COMPARED ANATOMY OF SPECIES OF *Calycophyllum* DC. (Rubiaceae)[1]

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ABSTRACT – The wood anatomy of three species and one variety of genus *Calycophyllum* DC. (*C. candidissimum*, *C. multiflorum*, *C. spruceanum* e *C. spruceanum* f. *brasiliensis*) is presently described according to IAWA (1989). Most of the observed characteristics agree with the common pattern described to family Rubiaceae, and allows grouping them in the "Type II" wood structure: predominantly radial multiple pores; axial parenchyma absent; large rays (2-4 or more cells), with few layers of square and upright cells in the margins; septate fibers with simple pits. These characters, observed in all studied species, do not agree with the anatomical pattern referred to Ixoroideae subfamily.

Keywords: Wood anatomy; Cluster Analysis; Ixoroideae.

ANATOMIA COMPARADA DE ESPÉCIES DE Calycophyllum DC. (Rubiaceae)

*RESUMO – São anatomicamente descritos os lenhos de três espécies e uma forma botânica do gênero Calycophyllum DC. (**C. candidissimum**, **C. multiflorum**, **C. spruceanum** e **C. spruceanum** f. **brasiliensis**), de acordo com IAWA (1989). A maior parte das características observadas concorda com o padrão típico em Rubiaceae, permitindo agrupá-las na estrutura de lenho "Tipo II": poros predominantemente em múltiplos radiais; parênquima axial ausente; raios grandes (2-4 células ou mais), com poucas camadas de células quadradas e eretas nas margens; e fibras septadas, com pontoações simples. Os caracteres, observados nas quatro espécies, discordam do padrão anatômico da subfamília Ixoroideae.*

Palavras-chave: Anatomia da Madeira; Análise de agrupamento; Ixoroideae

1. INTRODUCTION

The Rubiaceae Juss. family, which is the fourth in number of species in the world and surpassed only by Orchidaceae Juss., Asteraceae Bercht. & J.Presl and Fabaceae Lindl. (DELPRETE, 2004; GOVAERTS et al., 2007; JUDD et al., 2009; MÓL, 2010; FERREIRA JUNIOR; VIEIRA, 2015), covers about 13,000 species and 650 genera according to Delprete and Jardim (2012). Even with cosmopolitan distribution, most of the species is concentrated in the hottest regions of the world, especially in the tropics, where it is possible to find over 75% of them (CHIQUIERI et al., 2004).

The *Calycophyllum* DC. genus (sin.: *Enkylista* Benth. & Hook. f., *Eukylista* Benth. and *Semaphyllanthe* L. Andersson) is Neotropical, widely occurring from Mexico to Brazil (MENDOZA et al., 2004).

Due to frequent updates in botanical literature as well as the phylogenetic changes in the kingdom Plantae, the number of *Calycophyllum* species is controversial. Delprete (1996) recognizes the existence of only ten species; in the New York Botanical Garden herbarium (2014) nine are listed and, according to the International Plant Names Index (IPNI, 2014), the dichotomies are in number of 21, between valid and synonym species.

The *Calycophyllum* species, characterized for medium to big trees, present conspicuous foliar sepal in the flowers as a morphological peculiarity, which is responsible for the generic name. In addition to the acknowledged medicinal potential, the wood has high specific mass, dark brown color and good natural durability, aspects that highlight them in the international market, such as *Calycophyllum candidissimum* and *C. spruceanum* in tropical America, which are suitable for construction. The specific mass, ranging from 0.80 to 0.85 g/cm³, offers proper hardness and resistance to construction in general and to the manufacture of vehicles and tools.

Despite its economic importance, the genus has been poorly studied from the anatomical point of view and timber usage in Brazil up to present moment, which leads to a lack of literature for depth studies on the species. In this sense, this paper aims to describe, anatomically, the logs of *Calycophyllum candidissimum, C. multiflorum*, *C. spruceanum* and *C. spruceanum* f. *brasiliensis* and verify the similarity/dissimilarity of them, based on anatomical characters.

2. MATERIAL AND METHODS

The material in study consists of wood samples from three species of *Calycophyllum*: *Calycophyllum candidissimum* (Vahl) DC., *Calycophyllum multiflorum* Griseb. and *Calycophyllum spruceanum* (Benth.) K. Schum.) and from a botanical form of this genus *Calycophyllum spruceanum* f. *brasiliensis* K.Schum.

Wood samples were received by exchange of various institutions such as the Jodrell Laboratory (Kew Gardens, London), the Institute of Technological Research of the state of São Paulo (IPT) and the German university Black Forest Academy (BFA). Variable in number, according to species, all samples are listed in Table 1.

