Original article

Sucupira-branca (*Pterodon* - Fabaceae): does wood anatomy support the distinction among species and hybrid?

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ABSTRACT

Pterodon (Fabaceae) species known as "sucupira-branca" are traditionally used in Brazilian medicine. This South American monophyletic genus has species delimitation problems. To separate the recognized *Pterodon* species we described the wood anatomy of *P. abruptus*, *P. emarginatus*, *P. pubescens*, hybrid (between *P. emarginatus* and *P. pubescens*) and *P. apparicioi*, the latter two for the first time. Wood anatomical studies were undertaken, and qualitative and quantitative features statistically tested. Qualitatively the species are very similar, but in most quantitative features, differences were observed, especially axial parenchyma quantity and type. *Pterodon abruptus* was easily separated by high density narrow vessels allied to a low area of axial parenchyma but with confluent almost forming bands. Hybrid was more similar to *P. emarginatus* and had a significantly greater axial parenchyma area than other *Pterodon* species. PCA analysis separates only *P. abruptus*. Cluster analysis using qualitative and quantitative data were able to separate *P. abruptus* and *P. apparicioi* from the other species. Vessels, ray and axial parenchyma features enable to clarify the distinction in identification key.

Keywords: Caatinga, Cerrado, hybrid, *Pterodon*, secondary xylem, sucupira-branca, taxonomy, wood identification

Introduction

Pterodon is a genus of arboreal species distributed in South America in Brazil and Bolivia (Pinto *et al*. 2014; Tropicos.org 2021). The natural high resistance of *Pterodon* wood to xylophagous organisms makes this genus a good candidate for being used in civil construction and fences (Mainieri & Chimelo 1989). The phytoextracts are employed by traditional communities as anti-inflammatory (Hansen *et al*. 2010) and the ethanolic fruit extracts used as larvicides against *Aedes aegypti* (De Omena *et al*. 2007).

Pterodon is monophyletic, well-supported, and related to *Dipteryx*, *Taralea* and *Monopteryx* (Cardoso *et al*. 2015; Leite *et al*. 2015; Silva *et al*. 2018). However, there is no phylogeny for *Pterodon* genus as a whole; and according to Cardoso *et al*. (2015) *P. emarginatus* and *P. pubescens* are closer phylogenetically to each other than to *P. abruptus*.

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The circumscription of *Pterodon* species differs and varies among published databases and the exact number of species remains uncertain. For example, in ILDIS (2021) *P. apparicioi*, *P. pubescens* and *P. polygalaeflorus* are considered synonyms of *P. emarginatus*. In Tropicos.org (2021), *P. polygalaeflorus* and *P. apparicioi* are legitimate species, but *P. pubescens* is considered a synonym of *P. emarginatus*. In Flora do Brasil 2020 (2021), *P. polygalaeflorus* is considered a synonym of *P. emarginatus*, and *P. pubescens* and *P. apparicioi* are accepted. *Pterodon abruptus* is accepted in all databases. Five species are assigned to *Pterodon*: *P. abruptus*, *P. apparicioi*, *P. emarginatus, P. pubescens* and *P. polygaliflorus*. (*P. polygalaeflorus* variation in writing), according to IPNI (2021). In addition, *P. macrophylla* was cited in IPNI (2021), but should be considered a *nomen nudum* as it was only cited in Reisen in British-Guiana in den Jahren 1840-1844, volume 3 (page 1103), without description or type assigned and not reported again.

Part of this meandering history regarding the number of species in *Pterodon* dates back to Legumes of Bahia (Lewis 1987), in which the author indicated that *P. polygaliflorus* and *P. pubescens* are synonyms of *P. emarginatus*. *Pterodon emarginatus* as proposed by Lewis is a dimorphic species with individuals with pink flowers, pubescent leaves and retuse folioles apex as well as plants with purple flowers, glabrate leaves with emarginate to truncate folioles apex. These two phenotypes do not occur side by side, the populations of each form are parapatric (Rocha 2006). The same author employing RAPD (random polymorphic DNA) and morphological data recognized two distinct taxa. Comparison with phototypes and literature of the tree taxa (*P. emarginatus*, *P. polygaliflorus, P. pubescens*) allowed to conclude that *P. polygaliflorus* is a synonym of *P. emarginatus* (with purple flowers) and *P. pubescens* (with pink flowers) is a separate valid species. Rocha (2006), based on RAPD, also recognized some hybrids between *P. emarginatus* and *P. pubescens,* forming a narrow range in the Federal District, which suggests that the diverging time of these two species should be recent. The flower color of hybrid individuals has pink sepals and purple petals.

Baretta-Kuipers (1981) found that the different patterns of parenchyma and ray structure were the most significant anatomical features to identify Fabaceae genera. Gasson (1999), when comparing the genera within Dipterygeae, found that *Dipteryx* and *Pterodon* are anatomically more similar to each other than they are to *Taralea*. Despite having remarkably similar wood anatomy, *Dipteryx* and *Pterodon* differed due to the presence of irregularly or non-storied rays and axial parenchyma, as well as differences in intervessel pitting diameter.

Secondary xylem studies have proved useful in the taxonomy and highly significant in the elucidation of plant phylogeny (Baretta-Kuipers 1981; Gasson 1999; Nisgoski *et al*. 1998), by providing evidence for assigning a definite position to taxa of uncertain affinity (Sharma 2009).

According to Dickison (2000), in no other plant tissue the trends of structural evolution are so clearly defined, since the wood structure tends to be more conservative in many features than the external morphology (Baretta-Kuipers 1981). However, it is noteworthy that the quantitative characteristics will show more dissimilarities than the qualitative ones (see Loureiro *et al*. 1984; Nisgoski *et al*. 1998; Oliveira *et al*. 2001), which varies in amplitude within each species (Sonsin *et al*. 2012).

The aim of this study was to characterize the wood anatomy of *Pterodon* species, including the hybrid, to verify which anatomical features vary among species, and mapping for the first time the most important wood anatomical characters using multivariate data analysis, providing informative characters to help circumscribe the *Pterodon* species.

Material and Methods

Wood samples were collected at diameter at breast high (DBH) = 1,30 m with a hand-held drill (BT45-Still®), adapted with a hollow drill bit (non-destructive sampling method), in all *Pterodon* Vogel species (Tab. 1). All were deposited in the UBw wood collection accompanied by vouchers deposited in UB herbarium (acronym according to Thiers 2018). *Pterodon* species were always collected in flower, except for *P. abruptus*. The flowering period is especially important to identify hybrid trees. The plants were identified by DMS Rocha. *Pterodon abruptus* was collected in the Caatinga because it is endemic from this biome (Shimizu & Semir 2016).

Climate data is classified according to Köppen and Geiger and is given for each location: *P. apparicioi* collection site climate classification is Cwa, has an annual mean temperature (AMT) of 20.7 ºC, annual mean precipitation (AMP) of 1149 mm, with a dry season of five months with precipitation below 45 mm; *P. abruptus* climate classification is BSh, has an AMT of 26 ºC, AMP of 747 mm, with precipitation below 25 mm for five months; *P. pubescens* climate classification is AW, has an AMT of 21.9 ºC, AMP of 1443 mm, with precipitation below 45 mm for five months; *P. emarginatus* and hybrid climate classification is AW, has an AMT of 22.3 ºC, AMP of 1223 mm, with precipitation below 40 mm for five months. Climate data were obtained from the website Climate-Data.org (https://pt.climate-data.org, weather data between 1999 to 2019 and refreshed from time to time).

