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Comparative wood anatomy of species of *Psychotria* L. (Rubiaceae) in Atlantic Rainforest remnants of Rio de Janeiro State, Brazil

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ABSTRACT

Psychotria is the largest Rubiaceae genus and one of the most abundant in the Atlantic Rainforest of Rio de Janeiro State. The present work aims to characterize and compare the wood of nine of these species. The *Psychotria* wood were characterized by: slightly distinct growth rings; diffuse porosity; solitary vessels or on radial multiples of 2-6 or clusters of 3-5 vessel elements, with terminal and lateral simple perforation plates and vestured and alternate intervessel and vessel-ray pits; septate fiber-tracheids; and rare axial parenchyma. Although, the wood anatomy of the *Psychotria* may be considered homogeneous, the statistical analyses, based on qualitative and quantitative features, allowed the segregation of the species and indicated the importance of habit (arboreous or shrubby) on the wood anatomy of the species. It is worth mentioning that the sampling was realized in only one studied site (Ilha Grande), which contributed to the conclusion that the wood characteristics are more related to the specificities of each studied taxon than to with environmental variations.

Keywords: Ilha Grande, Psychotriae, Rubioideae, Tropical Forest, wood diagnostic characters

Introduction

The remaining remnants of Atlantic Forest in the state of Rio de Janeiro represent almost 20% of its original area in the state. One of the largest fragments is the island of Ilha Grande, the third largest Brazilian island and the largest in the state of Rio de Janeiro (190 km^2). Being one of the largest remaining tracks of Atlantic Forest, and comprised predominantly of Dense Ombrophyllous Forest, the forest of Ilha Grande is of particular value for scientific study (Araujo & Oliveira 1988; Oliveira & Coelho Netto 2001; Callado *et al.* 2009).

Rubiaceae is one of the most important plant families, in terms of species richness, in the Atlantic Forest remnants of the state of Rio de Janeiro (Silva Neto & Peixoto 2012). *Psychotria* is the largest genus of the Rubiaceae with approximately 2000 species (Davis *et al.* 2001). The genus is subdivided into three subgenera: *Psychotria* (pantropical), *Tetramerae* (some species in Africa and Madagascar) and *Heteropsychotria* (neotropical) based on morphological characters and geographical distribution (Steyermark 1972; Lopes *et al.* 2004). This genus is now considered paraphyletic and is found in both hemispheres in predominantly shady locations and in relatively moist soils in sub-woods (Taylor 1996; Bremer & Eriksson 2009).

Among the 19 species of *Psychotria* that occur in Dense Ombrophyllous Forest of Rio de Janeiro (List of Species of the Brazilian Flora 2015), 16 occur on Ilha Grande, including nine woody species (Manão 2011).

The taxonomy of *Psychotria* is complex, due not only to the large number of species but also to the absence of morphological characters available for defining taxa (Nepokroeff *et al.* 1999). Authors such as Koek-Noorman (1969a; b), Koek-Noorman & Hogeweg (1974), Rogers (1981; 1984), Ter Welle *et al.* (1983), Koek-Noorman & Puff (1983; 1991), and Jansen *et al.* (1997; 2002) have characterized the wood anatomy of Rubiaceae, examining its taxonomic, ecological and evolutionary aspects among the subfamilies, tribes, genera and species, and recognized the importance of wood anatomy for the taxonomic classification of this group. Koek-Noorman (1977) classified the wood of Rubiaceae as of two types, with type II characteristic of Rubioideae: solitary vessels, radial clusters of few vessel elements, septate libriform fibers and axial parenchyma absent or extremely rare.

This study aims to characterize the wood anatomy of nine species of *Psychotria* from the Atlantic Forest, in order to determine which characteristics are most useful for taxonomic study and which are more useful for ecological investigation. Ultimately this study aims to increase the understanding of anatomical features of this genus in order to better understand the adaptive strategies of its species.

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Materials and Methods

Sampling was performed on the southeast side of Ilha Grande, Rio de Janeiro State, Brazil, (23°10'45"S, 23°11'24" S, 44°11'01"W, 44°11'47"W) in an area of submontane Dense Ombrophyllous Forest of the Atlantic Forest biome. Five individuals of each of the following species were sampled: *Psychotria hoffmannseggiana* Müll. Arg., *Psychotria brasiliensis* Vell., *Psychotria carthagenensis* Jacq., *Psychotria deflexa* DC., *Psychotria leiocarpa* Cham. & Schlecht., *Psychotria nuda* Cham. & Schlecht., *Psychotria pubigera* Schltdl., *Psychotria ruelliifolia* Cham. & Schltdl., and *Psychotria vellosiana* Benth. (Tab. 1).

Wood samples were obtained by non-destructive methods. For arboreous specimens samples were taken using a Pressler probe at approximately 1.30 m above the ground, whereas for shrubby specimens samples were taken as stem disks using a machete. Part of the wood samples and permanent slides were deposited and registered in the wood collection of the Universidade Estadual do Norte Fluminense (UENF), whereas the remainder was used for anatomical identification.

Blocks were cut from each sample and 15-20 μm sections were made with a Leica SM 2010R sliding microtome (Nussloch, Germany) in transverse and longitudinal (radial and tangential) planes. The sectioned material was dehydrated in ethanol and double stained with Astra Blue and hydroalcoholic Safranin (Burger & Richter 1991). Macerations were prepared using Jeffrey solution (Johansen 1940), and stained with aqueous Safranin. All anatomical measurements were performed using a light microscope (Axioplan Zeiss, Oberkochen, Germany). Vulnerability (IV = tangential diameter of vessels/vessel frequency) and mesomorphy (IM = IV X length of vessel elements) were calculated following the method proposed by Carlquist (2001) (Tab. 1). In general, the measurement techniques and terminology follow IAWA guidelines (IAWA committee 1989).

For the scanning electron microscopy (SEM), material was prepared according to the methods of Jansen *et al.* (2008), and observations made using a ZEISS-DSEM 962 (Oberkochen, Germany) scanning electron microscope.

Statistica 7.0 Software (StatSoft 1993, Tulsa, Oklahoma, USA) and PAST version 2.01 (Hammer *et al.* 2001) for Windows XP/Vista were used to perform the statistical analyses. The Shapiro-Wilks W test was used to evaluate the normality of each parameter (Zar 1996).