Permanent and macerated slides were prepared for the microscopic study. Three test samples (3 x 3 x 3 cm) were extracted from the timber material and properly oriented to obtain anatomical sections in the transverse, longitudinal radial and longitudinal tangential planes. Plus, another block was taken for maceration purposes.

The assembly of histological slides followed the standard procedures recommended by Burger and Richter (1991). Maceration was performed by the Franklin method, which was modified (KRAUS; ARDUIN, 1997). Anatomical sections were stained with acridine red, chrysoidine and astra blue. Entellan was used in the assembly of permanent slides.

Wood descriptions followed the recommendations of IAWA (1989) in relation to quantitative characters, so, 25 repetitions were measured as indicated by the standard. Percentages of different tissues, ray height and width (including body and margins, in number of cells) as well as wall thickness of fibers and vessels were added to the measurements, as these aspects were not contemplated in the standard referred. In the case of tissue percentage, 600 determinations were carried out randomly with the aid of a cell counter, as proposed by Marchiori (1980). Wall thickness of vessels and fibers, measured in macerated slides during the study, was obtained according to the following formula: E = DT - DL/2, where E = wall thickness of vessels or fibers; DT = total diameter; and DL = diameter of the lumen.

To determine the abundance of pores (pores/mm²), we used a square which area was known, superposed on anatomical slides in transversal section. In this calculation, each pore was considered as a unit, not as series or clusters.

Anatomical structure measurements were carried out under a Carl Zeiss microscope in the Laboratory of Wood Anatomy of Federal University of Santa Maria. The photomicrographs were taken in a Leica DM 1000 microscope equipped with an Olympus Camedia CX-40 digital camera in the Laboratory of Wood Anatomy at the North Higher Education Center of Rio Grande do Sul (CESNORS, Frederico Westphalen campus) of Federal University of Santa Maria. The figures in parenthesis are equivalent to the minimum and maximum values observed in quantitative characteristics, the value that follows the mean is the standard deviation.

The Botanical classification used in this study follows the APG III (2009). Also, the scientific names of species were found at the IPNI, The International Plant Names Index - www.ipni.org (accessed in 09.23.2015).

In order to verify the formation of different groups, as well as to measure, explain and predict the degree of relation between the Rubiaceae presently investigated, we held an agglomerative hierarchical cluster analysis, based on the anatomical characteristics of the species

Table 1 – List of investigated samples.
Tabela 1 *– Lista das amostras investigadas.*

Species	Xylotheque registration	Total number of samples
Calycophyllum spruceanum f. *brasiliensis*	IPT (BCTw) – 1309	1
Calycophyllum candidissimum	IPT (BCTw) – 6009, BFA-10583	2
Calycophyllum multiflorum	BFA -16581, 8217 IPT (BCTw) – 9434, 128, 9661, 9542, 3717, 45 KEW (K) – 4187	9
Calycophyllum spruceanum	KEW (K) – 22228, 22450 IPT (BCTw) – 294 BFA- 13196	4
Sum		16

at where: KEW (K) – Jodrell Laboratory, The Royal Botanic Gardens (Kew Gardens, Londres); IPT (BCTw) - Xiloteca Dr. Calvino Mainieri, Instituto de Pesquisas Tecnológicas do estado de São Paulo; BFA – Black Forest Academy, Alemanha.

(Table 2). The analysis was generated from a matrix of binary data, which obtained the Jaccard distance, and these values were connected by proportional weight method (*Unweigthed Pair-Group Method Using Arithmetical Averages* - UPGMA). To validate the dendrogram, we calculated the cophenetic correlation coefficient (*r*). Data and analyzes were processed in the R software (R DEVELOPMENT CORE TEAN, 2011), vegan package (OKSANEN et al., 2012).

3. RESULTS

3.1 Anatomical description of the investigated species

Calycophyllum candidissimum (Vahl) DC.

Growth rings: distinct, visualized by layer of 5 or 6 rows of radially flattened fibers and with thicker walls in latewood, as well for the enlargement of the rays in the growth ring boundary (Figure 1A).

Vessels: extremely numerous (163 ± 19 (126-202) pores/mm²), occupying 20% of the wood volume. Diffuse, uniform and radially arranged porosity. Rounded section vessels, tending to oval (48 ± 9 (28-65) μm), thin walls (3 ± 1 (1-5) μm), predominantly in radial multiples of 2-9 (59%), less commonly solitary (34%) and raceme forms of 3-6 units (7%); contacts with ray cells are frequent (Figure 1A). Vascular elements of average length (656 ± 205 (190-1050) μm), with simple and oblique perforation and long appendages (102 ± 64 (30-340) μm) in both or in only one end. Small, round (5 ± 1 (4-8) μm) and alternate intervessel pits; horizontal, lenticular, included, ornamented opening. Ray-vascular pits, similar to the intervessel ones, although smaller (3 ± 1 (3-5) μm). Absent tilos, helical thickening, striations and deposits.