Wood samples were stored in 70 % alcohol with 50 ml of glycerin to soften the wood. Additionally, samples were placed in a pressure cooker for about two hours (separate periods of 30 minutes), since boiling them was not enough. We cut the wood into cubes (*ca*. 2 cm²) and microtome sectioned into transverse (TRS), tangential (TLS) and radial longitudinal (RLS) sections, with 15 to 20 µm, and mounted according to Kraus & Arduim (1997). Histological slides were permanently embedded in synthetic resin (Entellan®).

Table 1. *Pterodon* genera collection data. CN = collector number; Herb = Herbarium; H = height; DBH = Breast height diameter; Alt = altitude; Ce *s.s.*= cerrado *sensu stricto*; Ca = caatinga; MG = Minas Gerais; DF = Distrito Federal.

Species	Collection site / Habitat	Geographical coordinates	CN Wood / Herb	Collectors	H (m)	DBH (cm)	Alt (m)
P. apparicioi Pedersoli	Jaboticatubas (MG) / serra do cipó Ce *s.s.*	19°21'47.70''S 43°37'12.30''W	249/2389	Sonsin-Oliveira, J & Fagg, CW	12	39.2	884.9
		19°21'45.28''S 43°37'11.99''W	250/2390		8	33.4	880.5
		19°21'43.34''S 43°37'10.65''W	251/2391		12	34.7	897.6
		19°21'06.83''S 43°37'05.79''W	252/2393		14	30.2	803.3
		19°21'06.06''S 43°37'06.55''W	253/2394		15	40.1	791.8
P. abruptus Benth.	Jaíba (MG) Ca	14°56'06.01''S 43°54'01.59''W	271/3233	Sonsin-Oliveira, J & Oliveira RC	4.5	14.3	447.6
		14°56'06.94''S 43°54'01.95''W	272/3233		6	13.1	523
		14°56'07.26''S 43°54'01.61''W	273/3233		4	13.7	422.5
P. pubescens Benth	Fazenda Água Limpa (DF) Ce *s.s.*	15°56'34.02''S 47°54'35.29''W	164/3075	Sonsin-Oliveira, J & Rocha, DMS	4.5	14.6	1078
		15°56'32''S 47°54'35.22''W	165/3074		5.5	24.2	1078
		15°56'34.02''S 47°54'35''.29''W	166/3073		6.5	25.1	1078
		15°56'32''S 47°54'35.22''W	167/3072		7	19.4	1078
P. emarginatus Vogel	Planaltina (DF) Ce *s.s.*	15°36'03.23''S 47°39'32.61''W	174/3076	Sonsin-Oliveira, J; Oliveira, RC & Rocha, DMS	6	31.8	978
		15°35'59.07''S 47°39'15.90''W	177/3079		9.5	58.3	976
		15°35'53.91''S 47°39'23.89''W	178/3080		4	25.5	977
		15°35'42.47''S 47°39'46.03''W	179/3081		5	18.3	976
hybrid	Planaltina (DF) Ce *s.s.*	15°39'45.8''S 47°41'30.8''W	170/3083	Sonsin-Oliveira, J; Rocha, DMS; Santiago, VHD; Oliveira, RC	9	43.6	1035
		15°40'58.10''S 47°42'24.60''W	171/3084		8	37.9	1009
		15°40'58.70''S 47°42'43.10''W	172/3085		8	34.4	1063
		15°40'58.70''S 47°42'43.10''W	173/3086		4,8	41.7	1063

Cells were macerated according to Franklin's method (1945, modified by Kraus & Arduin 1997) and stained with alcoholic safranin and alcian blue dye 50 % (Sass 1958). Semi-permanent slides were mounted in glycerin diluted in water (1:1).

The wood qualitative and quantitative anatomical descriptions were based on IAWA Committee (1989), and COPANT (1974) was used only for ray width category (microscopy). All measurements were made with Image-Pro Plus ® (6.0). Thirty measurements per feature per individual were made, including height and ray width, vessel and axial parenchyma area. This last one was measured in 0.25 mm², by dividing 1mm² into four; using a Surface Microsoft notebook with a digital pen we were able to draw the area. Olympus light microscope was used, and the images were recorded with a Leica photomicroscope associated with a microcomputer with LAS EZ image capture system.

Statistical analyzes were performed with PAST v3 and Microsoft Excel software. The mean, standard deviation and coefficient of variation (CV) were calculated. A normality test was done, and the Log values of the anatomical characteristics were used to reduce the large differences in values of each measured structure. One-way ANOVA (several samples) and Tukey tests were performed on each anatomical feature to test if anatomical variables were significantly different among the *Pterodon* species.

We performed an UPGMA cluster analysis based on a binary matrix representing the presence (1) or absence (0) of a character state and employing Jaccard similarity coefficient for the qualitative and quantitative anatomical features, the latter transformed into classes given by IAWA variables. The characters employed in this matrix were: growth rings; intervessel pits shape and size; axial parenchyma strand; ray frequency, width and storied; presence of prismatic crystals; vessel diameter and density; fiber length. The axial parenchyma area was not included on cluster analysis because it did not have IAWA (1989) variable available, and we were not able to transform it into classes, since we do not have enough data to use as a parameter.

Principal Component Analysis (PCA) with correlation matrix was employed to quantitative characters in order to verify the differences among species, using average values of the IAWA List and Gasson *et al*. (2010) parameters (maximum and minimum vessel lumen diameter (μm), maximum and minimum ray height (cells)), in addition to maximum and minimum axial parenchyma area. All data were log transformed prior to PCA to standardize the dataset.

A dichotomous identification key was made to help separate taxa.

Results

In macroscopic analysis we found that all woods were hard to cut, with fine texture, irregular to straight grain, with no odor or distinct luster. Heartwood color varied from yellow brown to dark brown. *Pterodon abruptus* may be easily distinguished in macroscopic analyzes by highest vessel density (40-100/mm²), smaller vessels (< 0.05 mm) and predominance of the unilateral long confluent parenchyma forming bands. All other species had clearly wood characteristics of *Pterodon*, and could not be distinguished with macroscopy. Therefore, we

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made a description for the genus as follows (Figs. 1-3, S1). A description of the *P. apparicioi* wood anatomy and hybrid are presented here for the first time (Text S2).

Growth rings (Fig. 1A-F): well demarcated by marginal parenchyma. Vessels (Fig. 1A-F): diffuse-porous, solitary and multiple vessels; simple perforation plates (Fig. 3A); intervessel pits alternate, circular, vestured and sometimes polygonal (Fig. 3C); vessel-ray pitting similar to intervessel pits in size and shape throughout the ray cell (Fig. 3D); deposits occasionally present. Fibers: with simple to minutely bordered pits, very thick-walled (Fig. 1A-F, 3B). Axial parenchyma (Fig. 1A-F): short to long confluent (oblique), lozenge aliform, vasicentric, unilateral and in marginal lines; 2-5 cells per parenchyma strand (Fig. 2A-E);

also, few sclerified axial parenchyma cells were observed (Fig. 3B). Ray (Fig. 2A-F): predominantly uniseriate, and 1-2 cells wide (Fig. 2A-E); all ray cells procumbent (Fig. 2F); perforated ray cells in some individuals of *P. emarginatus*, *P. pubescens*, *P. abruptus* and hybrid (Fig. 3F). Storied structure (Fig. 2A-E): axial parenchyma (not observed in all individuals) and small rays storied (Fig. 2A-F). Mineral inclusions: prismatic crystals in axial parenchyma of *P. emarginatus* (Fig. 3E), hybrid and *P. abruptus*.