Qualitative analysis was performed on the nine species of *Psychotria* L., as well as *Coussarea friburguensis* M. Gomes., and *Coussarea congestiflora* Mull. Arg., as outgroup taxa, for which the wood anatomy had previously been described by Barros *et al.* (2001). For the qualitative analysis we created a binary matrix of presence and absence of wood anatomical characteristics, based on IAWA 1989, that differed among species. To ensure the segregation of species regardless of habit the analysis was performed excluding the variables of size, width and frequency of vessels. Cluster analysis and principal component analysis (PCA) were conducted using this matrix using the unweighted pair group method of analysis (UPGMA) (Sokal 1986).

To perform a quantitative analysis, fifteen wood anatomical features of the nine species shown in Tab. 2 were submitted to principal component analysis (PCA) (Sokal 1986). The Kruskal-Wallis test for nonparametric measurements was used to test the differences between the medians of each parameter analyzed among species.

Results

Among species comparisons of wood anatomy described below following the terminology of IAWA guidelines (IAWA committee 1989). The figures were from agreement with the rules and selected to present the most representative differences between characters and species. Figure 1 shows transversal (Fig. 1A-C), tangential (Fig. 1D-F) and radial sections (Fig. 1G-I). Figure 2 shows fibers (Fig. 2A-B), vessels (Fig. 2C-F) and ray cells (Fig. 2G). Figure 3 shows details of vessels (Fig. 3A-C), fibers (Fig. 3D) and ray cells (Fig. 3E-F).

Table 1. Characteristics of *Psychotria* species studied. N° Reg. = registration number in the UENF Wood collection, DBH = diameter at breast height in centimeters, H = height in meters, VL = vulnerability index, ME = mesomorphy index.

Species	N° Reg.	Habit	DBH	H	VL	ME
P. hoffmannseggiana	112, 113, 155, 156 e 157	Shrubby	4.2 (±1.09)	1.8 (±0.44)	0.127	141.4
P. brasiliensis	134, 135, 136, 137 e 138	Arborecent	11.8 (±2.04)	6.4 (±1.51)	0.592	678.3
P. carthagenensis	144, 145, 146, 147 e 148	Arborecent	18.4 (±2.88)	12.8 (±2.16)	2.172	2115.9
P. ruelliifolia	107, 115, 152, 153 e 154	Shrubby	6.4 (±1.14)	2.3 (±0.27)	0.385	344.7
P. deflexa	110, 111, 159, 160 e 161	Shrubby	4.6 (±1.14)	2.3 (±0.57)	0.156	125.5
P. leiocarpa	106, 114, 158, 162 e 163	Shrubby	5.6 (±1.34)	1.9 (±0.41)	0.136	96.3
P. nuda	108, 130, 131, 132 e 133	Arborecent	9.8 (±1.48)	5.6 (±1.51)	0.148	132.9
P. pubigera	109, 116, 149, 150 e 151	Arborecent	7 (±1.87)	6 (±1.58)	0.315	307.6
P. vellosiana	139, 140, 141, 142, e 143	Arborecent	18.8 (±2.58)	10 (±2.12)	2.653	2318.7

Table 2. Quantitative wood anatomical features of the nine *Psychotria* studied. *bar = P. hoffmannseggiana; bra = P. brasiliensis ; car = P. carthagenensis; rue = P. ruelliifolia; def = P. deflexa; lei = P. leiocarpa; nud = P. nuda; pub = P. pubigera; vell = P. vellosiana.* Q25 = quartile 25, μ = medians, Q75 = quartile 75, KW = Kruskal-Wallis test between the medians.