Axial parenchyma: absent.

Rays: numerous (14 ± 2 (9-20) rays/mm), heterogeneous and of two different sizes, representing 32% of wood volume. The uniseriate ones (15%), of square and erect cells, sometimes with procumbent cells; 288 ± 106 (150-650) μm in height and 5 ± 3 (1-12) cells and 13 ± 3 (8-20) μm in width. Multiseriate rays, mostly triseriate (57%), less commonly biseriate (16%) and tetraseriate (12%) (Figure 1E), composed of procumbent cells in the central body (5-19) and short marginal band (1-5 cells) of square and upright cells; 457 ± 106 (190-620) μm in height, with 16 ± 4 (8-25) cells and 33 ± 6 (15 - 50) μm in width. Present fused rays. Absent aggregated rays. Present perforated ray cells (Figure 1C); absent tile, oil and surrounding cells.

Fibers: libriform, septate; medium length (1435 ± 286 (910-2000) μm) and thin to thick walls (6 ± 1 (4-9) μm) - simple pits, vertical slit and large opening, restricted to radial faces of the wall. Fibrous tissue occupies 48% of wood volume. Absent gelatinous fibers, helical thickening and fiber tracheids.

Other characters: crystals in sand form (microcrystals) and deposits in the ray cells. Absent cell canals, glandular cysts, stratified structure, silica, included phloem, oil cells, mucilaginous cells and pith flecks.

Calycophyllum multiflorum Griseb.

Growth rings: distinct, marked by layers of fibers radially narrow, thicker and lignified walls in latewood and the small enlargement of rays in the ring boundary.

Vessels: frequently in contact with rays; rounded section, tending to oval (42 ± 9 (15-60) μm) and thick walls (4 ± 1 (1-10) μm), composing 38% of the wood volume. Diffuse and uniform porosity. Extremely

Table 2 – Anatomical characters used in the cluster analysis.
Tabela 2 – *Caracteres anatômicos utilizados na análise de agrupamento.*

ANATOMY CHARACTERS	*C. s.* f. *bras*	*C. cand*	*C. mult*	*C. spru*
Growth ring distict	1	1	1	1
Vessels in radial pattern	1	1	1	1
Wood diffuse-porous	1	1	1	1
60-150 vessels per square millimetre	1	0	0	1
150-250 vessels per square millimetre	0	1	0	0
250-550 vessels per square millimetre	0	0	1	0
Vessels thin-walled (1,0-3,5 µm)	0	1	0	0
Vessels thick-walled (3,5-10 µm)	1	0	1	1
Porous in frequent contacts with ray cells	1	0	0	0
Medium-sized appendages (25-95 µm)	0	0	1	0
Long-sized appendages (≥ 95 µm)	1	1	0	1
Contents in the vessel cavity	0	0	1	1
Simple perforation plates	1	1	1	1
Small intervessel pits	1	1	1	1
Vestured intervessel pits	1	1	1	1
Axial parenchyma absent	1	1	1	1
Heterogeneous rays	1	1	1	1
Uncommon uniseriate rays (8-12%)	1	0	0	1
Frequent uniseriate rays (≥ 12%)	0	1	1	0
Uniseriate rays with square and upright cells	1	0	1	1
Uniseriate rays with procumbent, square and upright cells	0	1	0	0
Uniseriate rays up to 13 µm wide	0	0	1	0
Uniseriate rays with more than 13 µm wide	1	1	0	1
Uniseriate rays up to 4 height cells	1	0	0	1
Uniseriate rays with more than 4 height cells	0	1	1	0
Multiseriates rays majority biseriate	0	0	1	0
Multiseriates rays majority triseriate	0	1	0	1
Multiseriates rays majority tetraseriate	1	0	0	0
Wider multiseriates rays	1	0	0	1
Multiseriates rays up to 20 body cells rows	0	1	1	0
Multiseriates rays with more than 20 body rows cells	1	0	0	1
Multiseriates rays with more than 2 marginal cells rows	1	1	0	1
Multiseriates rays up to 14 height cells	0	0	1	0
Multiseriates rays with 14-17 height cells	1	1	0	0
Multiseriates rays with more than 17 height cells	0	0	0	1
Multiseriates rays up to 26 µm wide	0	0	1	0
Multiseriates rays with 26-33 µm wide	1	1	0	0
Multiseriates rays with more than 33 µm wide	0	0	0	1
4-12 rays per millimetre	1	0	1	1
>12 rays per millimetre	0	1	0	0
Abundant contents in ray cells	1	1	1	1
Septate fibres present	1	1	1	1
Abundant contents in fibres	0	0	1	0