Some observed differences were related to environmental influences, such as growth ring variation (see Tab. 2). In addition, differences in axial parenchyma abundance, which was the most discriminant feature, and in ray storied
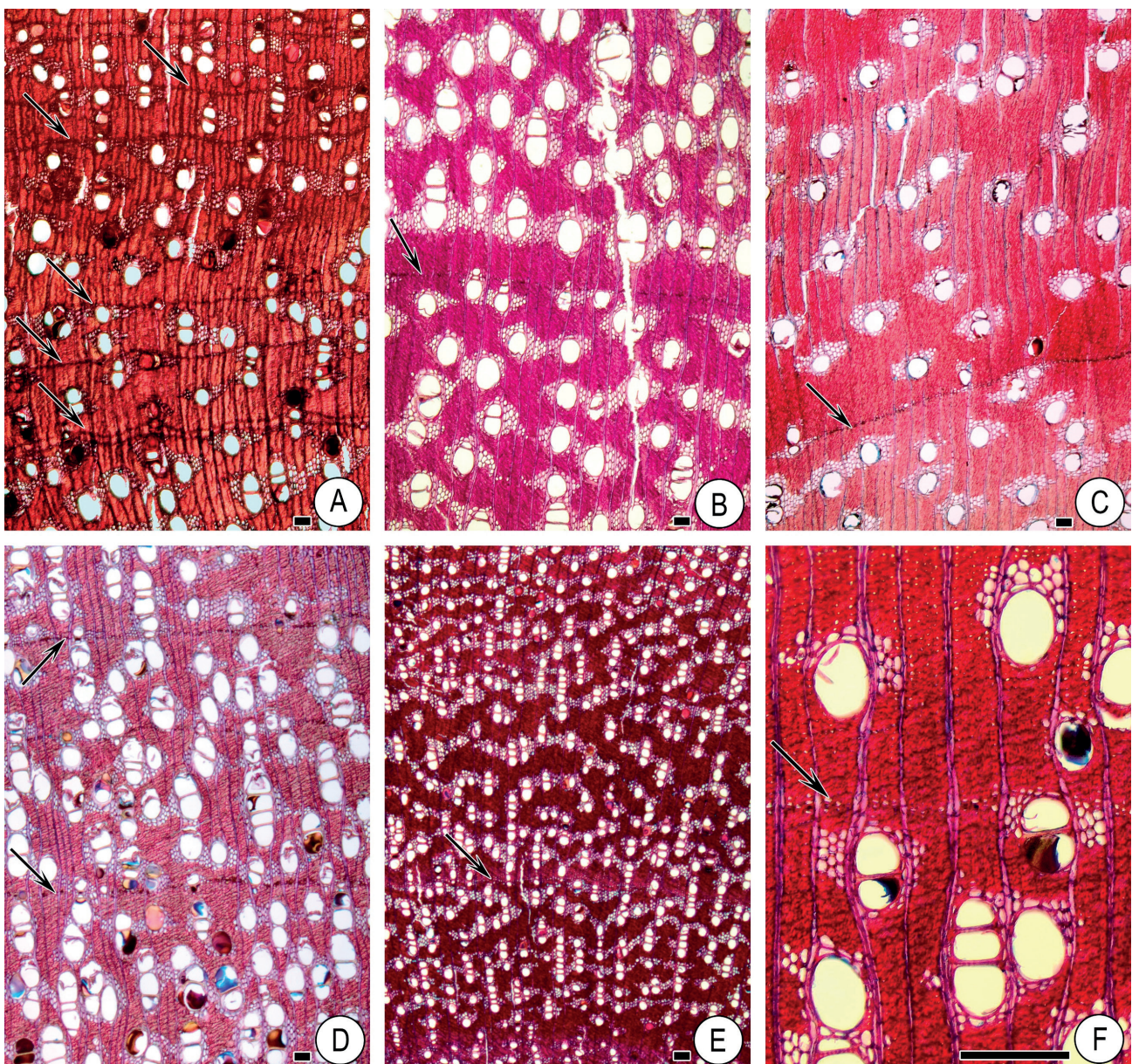