	Species		bar	bra	car	rue	def	lei	nud	pub	vel
Vessels		Q25	175	59	24	80	130	161	152	95	25
	Vessels/mm²	μ	181	66	26	91	141	178	178	102	27
	(N 50)	Q75	189	75	28	98	152	193	190	114	30
		KW	a	be	d	b	c	a	ac	bc	de
		Q25	931	1048	909	783	710	620	784	840	782
	Length(μm)	μ	1120	1141	965	888	772	734	921	956	815
	(N 125)	Q75	1245	1277	1000	984	838	789	993	1068	887
		KW	a	a	d	be	c	c	bde	bd	e
		Q25	19.4	32.2	48.1	28.4	20.4	21.4	22.3	28.5	60.5
	Tang. Diameter (μm)	μ	28.0	36.8	53.6	33.2	26.2	24.0	24.9	32.9	67.8
	(N 125)	Q75	34.6	41.0	60.8	38.5	28.0	25.8	28.4	35.8	74.7
		KW	a	b	d	b	a	ac	c	b	d
		Q25	22.3	32.0	60.5	33.0	24.0	25.8	24.0	26.7	62.2
	Rad. diameter (μm)	μ	25.1	36.5	65.8	37.3	27.6	27.7	26.7	29.4	71.1
	(N 125)	Q75	28.5	43.2	71.1	41.8	30.2	30.4	30.2	32.9	79.2
		KW	a	b	d	b	ac	ac	ac	c	d
		Q25	2.5	1.8	3.2	2.5	2.6	2.7	1.8	2.1	1.6
	Wall thickness (μm)	μ	2.8	2.0	3.6	2.9	3.0	3.0	2.1	2.4	1.7
	(N 125)	Q75	3.2	2.5	4.0	3.2	3.4	3.4	2.5	2.7	2.0
		KW	a	b	c	a	a	a	b	b	d
		Q25	2.8	3.4	3.7	3.5	2.8	2.9	3.3	2.8	4.6
	Intervessel pits (μm)	μ	3.1	3.9	4.1	4.2	3.1	3.2	3.6	3.0	4.9
	(N 125)	Q75	3.5	4.3	4.6	4.8	3.5	3.4	3.9	3.3	5.3
		KW	a	bd	b	b	ac	a	d	ac	e
		Q25	2.9	3.3	3.8	3.6	2.9	2.8	2.9	2.8	4.5
	Vessel-ray pits (μm)	μ	3.1	3.8	4.2	4.1	3.2	3.1	3.3	3.0	4.8
	(N 125)	Q75	3.5	4.3	4.6	4.7	3.5	3.5	3.8	3.3	5.2
		KW	ad	b	c	bc	ad	ad	a	d	e
Fiber		Q25	22.8	29.4	30.9	19.6	21.3	19.6	22.8	24.4	28.7
	Diameter (μm)	μ	23.4	32.0	34.8	22.1	22.2	21.3	27.2	26.9	33.6
	(N 125)	Q75	31.1	37.3	37.1	25.2	28.2	22.4	29.5	28.6	38.4
		KW	a	d	d	b	c	b	ac	ac	d
		Q25	14.7	16.8	17.8	13.8	12.9	11.2	16.4	14.4	19.6
	Lumen diam. (μm)	μ	19.8	18.9	19.4	15.1	15.6	12.9	18.8	16.3	24.8
	(N 125)	Q75	23.1	25.1	22.3	17.5	19.4	14.3	21.4	17.7	30.5
		KW	a	a	ac	b	b	d	a	b	c
		Q25	1388	1420	1459	1115	1046	1002	1278	1330	1199
	Length (μm)	μ	1506	1543	1553	1249	1170	1063	1363	1411	1271
	(N 125)	Q75	1647	1614	1711	1342	1289	1150	1481	1514	1388
		KW	a	a	a	b	b	d	c	c	b
		Q25	3.77	5.68	6.32	2.88	4.09	3.58	3.39	4.81	3.54
	Wall thickness (μm)	μ	4.2	6.4	7.3	3.5	4.5	4.1	3.8	5.3	4.0
	(N 125)	Q75	4.6	7.2	7.9	4.2	5.0	4.8	4.2	5.6	4.4
		KW	a	c	c	b	a	a	ab	d	ab
		Q25	3.20	4.36	3.68	2.71	3.48	2.43	2.85	2.14	6.58
	Pits (μm)	μ	3.56	4.88	4.19	3.34	4.02	2.79	3.36	2.38	7.17
	(N 125)	Q75	3.9	5.9	4.7	3.9	4.7	3.2	3.9	2.9	8.3
		KW	a	d	b	a	b	c	a	c	e
Rays		Q25	8	4	2	3	3	5	5	3	4
	Rays/mm'	μ	8	5	3	4	3	5	5	4	4
	(N 100)	Q75	9	5	3	4	4	6	6	4	5
		KW	a	d	c	b	bc	d	d	bc	e
		Q25	475	924	768	516	466	411	475	406	464
	Height (μm)	μ	597	1187	987	584	553	510	548	503	689
	(N 125)	Q75	750	1351	1102	742	685	633	747	668	823
		KW	a	b	b	a	a	a	a	a	a
		Q25	23.2	37.3	65.6	37.4	23.1	23.2	33.8	24.0	58.7
	Width (μm)	μ	28.4	44.5	69.5	42.8	26.7	26.7	39.2	26.7	69.3
	(N 125)	Q75	34.0	52.8	76.5	50.1	30.2	30.3	48.9	29.3	78.2
		KW	a	b	c	b	a	a	b	a	c