Legend: Anatomy character present (1). Anatomy character absent (0). *C. s.* f. *bras* (*Calycophyllum spruceanum* f. *brasiliensis*), *C. cand* (*Calycophyllum candidissimum*), *C. mult* (*Calycophyllum multiflorum*), *C. spru* (*Calycophyllapum spruceanum*). Font: authors

numerous vessels (434 ± 47 (322-549) pores/mm²) (Figure 1B) in radial multiples of 2-11 (70%), less commonly solitary (24%) and with scarce raceme forms of 3-10 units (6%). Vascular elements of average length (627 ± 122.0 (350-910) µm), with simple, oblique perforation plates, with no helical thickening and striations on the wall; long appendages (94 ± 56 (25-290) µm), in both or in only one end. Alternate, round, ornamented, small intervessel pits (5 ± 1 (4-8) µm); lenticular, horizontal, included opening. Ray-vascular pits, similar to the intervascular ones, but with slightly smaller pits (3 ± 1 (3-5) µm). Absent tilos.

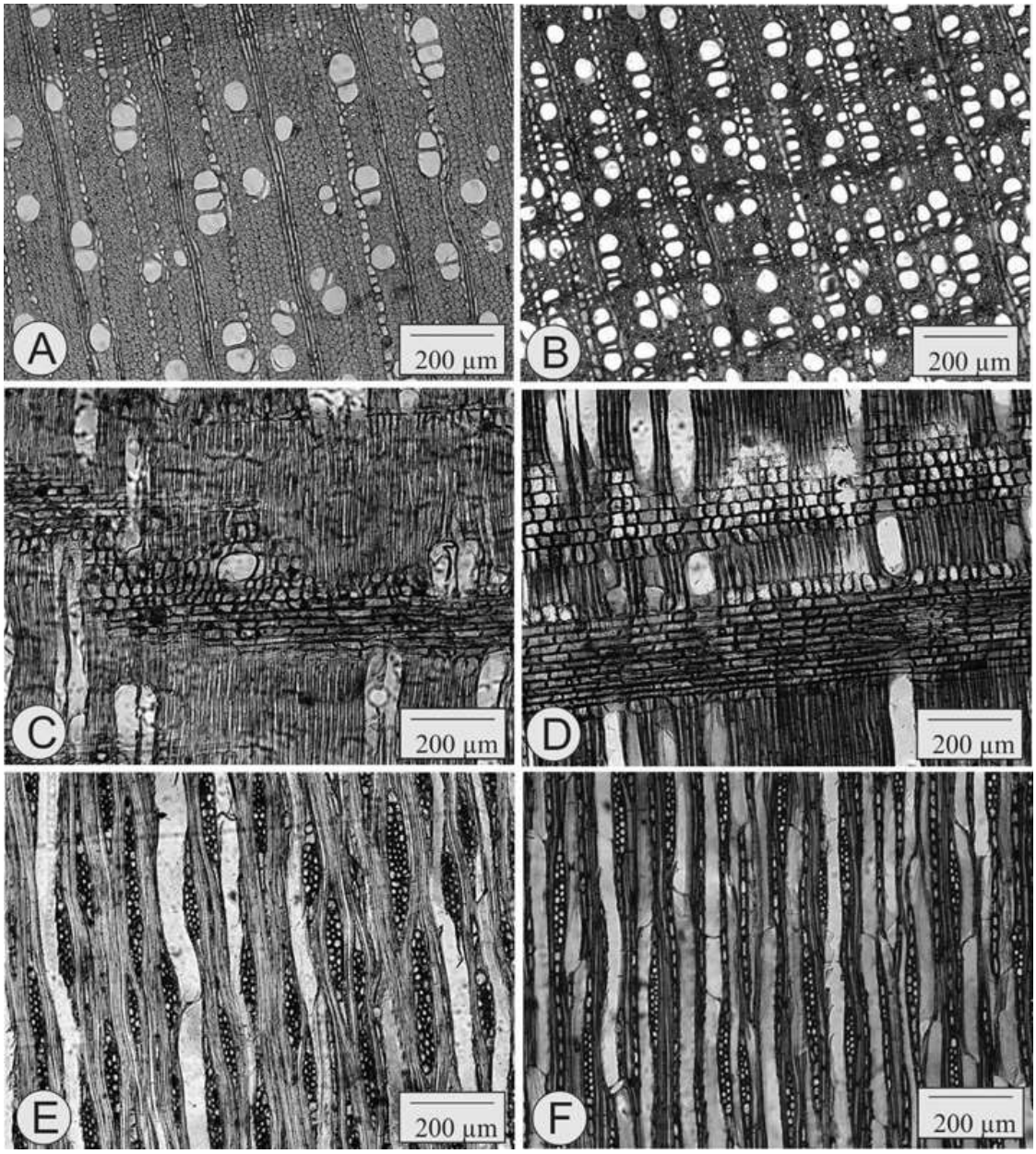

Figure 1 – A – Growth ring limit and vessels in frequent contact with ray cells (*Calycophyllum candidissimum* in cross section). B – *Calycophyllum multiflorum* with extremely numerous pores (cross section). C – Perforated ray cell in *C. candidissimum* (radial section). D – Heterogeneous rays in *C. multiflorum*, with procumbent cells in the center and square and upright cells at the margins (radial section). E – Multiseriate rays in *C. candidissimum* (tangential section). F – Uni and biseriate rays of *C. multiflorum* (tangential section).

Figura 1 – *A – Limite de anel de crescimento e vasos em contato frequente com células de raio (**Calycophyllum candidissimu**m em seção transversal). B – **Calycophyllum multiflorum** com poros extremamente numerosos. C – Célula perfurada de raio em **C. candidissimum** (seção longitudinal radial). D – Raios heterogêneos em **C. multiflorum**, com células procumbentes, no centro, e margens de células quadradas e eretas (seção longitudinal radial). E – Raios multiserriados em **C. candidissimum** (seção longitudinal tangencial). F – **C. multiflorum** com raios uni e bisseriados, em seção longitudinal tangencial.*

Axial parenchyma: absent.