Figure 1. Transverse section of the wood of *Pterodon* species. Arrows point to the growth ring. (**A**) *P. pubescens*. (**B**) Hybrid. (**C**) *P. emarginatus*. (**D**) *P. apparicioi*. (**E**) *P. abruptus*. (**F**) Detail of the growth ring in *P. apparicioi*. Bars: 100 μm.
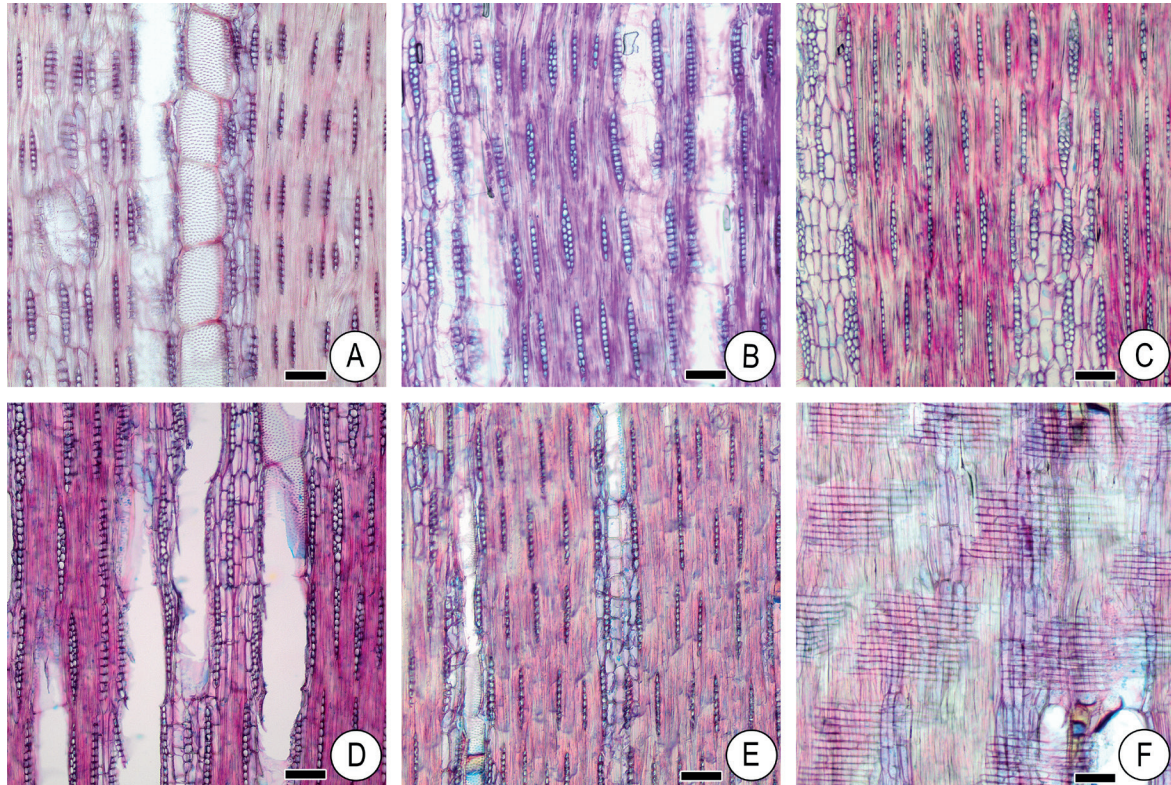
Figure 2. Longitudinal tangential section of the wood of *Pterodon* species. (**A**) *P. pubescens*. (**B**) Hybrid. (**C**) *P. emarginatus*. (**D**) *P. apparicioi*. (**E**) *P. abruptus*. (**F**) Longitudinal radial section in *P. apparicioi*. Bars: 100 μm.
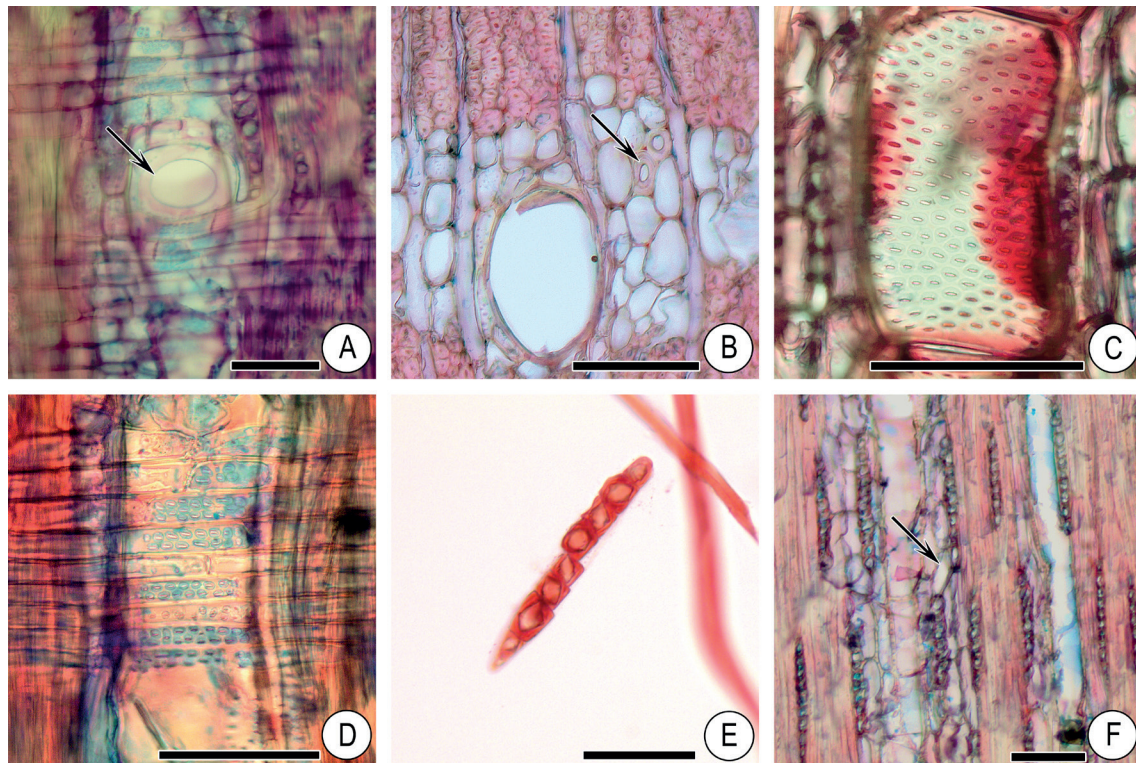

Figure 3. Wood of *Pterodon* species. (**A**) Radial section, *P. emarginatus*, simple perforation plate (arrow). (**B**) Transverse section, *P. apparicioi*, sclerified axial parenchyma (arrow). (**C**) Tangential section, hybrid, intervessel pits alternate, circular. (**D**) Radial section, *P. abruptus*, vessel-ray pitting. (**E**) Macerate, *P. emarginatus*, prismatic crystals in chambered axial parenchyma cells. (**F**) Tangential section, *P. abruptus*, perforated ray cells (arrow). Bars: 100 μm.

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Table 2. Comparative table of the main anatomical differences of *Pterodon* species. IVPA = Intervessel pits alternate; VDiSm = vessel diameter small (< 50µm); VDiMe = vessel diameter medium (50-100µm); VDiL = vessel diameter large (<100-200µm); VDeF = vessel density few (5-20/mm²); VDeMe = vessel density medium (20-40/mm²); VDeH = vessel density high (40-100/mm²); RWiET = width extremely thin (<15µm); RWiVT = width very thin (15-30µm); RWiT= width thin (31-50µm); RDeF = ray density few (4-12/mm); RDeN = ray density numerous (>12/mm); 1 = presence; 0 = absence; * = oblique; - = few places; + = predominantly; ba = forming bands.

	Anatomical features	hybrid	P. emarginatus	P. pubescens	P. apparicioi	P. abruptus
Growth rings	Demarcated by marginal lines of axial parenchyma	1	1	1	1	1
	Demarcated by fiber zones	-	0	-	-	-
Vessels	IVPA, circular	1	1	1	1	1
	IVPA, polygonal	+	1	+	+	+
	% VDiSm	2	1	0	0	77
	% VDiMe	62	42.5	41	48	23
	% VDiL	36	56.5	59	52	0
	% VDeF	91	82.5	94	28.5	1
	% VDeMe	7.5	17.5	6	70.5	97
	% VDeH	1.5	0	0	1	2
	Deposits	-	-	-	1	1
Axial parenchyma	Vasicentric	1	1	1	1	1
	Lozenge aliform	1	1	1	1	1
	Short confluent	1	1	+	1	1
	Long confluent	1*	1*	1*-	1*	1*+ba
	Unilateral	1	1	+	-	+
	Cells per strand	2-4 (+up to 3)	2-5 (up to 5)	3-4 (up to 5)	2-3 (up to 5)	2 (- up to 4)
Rays	Predominantly uniseriate	-	1	1	-	1
	1-2 cells wide	1	-	-	1	0
	Height (cells)	8.6 (up to 22)	11.2 (up to 25)	9.7 (up to 42)	10.9 (up to 36)	8.5 (up to 20)
	% RWiET	59	67	79	52.5	89
	% RWiVT	41	30	19	46.5	11
	% RWiT	0	3	2	1	0
	% RDeF	42	54	68	79	76
	% RDeN	58	46	32	21	24
	Perforated ray cells	1	1	1	0	-
Storied	Ray regular storied	1	1	1	1	1
	Ray irregular storied	-	-	-	-	0
	Prismatic crystals	1	1	0	0	1

structure were observed (Tab. 2). The variances are described below:

All growth rings were well to poorly demarcated by marginal parenchyma, and better seen in macroscopy. However, slight differences may occur, such as: marginal lines varied from one up to three cells wide; also, they were irregularly spaced when comparing the genus and within the species (Fig. 1A-F, S1). Only two samples of the hybrid (Fig. 1B, S1) and *P. pubescens*, and one of *P. apparicioi* and *P. abruptus* had fiber zones.