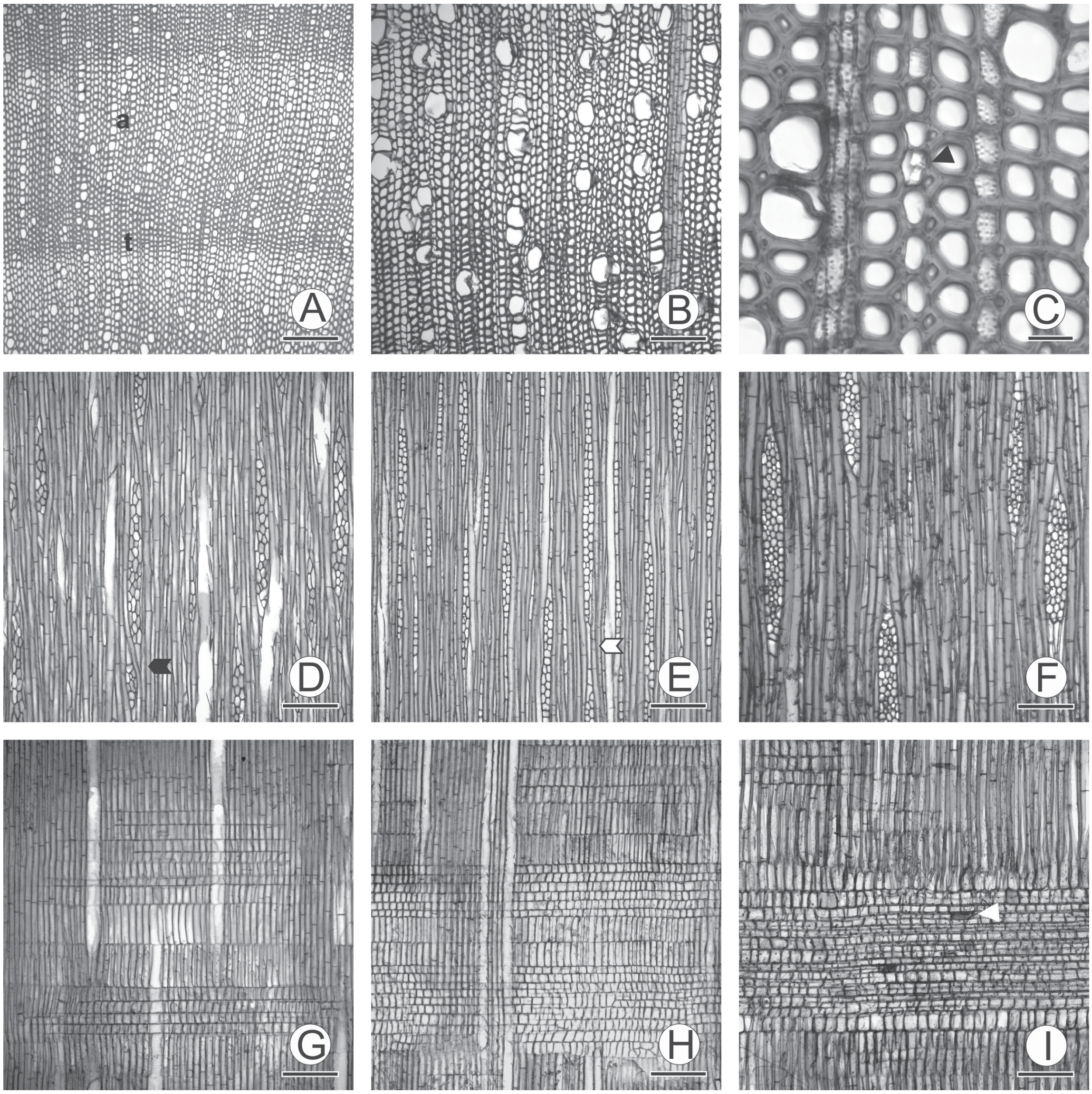

Figure 1. Light microscopy micrographs of *Psychotria* woods sections. A: *P. deflexa,* distinct growth rings, diffuse porosity and vessel arrangement in a radial pattern (e = initial wood, l = later wood). B: *P. vellosiana*, diffuse porosity and vessel arrangement in a radial pattern. C: *P. leiocarpa,* vessels with circular to oval outline, libriform fibers thin- to thick-walled and prismatic crystals inside ray cells (arrowhead). D: *P. nuda,* multiseriate and aggregate rays with uniseriate portions (arrowhead). E: *P. hoffmannseggiana,* uniseriate and biseriate rays, and fused rays (arrowhead). F: *P. vellosiana,* multiseriate rays with sheath cells and septate fiber-tracheids (arrow). G: *P. ruelliifolia,* rays composed of procumbent, square and upright cells. H: *P. hoffmannseggiana,* rays composed of square and/or upright cells. I: *P. carthagenensis,* rays composed of procumbent, square and upright cells, and raphides crystals inside procumbent ray cells (arrowhead). Scale bar: A, B and D to I = 100 μm; C = 20 μm.

Descriptions of studied species

Psychotria deflexa DC. (Figs. 1A, 2C, 3D)

Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.

Vessels: diffuse-porous, 140 vessels/mm^2; solitary, radial multiple of 2-6 or in cluster of 3-4 elements; circular to oval outline; mean length 829.56 μm; mean tangential diameter 25.36 μm; mean wall thickness 3.05 μm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; minute (<4 μm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits; tyloses and gums present.

Fibers: septate, mean length 1187.65 μm; mean diameter 22.25 μm; lumina 16.14 μm; thin- to thick-walled; mean size of pit 4.02 μm.

Axial Parenchyma: extremely rare.

Rays: average 3/mm; uniseriate and biseriate with uniseriate portions, composed of square and/or upright cells; mean width 26.84 μm, mean length 583.75 μm; fused rays and disjunctive cell wall present. One or more prismatic crystals per cell are present.

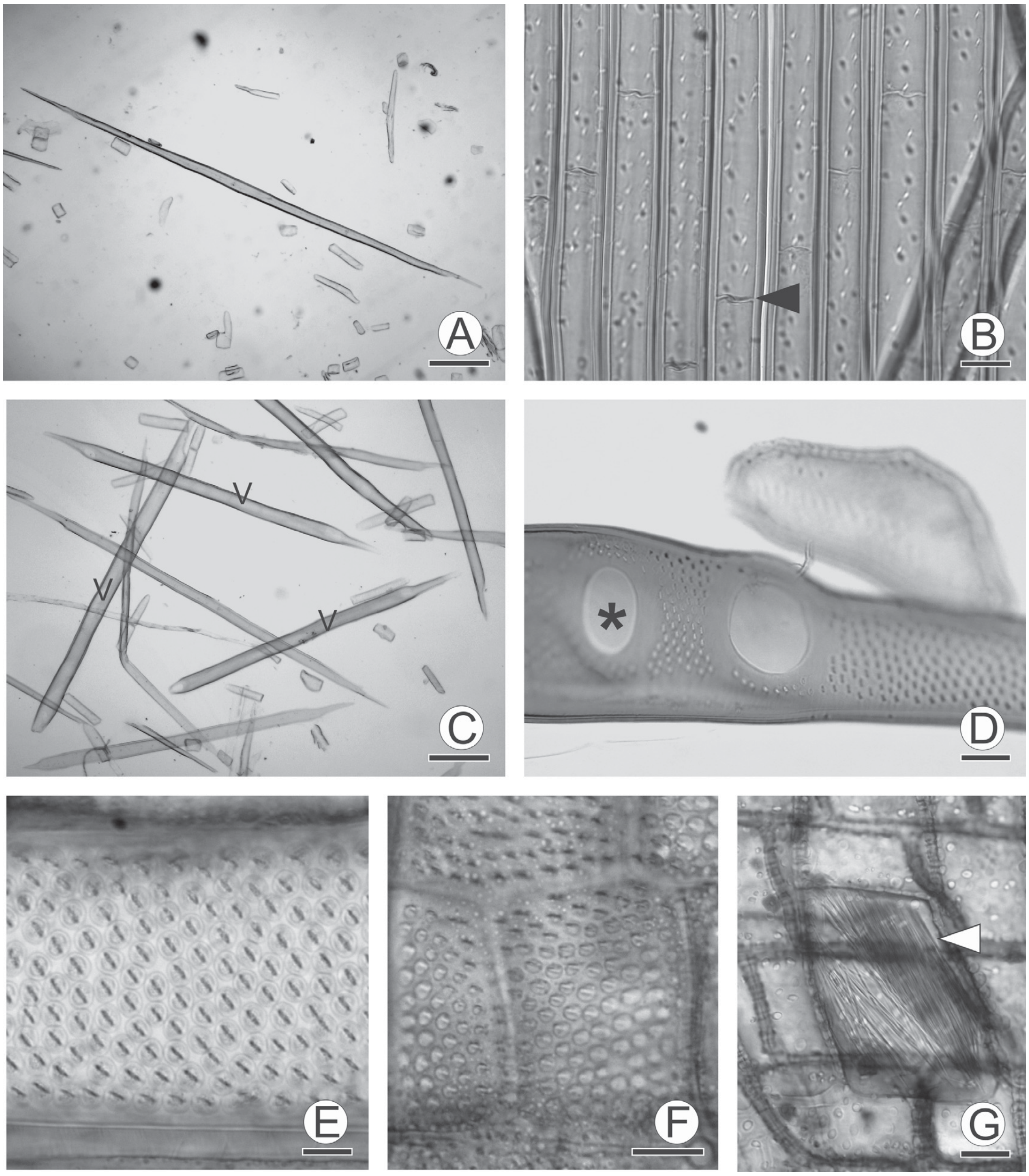

Figure 2. Light microscopy micrographs. A: *P. brasiliensis,* fiber-tracheids. B: *P. hoffmannseggiana,* septate fiber-tracheids (arrowhead), simple fiber pits and fiber-wall thickness. C: *P. deflexa,* vessel elements (V). D: *P. vellosiana,* appendices and simple perforation plates on terminal and lateral wall of vessel (*). E: *P. carthagenensis,* intervessel vestured pits. F: *P. vellosiana,* ray-vessel vestured pits. G: *P. carthagenensis*, raphides crystals inside the upright ray cell (arrowhead). Scale bar: A and C = 100 µm; B, D, E, F and G = 20 µm.