Rays: heterogeneous (Figure 1D) and of two different sizes, with plenty of 12 ± 2 (8-16) rays/mm. Radial tissue occupies 22% of the wood volume. Uniseriate rays (29%) of 267 ± 114 (80-660) μm in height, with 6 ± 3 (2-13) cells and 15 ± 3 (8-25) μm in width, composed of square and upright cells. The multiseriate ones, mostly with two cells wide (67%) and rare triseriate (4%) (Figure 1F); 384 ± 99 (210-650) μm of height, with 14 ± 4 (6-22) cells, and 26 ± 4 (15-38) μm in width, bringing procumbent cells in the center (2-19), and 1-5 marginal bands of square and upright cells. Present fused rays. Present perforated ray cells. Absent surrounding and tile cells.

Fibers: libriform, septate, with simple pits, in vertical slit, restricted to radial faces of the wall. Fibrous tissue occupies 40% of the wood volume. Medium fibers (1257 ± 269 (90-1780) μm), of thin to thick walls (6 ± 1 (3-10) μm). Absent helical thickening. Gelatinous fibers not observed.

Other characters: deposits and crystals in sand form (microcrystals), present in ray cells. Absent intercellular canals, cell canals, glandular cysts, stratified structure, silica, included phloem, mucilaginous cells and pith flecks.

Calycophyllum spruceanum f. *brasiliensis* K.Schum.

Growth rings: distinct, visualized by smaller radial diameter fibers and thicker walls in latewood and the tangential enlargement of rays in the ring transition.

Vessels: frequent contact with the ray cells, numerous (97 ± 8 (82-109) pores/mm²) (Figure 2A), round or oval (71 ± 11 (55-93) μm), thick walls (± 5 1 (3-8) μm) and in radial arrangement, composing 23% of wood volume. Vessels in diffuse, uniform porosity; solitary (50%), in radial multiples of 2-6 (45%), and few raceme forms (5%) of 3-6 units. Vascular elements of medium length (649 ± 170 (270-900) μm). Simple, oblique perforation plates. Long appendages (110 ± 74 (30-290) μm) in both or in only one end. Small, rounded (7 ± 1 (5-8) μm), alternate intervessel pits; with lenticular, horizontal, included, ornamented opening. Ray-vascular pits similar to the intervessel ones, although smaller (4 ± 1 (3-5) μm). Absent tilos, helical thickening, striations and deposits.

Axial parenchyma: absent.