The axial parenchyma predominant type was short to long confluent (oblique), lozenge aliform, vasicentric, unilateral and in marginal lines, but some differences were observed (Fig. S1), such as: in *P. pubescens* (Fig. 1A) there was a predominance of short confluent (mostly up to three vessels) and lozenge unilateral; hybrids had long and short confluent (oblique), with the cells being conspicuously larger when compared to the other species; in *P. abruptus* (Fig. 1E) the long confluent (oblique) forming bands exclusively unilateral was observed; in addition, we have noted a variation in the amount of axial parenchyma becoming only unilateral and lozenge and few short confluent near the growth ring (Fig. 1B).

Except for *P. abruptus*, all other *Pterodon* species and hybrids (Fig. 2B) had some individuals with irregular storied rays.

Major differences of qualitative and few quantitative analyzes were added according to IAWA and COPANT (for ray width) category in Table 2, for better comparison. Yet, some anatomical features which were associated remained in the Table.

The quantitative data are shown in Table 3, with some overlap among them. Of the 14 anatomical features analyzed, the hybrid was similar to *P. emarginatus* in seven, to *P. apparicioi* in five, to *P. pubescens* in five, and to *P. abruptus* in eight (Tab. 3). In addition, the hybrid shared three anatomical characteristics (statistically similar) with both *P. emarginatus* and *P. pubescens* (intervessel pit diameter and fiber diameter and thickness), while was similar to *P. emarginatus* and *P. apparicioi* in four anatomical features (vessel ray pit diameter, fiber diameter and wall thickness and ray width) (Tab. 3).

Table 3. Quantitative data of *Pterodon* species. A=average; SD= standard deviation; CV= coefficient of variation (%). VL = Vessel element length; VDi = Tangential diameter of vessel lumina; VDe = vessel density/mm²; IVPD = intervessel pits diameter; VRPD = vessel-ray pits diameter; VeA= vessel area; SV = solitary vessel; FL = fiber length; FDi = fiber diameter; FLu = fiber lumen diameter; FWT = fiber wall thickness; R/mm = ray/mm; RH = ray height; RW = ray width; PaA = axial parenchyma area. Averages followed by the same letters in line do not differ statistically in ANOVA and Tukey test (< 0.05).

Anatomical features		hybrid Ce s.s.	*P. emarginatus* Ce s.s.	*P. pubescens* Ce s.s.	*P. apparicioi* Ce s.s.	*P. abruptus* Ca
VL (µm)	A	207[e]	240[c]	274[a]	260[b]	222[d]
	SD	29.9	26.6	34.2	31.1	32.6
	CV	14.5	11.1	12.5	12	14.7
VDi (µm)	A	94.8[b]	108[a]	109[a]	102[a]	46.8[c]
	SD	24.3	23.4	24.3	19.5	5.9
	CV	25.6	21.6	22.3	19.1	12.5
VDe /mm²	A	15.4[d]	17.7[c]	14.2[d]	23.7[b]	62[a]
	SD	5.5	3.42	4	5.7	13.8
	CV	35.8	19.4	28.2	23.9	22.3
IVPD (µm)	A	9.6[a]	10[a]	9.8[a]	8.4[b]	7.2[c]
	SD	1.2	1.6	2.1	1	0.9
	CV	12	15.7	21	11.6	12.3
VRPD (µm)	A	8[a]	7.9[a]	7[b]	7.6[a]	7.5[a]
	SD	1.2	1.9	2.2	1.2	1.1
	CV	15.4	23.6	31.3	16	15.3
VeA (µm²)	A	9576[c]	12155[a]	11613[b]	9279[c]	2104[d]
	SD	4949	4742	5564	3794	539
	CV	51.7	39.0	47.9	40.9	25.6
SV / mm²	A	5.9[c]	6.1[c]	7.1[b]	8.1[b]	23.7[a]
	SD	2.6	2.2	2.6	2.2	6.4
	CV	44.5	35.6	44.5	30.6	27.1
SV %	A	61.9[bc]	56.1[cd]	71.7[a]	55.7[d]	62.3[b]
	SD	17.3	14.6	15.6	17.1	11.6
	CV	28.0	26.1	21.8	30.7	18.6
FL (µm)	A	904[b]	1016[a]	1038[a]	988[a]	847[c]
	SD	108	113	204	149	97
	CV	11.9	11.1	19.6	15.1	11.5
FDi (µm)	A	13.9[a]	14.8[a]	14.6[a]	13.8[a]	12.8[b]
	SD	2.7	2.9	2.5	2.5	2.9
	CV	19.6	20.0	16.9	18.2	22.8
FLu (µm)	A	2.5[c]	2.8[ab]	2.6[bc]	3.1[a]	2.6[bc]
	SD	0.76	0.78	0.71	1.2	0.7
	CV	30.5	27.9	27.7	37.8	26.1
FWT (µm)	A	5.7[ab]	6[a]	6[a]	5.4[bc]	5.1[c]
	SD	1.2	1.3	1.1	1.1	1.3
	CV	20.9	22.4	18.8	20	24.8
R / mm	A	13.3[a]	12.4[ab]	11.6[b]	11.2[c]	11.5[bc]
	SD	3.1	2.5	1.6	1.8	1.5
	CV	23.3	19.9	14	15.9	13.1
RH (µm)	A	153[c]	195[ab]	179[b]	206[a]	150[c]
	SD	44.6	65.9	86.5	69.2	35.4
	CV	29.2	33.8	48.2	33.6	23.6
RW (µm)	A	15.2[a]	14.1[ab]	13.5[b]	15.2[a]	11.3[b]
	SD	5.8	7.2	6	5.3	2.9
	CV	38	50.8	44.8	35.1	25.3
PaA / 0.25 mm²	A	5403[a]	4285[b]	3727[bc]	3163[c]	2382[d]
	SD	2164	3163	1948	1455	912
	CV	39	73	52	45	38

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When comparing the species, *P. pubescens* shared ten similar quantitative data with *P. emarginatus*, five with *P. apparicioi*, and only three with *P. abruptus*. *Pterodon emarginatus* had eight anatomical features similar to *P. apparicioi* and only four with *P. abruptus* (Tab. 3). In fact, the most distinct species was *P. abruptus*, collected in Caatinga biome, because it had the highest vessel density, with smaller vessels than all other species; smaller ray-vessel pit diameter, axial parenchyma area, fiber length, ray height and width. It is worth noting that the hybrid had a significantly higher area of axial parenchyma.

PCA, made only with quantitative anatomical features, showed 64 % of the total variance explained by component 1 and 2 (Fig. 4; Tab. S3). Component 1 explained 41 % of the variance, separated *P. abruptus* from the other species and was influenced mostly by vessel density, area, diameter. Component 2 explained 23 % but is not sufficient to separate the remaining taxa. *Pterodon abruptus* was the only species that remained totally separated from the other *Pterodon* species.
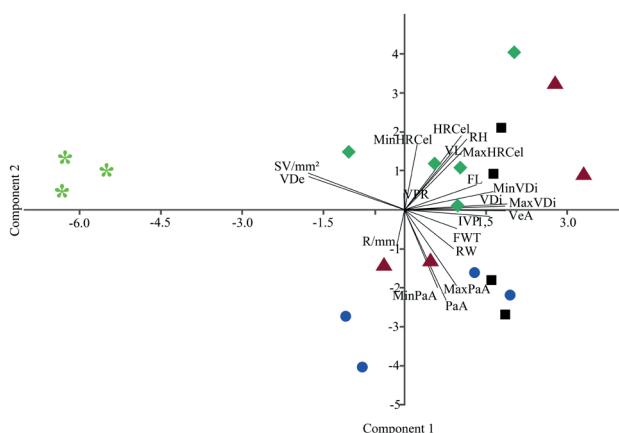

Figure 5. Cluster analysis – UPGMA (Jaccard). Pab = *P. abruptus*; Pap = *P. apparicioi*; Pe = *P. emarginatus*; Pp = *P. pubescens*; H = hybrid.