Psychotria vellosiana Benth. (Figs. 1B, 1F, 2D, 2F)

Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.

Vessels: diffuse-porous, 28 vessels/mm^2; solitary, radial multiple of 2-5 or in cluster of 3-4 elements; circular to oval outline; mean length 860.11 µm; mean tangential diameter 68.58 µm; mean wall thickness 1.81 µm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; small (4-7 µm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits.

Fibers: septate, mean length 1281.81 µm; mean diameter 32.92 µm; lumina 24.92 µm; thin- to thick-walled; mean size of pit 7.17 µm.

Axial Parenchyma: extremely rare.

Rays: average 4/mm; multiseriate (3-6 cells wide), with uniseriate portions, composed of procumbent, square and upright cells; mean wide 68.54 µm; mean length 665.65 µm; sheath cells and disjunctive cell wall present, fused and aggregate rays present.

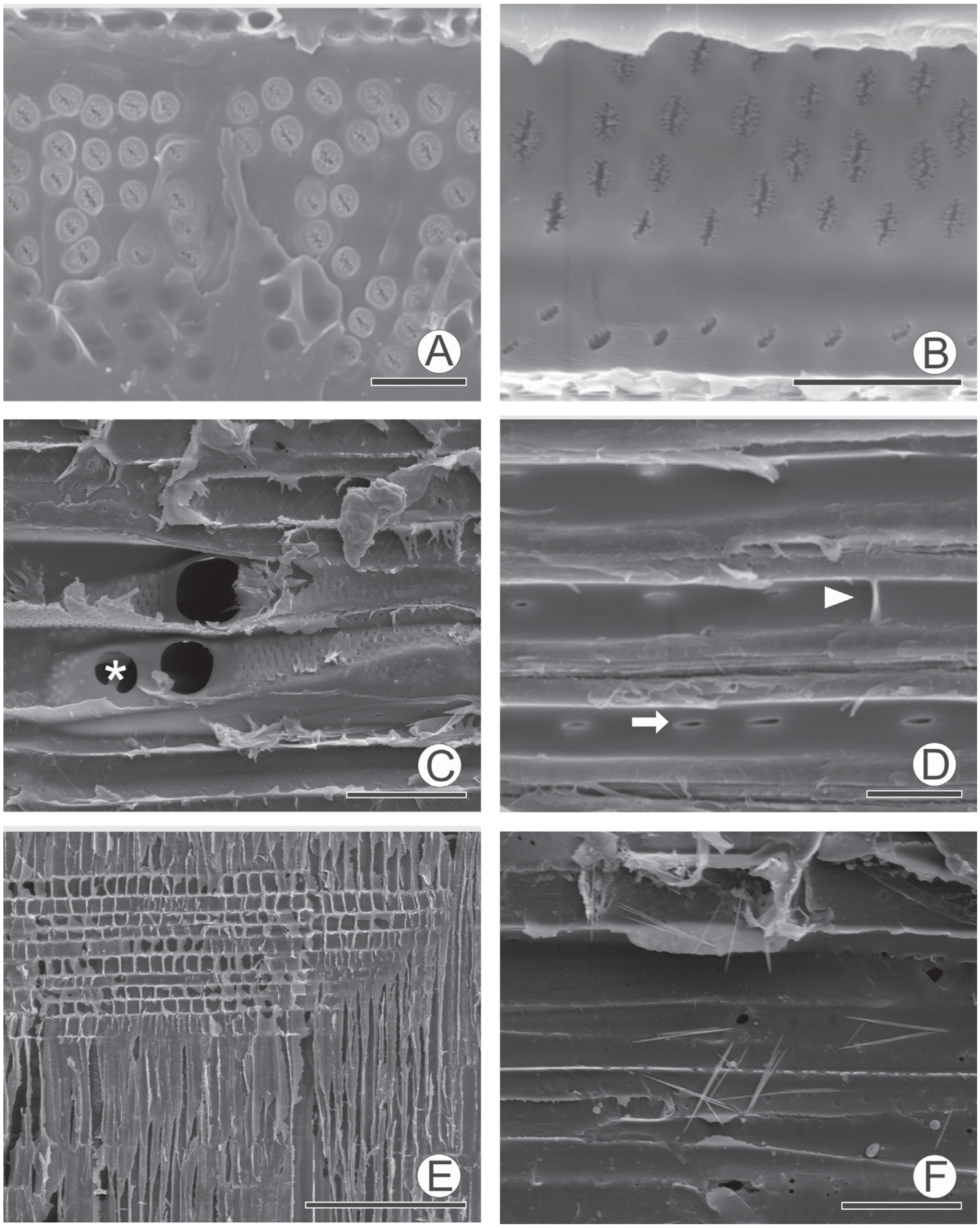

Figure 3. Scanning electron microscopy micrographs. A: *P. brasiliensis,* vestured intervessel pits viewed from outside the vessel. B: *P. pubigera,* vestured intervessel pits viewed from inside vessel. C: *P. ruelliifolia,* vessel with a simple perforation plate on terminal and lateral wall (*) and vessel appendices. D: *P. deflexa*, septate fiber-tracheids (arrowhead) with simple fiber pits (arrow). E: *P. pubigera,* ray composition of only square and upright cells. F: *P. ruelliifolia,* raphides crystals coming out from upright ray cells (arrowhead). Scale bar: A and B = 10 μm; C, D and F = 50 μm; E = 200 μm.

Psychotria leiocarpa Cham. & Schlecht. (Fig. 1C)

Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.