Rays: heterogeneous, of two different sizes and in abundance (12 ± 1 (10-15) rays/mm), representing 31% of the wood volume. The uniseriate rays, scarce (8%), of square and upright cells; 303 ± 126 (110-590) μm of height, with 4 ± 2 (2-9) cells and 15 ± 3 (10-20) μm in width. The multisseriate ones, mostly tetraseriate (62%) (Figure 2E), triseriate (22%), less commonly with more than 5 cells (7%) and rare biseriate (1%), composed of procumbent cells (6-21) in the central body, and 1-3 marginal bands of upright and square cells (Figure 2C); 467 ± 91 (300-660) μm of height, with 17 ± 4 (9-24) cells and 30 ± 9 (30-75) μm in width. Present fused rays. Absent aggregated rays. Present perforated ray cells; absent tile, surrounding and oil cells.

Fibers: libriform, septate, medium length (1371 ± 249 (850-1780) μm) and thin to thick walls (6 ± 2 (3-15) μm); with simple pits, restricted to radial faces of the wall. Fibrous tissue occupies 46% of the wood volume. Absent gelatinous fibers, helical thickening and fiber tracheids.

Other characters: crystals in sand form (microcrystals) and deposits present in ray cells. Absent cell canals, stratified structure, silica, included phloem, oil cells, mucilaginous cells and pith flecks.

Calycophyllum spruceanum (Benth.) K. Schum.

Growth rings: distinct, marked by radially narrow fibers in latewood and for the frequent ray enlargement in the ring transition.

Vessels: round or oval (83 ± 13 (40-125) μm), thick walls (4 ± 2 (1-8) μm) in radial arrangement, and with plenty of 98 ± 16 (69-130) pores/mm², occupying approximately 29% of the wood volume; pores in radial multiples of 2-6 (54%), solitary (40%), less commonly in racemes of 3-5 units (6%) (Figure 2B). Vessels in frequent contact with ray cells. Diffuse, uniform porosity. Vascular elements of 589 ± 225 (210-1013) μm in length, with simple oblique perforation plates and long appendages (101 ± 64 (20-420) μm) in both, less commonly in one end. Helical thickenings, striations and tilos not observed. Abundant content in the vessel cavity. Small, round (5 ± 1 (3-8) μm), alternate intervessel pits; with lenticular, horizontal, included, ornamented opening. Ray-vascular pits similar to intervessel pits, but smaller (3 ± 1 (3-5) μm).