An identification key using wood anatomy characteristics permitted the separation of *P. abruptus* and *P. apparicioi* (Tab. 4). However, wood characteristics were not enough to distinguish *P. pubescens*, *P. emarginatus* and hybrid between these species. Despite that, we could observe some wood anatomical details that may help to distinguish *P. pubescens*, *P. emarginatus* and hybrid, as follows: *P. pubescens* have over 70 % of solitary vessels/mm², while the other two have below 62 %; large vessels (100-200 μm) were present in 59 % of *P. pubescens* and 57 % in *P. emarginatus*, while the hybrid had 62 % of vessels of medium diameter (51-100 μm); 68% of rays density predominantly few (4-12/mm) in *P. pubescens* while *P. emarginatus* and hybrid had below 48 %; vessel area average was statistically different in the three species (Tab. 3); axial parenchyma area average of hybrid was significant higher than the other two (Tab. 3); prismatic crystals in chambered axial parenchyma cells were only observed in *P. emarginatus* and the hybrid.

Discussion

In general, the qualitative anatomical description of secondary xylem made here for the genus *Pterodon* is similar to the bibliography consulted (Mainieri *et al*. 1983; Gonçalez *et al*. 1985; Mainieri & Chimelo 1989; Paula & Cardoso 1995; Gasson 1999; InsideWood 2004). For *P. abruptus* there were only available images from three sections in "InsideWood" website (https://insidewood.lib.ncsu.edu/), and a description for the *Pterodon* genus based on this species and *P. pubescens*. When comparing the image from

Figure 4. Principal component analysis. Component 1 explained 41 % of the variance, and component 2 explained 23 %. ● = hybrid; ■ = *P. emarginatus*; ▲ = *P. pubescens*; ♦ = *P. apparicioi*; * = *P. abruptus*. The percentage of variability explained by each component and the contributions of single characters are indicated in Table S3. VL = Vessel element length (μm); VDi = Tangential diameter of vessel lumina (μm); MaxVDi = Maximum tangential diameter of vessel lumina (μm); MinVDi = Minimum tangential diameter of vessel lumina (μm); VDe = Vessel density/mm²; IVP = Intervessel pits diameter (μm); RVP = Vessel-ray pits diameter (μm); VeA = Vessels area (μm²); SV/mm² = Solitary vessels (mm²); FL = Fiber length (μm); FWT = Fiber wall thickness (μm); R/mm = Ray/mm; RW = Ray width (μm); RH = Ray height (μm); RHCel = Ray height (nº cell); MaxRHCel = Maximum ray height (nº cell); MinRHCel = Minimum ray height (nº cell); PaA = Axial parenchyma area (in 0.25 mm²); MaxPaA = Maximum axial parenchyma area (in 0.25 mm²); MinPaA = Minimum axial parenchyma area (in 0.25 mm²).

Cluster analyses (Fig. 5) clearly separated *P. abruptus*. *Pterodon apparicioi* although clustered together with the other species and hybrid, seems to consist of a separated group inside this major one.
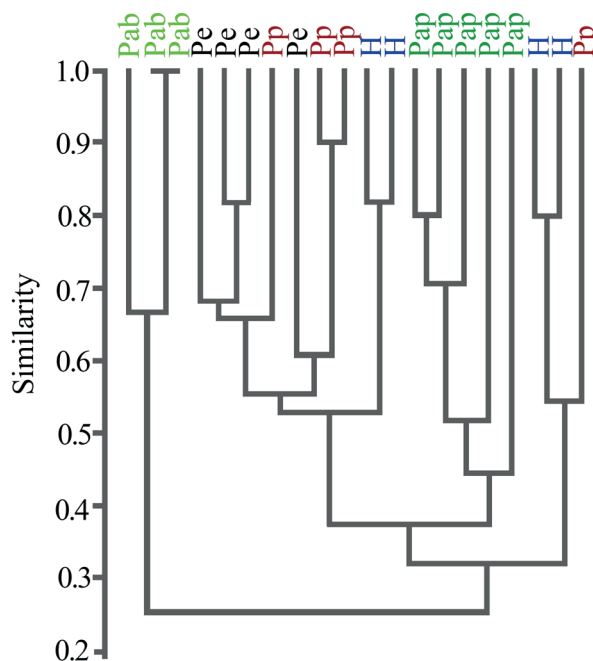
Table 4. Identification dichotomous key.

1a	axial parenchyma long confluent (oblique) forming bands exclusively unilateral; 75 % of small vessels (< 50 m) in radial multiple and 97 % with high density (40-100/mm²); rays exclusively uniseriate	*Pterodon abruptus*
1b	axial parenchyma short to long confluent (oblique), occasionally unilateral; 98 % of vessels were medium to large (50-200 μm); 98 % of vessels had low density (5-40 v/mm²); rays uniseriate and multiseriate	2
2a	more than 70 % of the vessels with medium density per mm² (20-40 v/mm²); intervessel pits diameter less than 8.4 μm	*P. apparicioi*
2b	over 80 % of vessels had low density (5-20 v/mm²); intervessel pits diameter larger than 9.6 μm	*P. pubescens, P. emarginatus* and hybrid

InsideWood with *P. abruptus* from our study, the wood anatomy in general is quite similar, differentiating only regarding the quantity of multiple vessels, that was clearly lower than ours, but there were only two imagens from the TRS (transverse section). Despite the observed differences this species was the same as *P. abruptus* collected by us.

In both PCA and cluster analyses, *P. abruptus* was easily separated from the other species either in macroscopy and/or microscopy, because it showed a high density of narrow vessels in multiple radial parenchyma, and the unilateral long confluent axial parenchyma forming bands. Despite the presence of axial parenchyma in long bands found only in this species, *P. abruptus* had the lowest axial parenchyma area when compared with the other taxa. Also, it had the lowest maximum number of ray height cells (up to 20), an important feature indicated by Gasson (1999) for identification.

Please note that quantitative features such as vessels and axial parenchyma abundancy may be influenced by both environmental (Baas & Wheeler 1991; Alves & Angyalossy-Alfonso, 2002) and genetic characteristics (Schweingruber *et al.* 2008). For example, small vessels are related to drier environments (Wheeler & Baas 1993; Carlquist 2001), here reflected in *P. abruptus* collected in the drier caatinga. However, parenchyma type is one of the most relevant anatomical features when identifying a family, genus or species as it is considered a conservative characteristic within a taxon. Nevertheless, the quantitative data of the species vary within the range for each taxon (Sonsin *et al.* 2012); so, those anatomical features were very useful to separate the species.

When analyzing *P. apparicioi* we observed that while using only quantitative anatomical features, even the ones proposed by Gasson *et al.* (2010), in PCA, it remains strongly related to *P. pubescens* and *P. emarginatus*, but not much with the hybrid. However, in cluster analysis, when qualitative data was also used, *P. apparicioi* was distinct from all other taxa (Fig. 5). Probably, if we were able to put the axial parenchyma into classes to do the analyzes, as we did with vessels, fiber and ray features, the hybrid distinction would be more evident.