Vessels: diffuse-porous, 178 vessels/mm^2; solitary, radial multiple of 2-5 or in cluster of 3-4 elements (rare); circular to oval outline; mean length 716.93 μm; mean tangential diameter 23.75 μm; mean wall thickness 3.12 μm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; minute (<4 μm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits; tyloses present.

Fibers: septate, mean length 1076.12 μm; mean diameter 21.04 μm; lumina 12.75 μm; thin- to thick-walled; mean size of pit 2.79 μm.

Axial Parenchyma: extremely rare.

Rays: average 5/mm; multiseriate (2-4 cells wide) with uniseriate portions, composed of square and/or upright cells; mean width 27.27 μm mean length 552.73 μm; aggregate and fused rays present. One or more prismatic crystals per cell and raphides crystals are present in upright cells.

Psychotria nuda Cham. & Schlecht. (Fig. 1D)
Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.
Vessels: diffuse-porous, 173 vessels/mm^2; solitary, radial multiple of 2-6 or in cluster of 3-5 elements; circular to oval outline; mean length 892.18 µm; mean tangential diameter 27.03 µm; mean wall thickness 2.18 µm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; minute (<4 µm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits.
Fibers: septate, mean length 1376.74 µm; mean diameter 25.63 µm; lumina 19.39 µm; thin- to thick-walled; mean size of pit 3.36 µm.
Axial Parenchyma: extremely rare.
Rays: average 5/mm; multiseriate (2-4 cells wide) with uniseriate portions, composed of procumbent, square and upright cells; mean width 40.70 µm; mean length 616.38 µm; aggregate and fused rays present.

Psychotria hoffmannseggiana Müll. Arg. (Figs. 1E, 1H, 2B)
Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.
Vessels: diffuse-porous; 183 vessels/mm^2; solitary, radial multiple of 2-5 or in cluster of 3-4 elements; circular to oval outline; mean length 1102.94 µm; mean tangential diameter 28.37 µm; mean wall thickness 2.87 µm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; minute (<4 µm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits; tyloses present.
Fibers: septate, mean length 1506.64 µm; mean diameter 22.57 µm; lumina 19.88 µm; thin- to thick-walled; mean size of pit 3.56 µm.
Axial Parenchyma: extremely rare.
Rays: average 8/mm; uniseriate and biseriate with uniseriate portions, composed of square and/or upright cells; mean width 28.55 µm; mean length 617.79 µm; fused rays present.

Psychotria ruelliifolia Cham. & Schltdl. (Figs. 1G, 3C, 3F)
Growth Rings: Indistinct.
Vessels: diffuse-porous, 90 vessels/mm^2; solitary, radial multiple of 2-5 or in cluster of 3-5 elements; angular outline; mean length 898.13 µm; mean tangential diameter 33.95 µm; mean wall thickness 2.87 µm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; small (4-7 µm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits.
Fibers: septate, mean length 1243.90 µm; mean diameter 22.54 µm; lumina 15.47 µm; thin- to thick-walled; mean size of pit 3.34 µm.
Axial Parenchyma: extremely rare.
Rays: average 4/mm; multiseriate (2-4 cells wide) composed of body cells procumbent and square with marginal rows of or upright cells; mean width 44.70 µm; mean length 629.81 µm; fused and aggregate rays present. Raphides crystals are present in procumbent and upright cells.

Psychotria carthagenensis Jacq. (Figs. 1I, 2E, 2G)
Growth Rings: Indistinct.
Vessels: diffuse-porous, 26 vessels/mm^2; solitary, radial multiple of 2-5 or in cluster of 3-4 elements; circular to oval outline; mean length 952.48 µm; mean tangential diameter 54.57 µm; mean wall thickness 3.64 µm; simple perforation plates and lateral perforation plates; appendices on both ends; small (4-7 µm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits; tyloses and gums present.
Fiber: septate, mean length 1571.12 µm; mean diameter 35.15 µm; lumina 20.86 µm; thin- to thick-walled; mean size of pit 4.19 µm.
Axial Parenchyma: extremely rare.
Rays: average 8/mm; uniseriate and biseriate with uniseriate portions, composed of square and/or upright cells and multiseriate sections (3-8 cells wide) composed of body cells procumbent and square with marginal rows or upright cells; mean width 73.63 µm; mean length 617.79 µm; sheath cells and disjunctive cell wall present, fused and aggregate rays present. Raphides crystals are present in procumbent, square and upright cells.

Psychotria brasiliensis Vell. (Figs. 2A, 3A)
Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.
Vessels: diffuse-porous, 67 vessels/mm^2; solitary, radial multiple of 2-5 or in clusters of 3-4 elements; circular to oval outline; mean length 1152.84 µm; mean tangential diameter 37.10 µm; mean wall thickness 2.16 µm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; minute (<4 µm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits.
Fibers: septate, mean length 1507.47 µm; mean diameter 33.18 µm; lumina 20.38 µm; thin- to thick-walled; mean size of pit 4.88 µm.
Axial Parenchyma: extremely rare.
Rays: average 5/mm; multiseriate (2-4 cells wide) with uniseriate portions, composed of body cells procumbent

with marginal rows of square or upright cells; mean width 44.85 μm; mean length 1167.24 μm; disjunctive cell wall and fused rays present.

Psychotria pubigera Schltdl. (Fig. 3B, 3E)

Growth Rings: distinct or slightly distinct, marked by thick-walled and radially flattened latewood versus thin-walled earlywood fibers.

Vessels: diffuse-porous, 105 vessels/mm²; solitary, radial multiple of 2-5 or in cluster of 3-4 elements (rare); angular outline; mean length 952.68 μm; mean tangential diameter 32.46 μm; mean wall thickness 2.44 μm; simple perforation plates and 1-2 lateral perforation plates; appendices on both ends; minute (<4 μm), alternate, circular and vestured intervessel pits with distinct borders; vessel-ray pits similar to intervessel pits; tyloses present.

Fibers: septate, mean length 1408.77 μm; mean diameter 26.49 μm; lumina 16.05 μm; thin- to thick-walled; mean size of pit 2.38 μm.

Axial Parenchyma: extremely rare.

Rays: average 4/mm; exclusively biseriate, composed of square and upright cells; mean width 26.60 μm; mean length 556.70 μm; fused rays present.