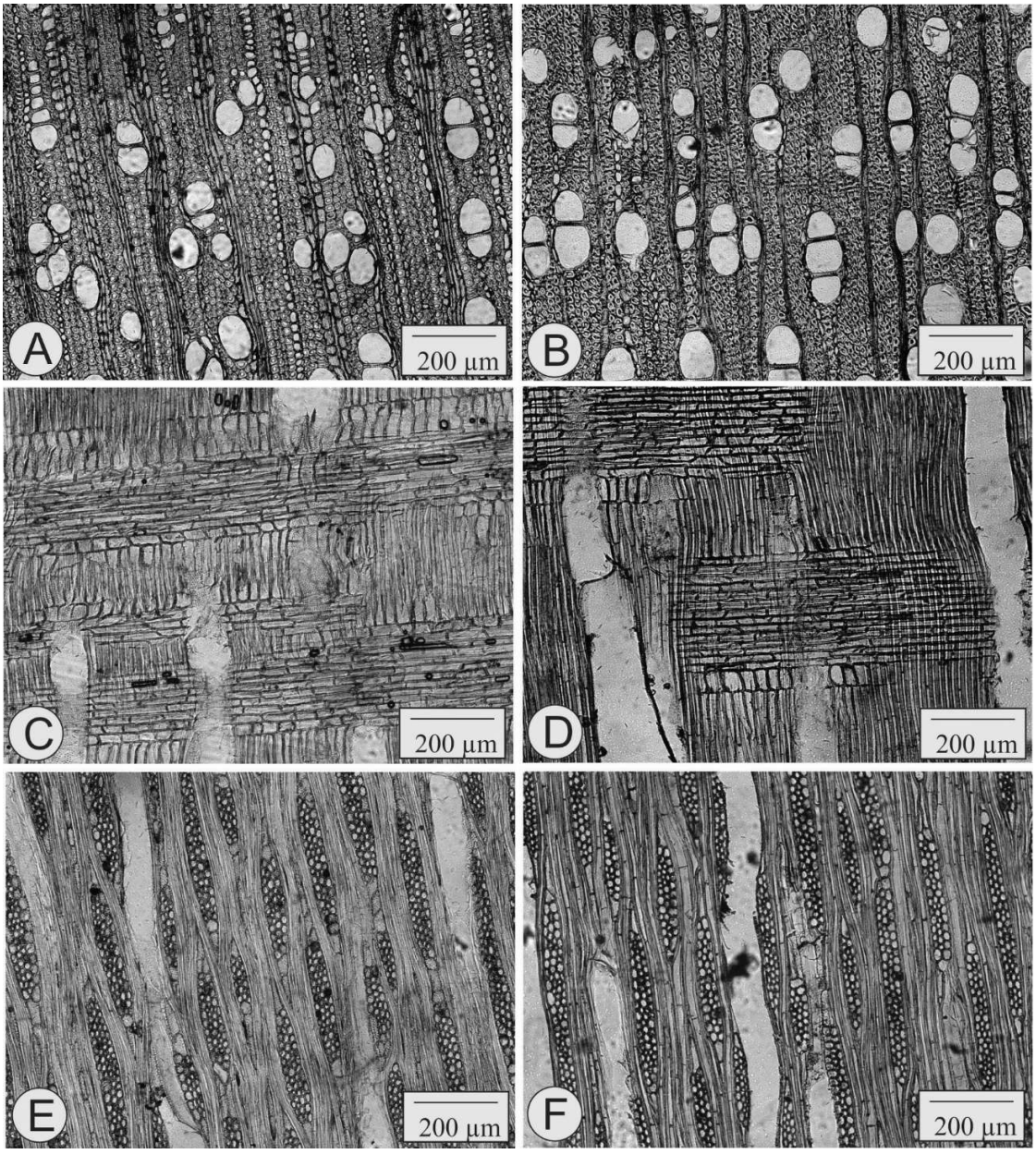

Figure 2 – A – Very numerous pores in *Calycophyllum spruceanum* f. *brasiliensis*, with frequent contacts with ray cells (cross section). B – Solitary and radial multiples pores (transverse section in *Calycophyllum spruceanum*). C – Radial section in *C. spruceanum* f. *brasiliensis*, with procumbent square and upright cells. D –Heterogeneous rays of *C. spruceanum* (radial section). E – Rays mainly tetraseriate in *C. spruceanum* f. *brasiliensis* (tangential section). F – Ray with 1 to 5 (or more) cells wide in *C. spruceanum* (tangential section).

Figura 2 – *A – Poros muito numerosos em* ***Calycophyllum spruceanum*** *f.* ***brasiliensis****, com frequentes contatos com células de raio (seção transversal). B – Poros solitários e em múltiplos radiais (seção longitudinal transversal em* ***Calycophyllum spruceanum****). C – Seção longitudinal radial de* ***C. spruceanum*** *f.* ***brasiliensis****, com células radiais procumbentes, quadradas e eretas. D –* ***C. spruceanum*** *com raio heterogêneo, em seção longitudinal radial. E – Raios predominantemente tetrasseriados em* ***C. spruceanum*** *f.* ***brasiliensis*** *(seção longitudinal tangencial). F – Raios com 1 a 5 (ou mais) células de largura, em* ***C. spruceanum*** *(seção longitudinal tangencial).*

Axial parenchyma: absent.

Rays: plenty of (11 ± 2 (8-14) rays/mm), representing 24% of the wood volume; heterogeneous (Figure 2D) and of two different sizes, they gather procumbent, square and upright cells. Frequent fused rays; absent aggregated rays. The uniseriate rays are scarce (12%), with square cells in the central body (1-8 bands), and 1 band of upright cells in the margins; 279 ± 110 (110-620) μm of height, with 4 ± 2 (1-5) cells and 14 ± 4 (5-25) μm in width. The multiseriates one, mostly triseriate and tetraseriate (43 and 38%, respectively), less commonly biseriate (4%) and scarce rays (3%) with 5 or more cells wide (Figure 2F); Compounds of procumbent cells in the central part (5-52) and 1-5 marginal bands of upright and square cells; 554 ± 161 (200-1230) μm of height, with 22 ± 8 (7-54) cells and 39 ± 15 (13-85) μm in width. Perforated ray cells, frequently among the square and upright. Absent surrounding, crystal and tile cells.

Fibres: libriform, representing approximately 47% of the wood volume. Septate fibers with simple pits, and opening in vertical slit, restricted to radial faces of the wall. Medium length fibers (1441 ± 366 (720-2400) μm) and thin to thick walls (7 ± 2 (3-21) μm). Absent spiral thickenings, gelatinous fibers and fiber tracheids,

Other characters: crystals in the form of crystalline sand and deposits present in the ray cells. Absent exchange rate variations, latex and tanninipherous tubes, intercellular canals, oil cells, mineral inclusions, mucilaginous cells and stratifications. Pith flecks observed in the material examined.