In InsideWood, *P. pubescens* is considered a synonym of *P. emarginatus* and they are probably genetically similar because where their distributions overlap, they form a zone

of hybridization (Rocha 2006) and are phylogenetically related (Cardoso *et al.* 2015). Therefore, we here compare *P. pubescens* InsideWood descriptions with anatomical data of *P. pubescens*, *P. emarginatus* and the hybrid in our study.

These species and the hybrid are all similar regarding quantitative and qualitative features, except for: presence of growth rings observed in both species studied by us, which are absent in the InsideWood description, however it is possible to observe in the TRS image; the axial parenchyma is two seriate in InsideWood, while the two studied species and the hybrid had a variation from 2 to 5 cells per strand; exclusively uniseriate rays is mentioned in InsideWood, but in both species studied by us they were predominantly uniseriate, with some biseriate rays, however the hybrid was predominantly multiseriate; only for *P. pubescens* and the hybrid the intervessel pit diameter were smaller than <10 μm, while in InsideWood, it is reported to be larger than 10 μm; also we observed irregular storied rays in some individuals of both species and hybrid, and prismatic crystals only in *P. emarginatus* and hybrid, all features not observed in InsideWood website description.

Gasson (1999) described intervessel pitting fine (up to 10 μm), clearly vestured for *Pterodon* genera, based on *P. abruptus* and *P. emarginatus*. Here, only *P. emarginatus*, *P. pubescens* and hybrids had intervessel pit diameters varying from 9.6 and 10 μm. The other two species had smaller pits, about 7.2 μm (*P. abruptus*) and 8.4 μm (*P. apparicioi*). Therefore, for the genus intervessel pit diameter should be of medium size category, 7-10 μm.

Fiber walls were statistically similar in all studied species, similar to Gasson (1999) for *Pterodon* genus. Notwithstanding the significant differences between precipitation rates of species collection sites, the thickness of fibers in this genus could be genetically intrinsic and not variable with climatic conditions (see Schweingruber *et al.* 2008), as seen in other studies for different Fabaceae species (*e.g.* Alves & Angyalossy-Alfonso 2002; Sonsin *et al.* 2012).

According to Gasson (1999), the axial parenchyma was mainly aliform and confluent, strands storied and 2-4-celled. The axial parenchyma type is consistent for the species studies here (predominantly short to long confluent (oblique), lozenge aliform and 2-5 celled), except for *P. abruptus* which had a distinctive axial parenchyma

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(predominantly unilateral long confluent). Different from Gasson (1999), the marginal parenchyma (not always easily observed in microscopy) was easily seen macroscopically for all species.

Rays height had similar mean values from 8 to 11 cells for all species, although occasionally we observed tall rays of up to 25 cells in *P. emarginatus*, 42 cells in *P. pubescens* and 20 cells in *P. abruptus*, and width 1 to 2 cells respectively, except for exclusively uniseriate in *P. abruptus*. Gasson (1999) reports that rays are uniseriate, very occasionally biseriate, usually up to 12 cells high, but occasionally up to 22 cells high in *P. emarginatus*. Moreover, it is interesting that only *P. abruptus* from Caatinga has more regular storied rays in all individuals, for the remaining species, intraspecific variation consisting of the presence of both, regular and irregular storied rays were detected; Dória *et al*. (2016) observed the same pattern when comparing two *Tabebuia* species from Cerrado and Caatinga biomes, reinforcing the difficulty in distinguishing those species based only on qualitative characters.

Some authors affirm that ray parenchyma cells respond to climatic conditions (Olano *et al*. 2013), but others have found contradictory results (Outer & Veenendaal 1976; Barajas-Morales 1985). As mentioned by Morris *et al*. (2016), this feature responds to both phylogenetic and environmental factors. We collected in seasonally dry environments, with the Caatinga drier than the Cerrado biome. Even so, there was no pattern observed for ray frequency, height or width, and despite being thinner and smaller in caatinga, only few species have significant differences, helping us, together with other anatomical features to distinguish some of the species.

We only observed prismatic crystals in hybrid, *P. emarginatus* and *P. abruptus* in histological slides and macerate. Gasson (1999) also reports that prismatic crystals were abundant in *P. abruptus*, but not in *P. emarginatus*. After comparing our data on anatomical features with those described by Gasson, it seems possible, due to the confusion in circumscribing *P. emarginatus* after Lewis publication, that the specimen examined by Gasson was in fact *P. pubescens*.

Tukey's test, as well as PCA and cluster analyses showed that all *Pterodon* species are very similar and only *P. abruptus* is clearly distinguished on anatomical characters, although cluster analysis also showed some separation of *P. apparicioi*, which indicated that qualitative characteristics are important to help differentiate this species from the other taxa. Gasson *et al*. (2010) had a similar problem in *Dalbergia nigra*, where the PCA was not able to provide a set of distinguishing characters from other commercially important members of the genus, because the majority of variation is within and not among species. This is similar to what we observed (see Fig. S1).

Despite few differences commented at results, quantitative and qualitative wood anatomical features analyzed did not distinguish the hybrid from *P. pubescens*

and *P. emarginatus*, as they have characteristics of both parental species. Gasson *et al*. (2010) also observed that qualitative features alone could not be used to separate *Dalbergia* genus. Even the presence of crystals in *P. emarginatus* and hybrid may not be sufficient to separate these taxa from *P. pubescens* as, according to IAWA (1989), crystals may be present in some individuals, but absent in others. Yahya *et al*. (2010) compared fiber, vessel, axial and ray parenchyma cells proportion, wood density and fibers dimensions among *Acacia* hybrid and parental species (*A. mangium* and *A. auriculiformis*) and only found statistical differences regarding fiber length and proportion of fiber and vessels, which is similar to our results.

According to Gasson *et al*. (2010), the wood anatomy alone is sometimes insufficient for species identification, as in the case of *Dalbergia nigra*, in relation to many important commercial *Dalbergia* species. Yet, we were able to distinguish *P. abruptus* and *P. apparicioi* when we gather both qualitative and quantitative anatomical features in a cluster analysis and identification key, and point out the few differences in *P. pubescens*, *P. emarginatus* and the hybrid. The anatomical variation observed within species that makes it difficult to separate *P. emarginatus* from *P. pubescens* is consistent with what is expected for allogamous (Rocha 2006) and closely related species (Cardoso *et al*. 2015).

Acknowledgments

References

Alves ES, Angyalossy-Alfonso V. 2002. Ecological trends in the wood anatomy of some Brazilian species. 2. Axial parenchyma, rays and fibres. IAWA Journal 23: 391-418.

Baas P, Wheeler EA. 1991. A survey of the fossil record for dicotyledonous wood and its significance for evolutionary and ecological wood anatomy. IAWA Journal 12: 275-318.

Barajas-Morales J. 1985. Wood structural differences between trees of two tropical forests in Mexico. IAWA Journal 6: 355-364.

Baretta-Kuipers T. 1981. Wood Anatomy of Leguminosae: its relevance to taxonomy. In: Polhill RM, Raven PH. (eds.) Advances in legumes systematics. London, Royal Botanic Gardens. p. 677-705.

Cardoso D, São-Mateus WMB, Cruz DT, *et al*. 2015. Filling in the gaps of the papilionoid legume phylogeny: the enigmatic Amazonian genus *Petaladenium* is a new branch of the early-diverging Amburaneae clade. Molecular Phylogenetics and Evolution 84: 112-124.

