et al.* 2018, Stüpp *et al.* 2021).

Ávila *et al.* (2017) noted that the trait of ray frequency is variable within a species. They observed values for *Schinus polygamous* that were similar to those described for wood. However, for *Lithraea brasiliensis* and *Ocotea pulchella,* ray frequency in charcoal was greater than in wood by as much as 25 %, while it was lower for *Myrcia palustris* and *Casearia sylvestris*.

CONCLUSION

After carbonization, no significant degradation of anatomical structure and cell arrangement occurred, enabling the identification of samples through comparison with wood description references. Radial position in the trunk did not exhibit a consistent pattern of influence on anatomical characteristics; rather, it was species-dependent. Wood from the Lauraceae family exhibited greater vessel diameter and lower vessel frequency compared to samples from the Rutaceae and Sapotaceae families. Discriminators such as vessel grouping, ray width and ray frequency proved effective in distinguishing *Chrysophyllum marginatum* from the other evaluated species. These results contribute to a charcoal database of Atlantic Forest species applicable in practical scenarios of forestry supervision.

AUTHOR CONTRIBUTIONS

Analysis, or interpretation of data: Rafaela Stange, Angela Luiza Schutz. Drafting of the manuscript: Rafaela Stange, Helena Cristina Vieira, Simone Ribeiro Morrone, Polliana D'Angelo Rios, Silvana Nisgoski. Critical revision of the manuscript for important intellectual content: Graciela Ines Bolzon de Muniz. Statistical analysis: Helena Cristina Vieira, Lia Wilma Stiegler

Technical, or material support: Polliana D'Angelo Rios. Supervising the work: Silvana Nisgoski. Coordinating the research Project: Silvana Nisgoski

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