Qualitative Comparative Analysis

Cluster analysis (Fig. 4) separated *Coussarea friburguensis* from the remaining species, which separated into three groups with more than 40% similarity. The group with greatest similarity was composed of *P. hoffmannseggiana*, *P. pubigera*, *P. deflexa* and *P. leiocarpa*. A second group was contained *P. brasiliensis*, *P. nuda* and *P. ruelliifolia*, and a third group contained *P. carthagenensis, P. vellosiana* and *Coussarea congestiflora.*

The principal component analysis (Fig. 5) separated *Coussarea friburguensis* from the remaining species, and the closest group was again *P. hoffmannseggiana*, *P. pubigera*, *P. deflexa* and *P. leiocarpa*. The other species did not form a cohesive group despite *P. vellosiana* and *C. congestiflora* being closer than in the cluster analysis. The first two axes of the principal component analysis of the qualitative data explained 47.35% of the total variance among species. Axis 1 accounted for 27.44% of the total variance and separated species mainly according to scalariform or simple perforation plates, angular or circular vessel outline, intervessel pit size, ray width, septate fiber presence, fiber length and growth ring distinctiveness. Axis 2 explained 19.91% of the total variance and separated species mainly by width of the fiber wall and the fiber pits, presence of sheath cells in rays and cellular composition of rays.

Quantitative Comparative Analysis

The first two axes of the principal component analysis of the quantitative data explained 55.29% of the total variance among species (Fig. 6). Axis 1 accounted for 40.37% of the total variance and separated the species mainly by vessel frequency, ray frequency, vessel mean wall thickness and vessel length. Axis 2 explained 14.92% of the total variance and separated the species mainly by intervessel pit size and vessel mean tangential diameter.

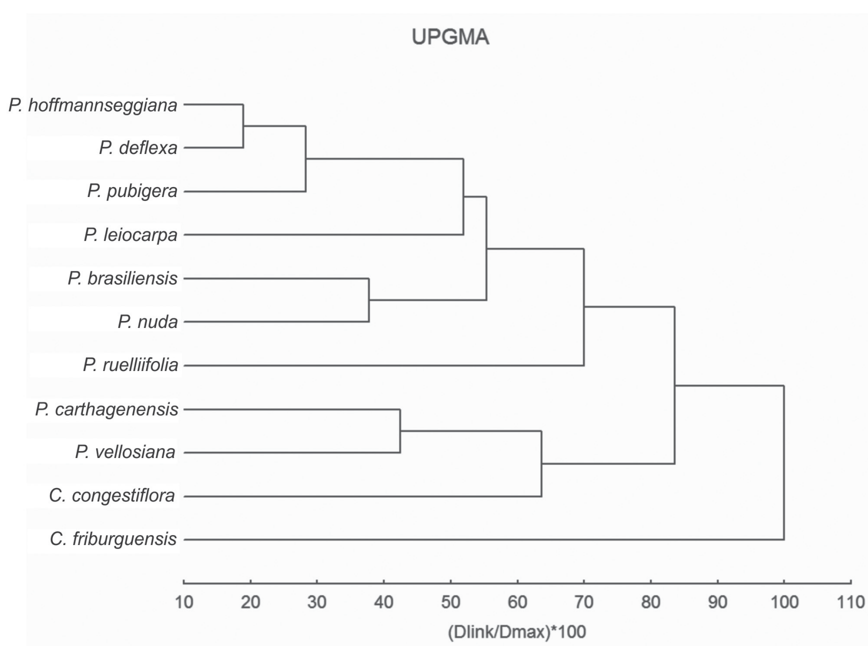

Figure 4. Cluster similarity analysis of *Psychotria* and *Coussarea* qualitative characters.

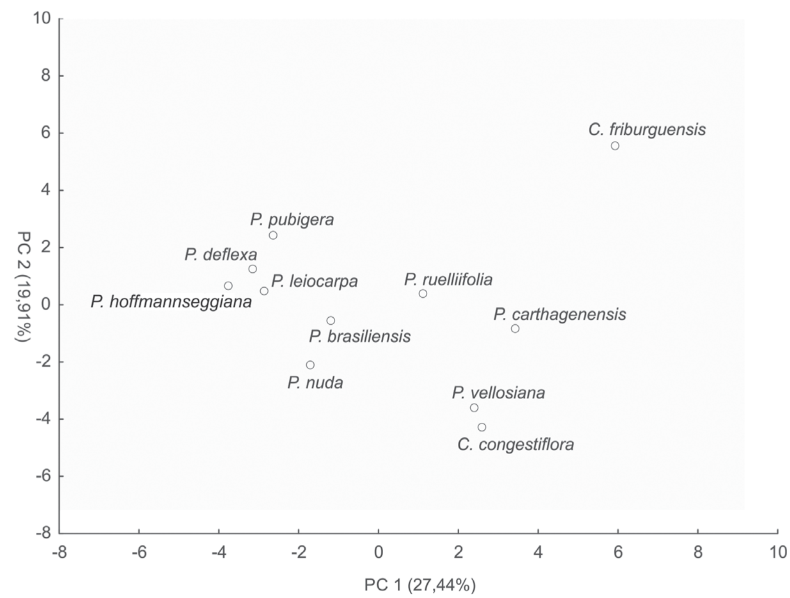

Figure 5. Principal Component Analysis of *Psychotria* and *Coussarea* qualitative characters.