Carlquist S. 2001. Comparative Wood Anatomy: systematic, ecological, and evolutionary aspects of dicotyledon wood. 2nd. edn. Berlin, Springer Berlin Heidelberg.

COPANT - Comission Panamericana de Normas Técnicas. 1974. Descrição macroscópica, microscópica e geral da madeira. COPANT 30:1-19.

De Omena M, Navarro D, Paula J, Luna J, Lima M, Santana A. 2007. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. Bioresource Technology 98: 2549-2556.

Dickison W. 2000. Integrative Plant Anatomy. San Diego, Harcourt Academic Press.

Dória LC, Podadera DS, Batalha MA, Lima RS, Marcati CR. 2016. Do woody plants of the Caatinga show a higher degree of xeromorphism than in the Cerrado? Flora 224: 244-251.

Flora do Brasil 2020. http://reflora.jbrj.gov.br/reflora/ floradobrasil/ FB29840. 13 dec. 2021.

Gasson P, Miller R, Stekel DJ, Whinder F, Zieminska K. 2010. Wood identification of *Dalbergia nigra* (CITES Appendix I) using quantitative wood anatomy, principal components analysis and naive Bayes classification. Annals of Botany 105: 45-56.

Gasson P. 1999. Wood Anatomy of the Tribe Dipterygeae with comments on related Papilionoid and Caesalpinioid Leguminosae. IAWA Journal 20: 441-455.

Gonçalez JC, Bezerra MHD, Lima MF. 1985. Contribuição para caracterização anatômica e química das madeiras de *Pterodon pubescens* Benth e *Vochysia thyrsoidea* Pohl. Revista Floresta 15: 43-48.

Hansen D, Haraguchi M, Alonso A. 2010. Pharmaceutical properties of "sucupira" (*Pterodon* spp.). Brazilian Journal of Pharmaceutical Sciences 46: 607-616.

IAWA Committee. 1989. IAWA list of microscopic features for hardwood identification. Iawa Bulletin 10: 219-332.

ILDIS (International Legume Database & Information Service). www.ildis. org/LegumeWeb/. 13 Dec. 2021.

InsideWood. 2004. onwards. http://insidewood.lib.ncsu.edu/search. 20 Mar. 2021.

IPNI - International Plant Names Index. 2021. Kew, Harvard University Herbaria & Libraries and Australian National Botanic Gardens. http:// www.ipni.org. 13 Dec. 2021

Kraus JE, Arduin M. 1997. Manual básico de métodos em morfologia vegetal. EDUR, Seropédica.

Leite VG, Teixeira SP, Vidal FM, Gerhard P. 2015. Floral development of the Early-Branching Papilionoid Legume *Amburana cearensis* (Leguminosae) reveals rare and novel characters. International Journal of Plant Sciences 176: 94-106.

Lewis G. 1987. Legumes of Bahia. Kew, Royal Botanic Gardens.

Loureiro A, Silva MF, Vasconcellos FJ. 1984. Contribuição ao estudo anatômico do lenho de 7 espécies de *Dimorphandra* (Leguminosae-Caesalpinioideae). Acta Amazonica 14: 289-313.

Mainieri C, Chimelo JP, Angyalossy-Alfonso V. 1983. Manual de identificação das principais madeiras comerciais brasileiras - Instituto de Pesquisas Tecnológicas. 1st. edn. São Paulo, Companhia de Promoção de Pesquisa Científica e Tecnológica do Estado de São Paulo.

Mainieri C, Chimelo JP. 1989. Fichas de características das madeiras brasileiras. 2nd. edn. São Paulo, Instituto de Pesquisas Tecnológicas – Divisão de Madeiras.

Morris H, Plavcová L, Cvecko P, *et al*. 2016. A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. New Phytologist 209: 1553-1565.

Nisgoski S, Muñiz GIB, Klock U. 1998. Diferenciação anatômica da madeira de 4 espécies do gênero *Caryocar*. Ciência e Natura 20: 85-99.

Olano JM, Arzac A, García-Cervigón AI, von Arx G, Rozas V. 2013. New star on the stage: Amount of ray parenchyma in tree rings shows a link to climate. New Phytologist 198: 486-495.

Oliveira CW, Callado CH, Marquete O. 2001. Anatomia do lenho de espécies do gênero *Nectandra* Rol. ex Rottb. (Lauraceae). Rodriguésia 52: 125-134.

Outer RW, Veenendaal WLH. 1976. Variation in wood anatomy of species with a distribution covering both rain forest and savanna areas of the Ivory Coast, West-Africa. In: Baas P, Bolton AJ, Catling DM. (eds) Wood structure in biological and technological research. Leiden, Leiden University Press. p. 182-195.

Paula JE, Cardoso EN. 1995. Anatomia e dendrometria da madeira de *Pterodon polygalaeflorus* e *P. pubescens*. Pesquisa Agropecuária Brasileira 30: 15-29.

Pinto RB, Francisco VMCR, Mansano VF. 2014. Morphological study of fruits, seeds and embryo in the tropical tribe Dipterygeae (Leguminosae-Papilionoideae). Rodriguésia 65: 89-97.

Rocha DMC. 2006. Aspectos taxonômicos, genéticos e reprodutivos de *Pterodon pubescens* (Benth) Benth e *P. emarginatus* Vog. (Leguminosae, Dipterygeae). PhD, Universidade Estadual de Campinas, Campinas.

Sass JE. 1958. Botanical microtechnique. 3rd. edn. Ames, Iowa State University Press.

Schweingruber FH, Börner A, Schulze E-D. 2008. Atlas of Woody Plant Stems: Evolution, Structure, and Environmental Modifications. 2nd. Edn. Berlin, Springer-Verlag.

Sharma OP. 2009. Plant taxonomy. 2nd edn. New Delhi, Tata McGraw-Hill Education Pvt. Ltd.

Shimizu GH, Semir J. 2016. Dipterygeae. In: Flora fanerogâmica do Estado de São Paulo. Tozzi AMGA, Melhem TS, Forero E, *et al*. (eds.) São Paulo, Instituto de Botânica. p 254-256.

Silva NF, Arruda RCO, Alves FM, Sartori ALB. 2018. Leaflet anatomy of the Dipterygeae clade (Faboideae: Fabaceae): Evolutionary implications and systematics. Botanical Journal of the Linnean Society 187: 99-117.

Sonsin JO, Gasson PE, Barros CF, Marcati CR. 2012. A comparison of the wood anatomy of 11 species from two cerrado habitats (cerrado *s.s.* and adjacent gallery forest). Botanical Journal of the Linnean Society 170: 257-276.

Thiers B. 2018. continuously updated: Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. http://sweetgum.nybg.org/science/vh/. 13 Dec. 2021.

Tropicos.org. 2021. Missouri Botanical Garden. https://tropicos.org. 13 Dec. 2021.

Wheeler EA, Baas P. 1993. The potentials and limitations of dicotyledonous wood anatomy for climatic reconstructions. Paleobiology 19: 48-498.

Yahya R, Sugiyama J, Silsia D, Gril J. 2010. Some anatomical features of an *Acacia* hybrid, *A. mangium* and *A. auriculiformis* grown in Indonesia with regard to pulp yield and paper strength. Journal of Tropical Forest Science 22: 343-351.