3.2 Comparative analysis of the wood

In the dendrogram of Figure 3, the level of distance that the species move away or approach 1.0 can be directly read in the left column, in dissimilarity. The higher the index, the lower the proximity of the species. The cophenetic correlation coefficient value of 0.9 indicates, according to Valentin (2000), that the dendrogram is relevant in the analysis.

4. DISCUSSION

A general analysis of the anatomical characteristics of the four dichotomies of *Calycophyllum* allows grouping them in wood structure "Type II" of Rubiaceae, according to Koek-Noorman (1977) and Jansen et al. (2002), due to the presence of pores, predominantly in radial multiples; absent axial parenchyma; large rays (2-4 cells or more), with a few layers of square and upright cells at the margin and septate fibers with simple pits.

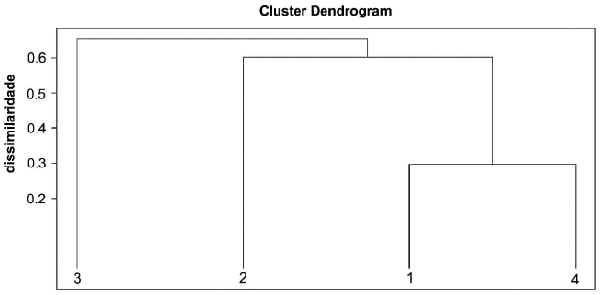

Figure 3 – Dendrogram of investigated species, using the Jaccard distance and UPGMA method. 1 – *Calycophyllum spruceanum* f. *brasiliensis*; 2 – *Calycophyllum candidissimum*; 3 – *Calycophyllum multiflorum*; 4 – *Calycophyllum spruceanum*. r = 0,9.

Figura 3 *– Dendrograma das espécies investigadas, utilizando a distância de Jaccard e método UPGMA. 1 –* ***Calycophyllum spruceanum*** *f.* ***brasiliensis****; 2 –* ***Calycophyllum candidissimum****; 3 –* ***Calycophyllum multiflorum****; 4 –* ***Calycophyllum spruceanum****. r = 0,9.*

As for delimitations in the subfamily level, the genus does not meet the standards cited by Koek-Noorman (1972) and Jansen et al. (1997, 1999) to Ixoroideae: solitary vessels, less commonly in small radial multiples; fiber tracheids and diffuse axial parenchyma, diffuse-in-aggregates axial parenchyma or axial parenchyma in short marginal lines. The *Calycophyllum* genus, according to the anatomy of wood, seems to be better positioned in the Condamineeae tribe, since most of their representatives gather the characteristics previously referred to as "Type II", according to Koek-Noorman and Hogeweg (1974). However, there is a disagreement as to the uniformity of wood, since the same authors attribute heterogeneous wood for the Condamineeae tribe, differing to the results observed in this study and referred to by Record and Hess (1949) for this aspect for all the Rubiaceae, as well as to the *Calycophyllum* genus, which has essentially homogeneous wood.

Although very similar, the logs show sufficient anatomical characteristics to distinguish the species,

especially the ones that follow: volume occupied by different tissues in the wood; diameter, abundance and pore clustering; length of vascular elements; presence of content in vessels; width and height of the rays, as well as the central body and margins and length of fibers.

During the clustering of the four investigated dichotomies, the lowest dissimilarity (0.3) was found between *Calycophyllum spruceanum* and *Calycophyllum spruceanum* f. *brasiliensis*. These results confirm the convenience of K. Schum and Müll. Arg. (CHIQUIERI et al., 2004) proposition, authors who considered the first as a variety of the second taxon. The anatomical characteristics that reinforce this understanding are: pores with average tangential diameter (50-100 µm), in large number and in radial multiples; heterogeneous rays, infrequent and predominantly triseriate and tetraseriate.

5. CONCLUSIONS

Based on microscopic characteristics of wood and agglomerative hierarchical clustering analysis of species, it follows that:

· The genus *Calycophyllum* is homogeneous when it comes to the anatomical structure of the wood, considering the number of species described to date;

· *Calycophyllum spruceanum* and *Calycophyllum spruceanum* f. *brasiliensis* are more similar to each other (low dissimilarity) than to the other two investigated dichotomies;

· The anatomy of secondary xylem of the four species studied shown to be highly similar (low dissimilarity);

· Despite the occurrence of many common structural features, the four investigated species have sufficient anatomical characteristics to differentiate them one from the other.

6. REFERENCES

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