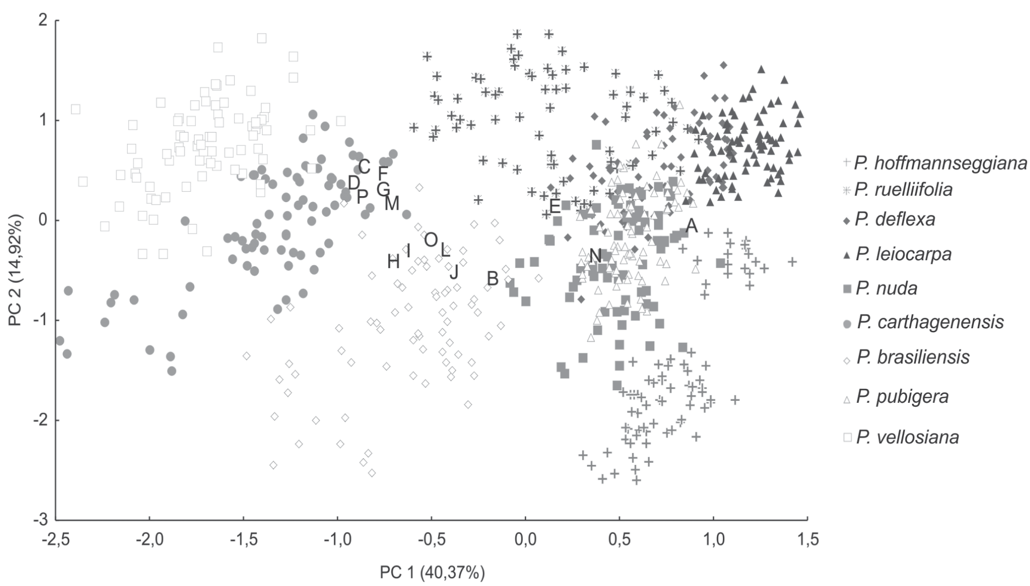

Figure 6. Principal Component Analysis of *Psychotria* quantitative characters. A = Vessels/mm², B = vessel length, C = tangential diameter of vessel, D = radial diameter of vessel, E = vessel wall thickness, F = intervessel pits, G = vessel-ray pits, H = fiber diameter, I = fiber lumina, J = fiber length, L = fiber wall thickness, M = fiber pits, N = rays/mm, O = ray wide, P = ray length.

Discussion

In general, the anatomical characters observed in the present study are classified as type II (Koek-Noorman 1977), corroborating the results of Jansen *et al*. (2002) who defended the placement of the genus *Psychotria* in the tribe Psychotrieae and the subfamily Rubioideae. The type II characters observed in the studied species were: vessel elements solitary, in radial multiples and clusters of a few elements; libriform and septate fibers; axial parenchyma absent or extremely rare; simple perforation plates; vestured vessel-ray parenchyma and intervessel-ray pits.

Although the species of *Psychotria* mainly occur in humid areas of the understory, the presence of narrow and numerous vessel elements is a characteristic of the genus and indicative of adaptation to conditions of water deficit with a preference for conductive security instead of efficiency.

The presence or absence of different types of mineral inclusions in the ray cells is an important parameter for identifying wood and serves as a taxonomic and systematic marker for Rubiaceae anatomy (Jansen *et al*. 2002; Callado & Silva Neto 2003). Raphide crystals are commonly found in the wood of the species of *Psychotria* and they are typical of Rubioideae (Jansen *et al*. 2001). Raphide crystals were found in the radial cells of *P. carthagenensis, P. ruelliifolia* and *P. leiocarpa*. In *P. deflexed* and *P. leiocarpa* one or more prismatic crystals in large ray cells were found. The ecological role of inorganic inclusions in wood is not very clear, but the process of formation and the shape of crystals are directly influenced by genetic factors (Luchi 1990; Nakata 2012), which strengthens their taxonomic significance.

Vestured intervessel pits are important features of Rubiaceae and their structure and size are of great diagnostic value for the family (Jansen *et al*. 2001). The presence of vestured pits is important in making a plant less vulnerable to embolism and consequent disruption in water transport (Tyree & Sperry 1989; Dickison 2000). However, more important than the size is the complex structure of the membrane pit (Sano 2004). According to Williams & Leon (2005), the size of pits and their structure are more related to the taxonomic group than to environmental conditions. All the species studied here exhibited vestured pits, but no correlation was observed regarding their size or structure.

The presence of septate fibers in the studied species may also be related to the high frequency and small-diameter of vessels and the extremely rare axial parenchyma. Substance transport and water conduction explain the presence of fibers with large lumen, thin walls and relatively large pits among the studied species (Jansen *et al*. 2002).

The habit of an individual plant can exert significant influence on the anatomical features of their wood. According to Lens *et al*. (2004), plants of shrubby habit have some anatomical features similar to those of plants of arboreous habit that are adapted to water deficit. Nevertheless, such features should not be interpreted as having adaptive value to xeromorphy, but instead to the habit of the species. When compared to shrubs, trees generally have larger, longer and less frequent vessels (Lens *et al*. 2004). In this work, the diameter and frequency of the vessels can also be related to the habit of the species, and among the nine species studied, *P. carthagenensis* and *P. vellosiana* are trees, while the remainder are small treelets or shrubs.

The width of rays may also be related to habit. According to Jansen *et al*. (2002), the presence of biseriate and multiseriate rays 2-4 cells wide and uniseriate portions at the margins is common among species of *Psychotria,* whereas species with exclusively uniseriate rays and multiseriate rays of more than four cells wide, as observed in *P. carthagenensis* and *P. vellosiana,* is less common. Since arboreous habit is observed only in these two species, it is possible that the structure of the rays can also be influenced by size of the species.

The results of the principal component analyses of qualitative and quantitative characters were similar, both separating *P. carthagenensis* and *P. vellosiana* from the remaining species. This separation is mainly due to these two species having less frequent and larger diameter vessels, and consequently greater vulnerability and mesomorphic indices. It is possible that these attributes are related to the maximum height of these two species compared to that of the others. *P. nuda*, *P. hoffmannseggiana*, *P. pubigera*, *P. deflexa* and *P. leiocarpa* were grouped together mainly because they have small diameters and high-frequency vessels.

The nine species of *Psychotria* were segregated by wood anatomy. Although, the characters of vessel frequency, length and diameter, and vulnerability and mesomorphic indices can be susceptible to environmental changes, and most likely have a direct relationship with growth habit (Carlquist 2001; Sperry 2003; Lens *et al*. 2004), they were useful as diagnostic characters in the present study since all samples were obtained from the same study site. However, the use of these characters as diagnostic must be done with caution and should not be used in phylogenetic analyses of the group.

The use of the two species of *Coussarea*, belonging to Rubioideae, as outgroups for cluster analysis allowed a clearer recognition of the differences between the species of *Psychotria* because *Coussarea* is phylogenetically close to *Psychotria*. The cluster analysis separated *Coussarea friburguensis* from the other species, and the remaining species were separated into three groups with more than 40% similarity. Cluster analysis showed certain differences in the proximity of species of *Psychotria* from the principal component analysis, especially with regards to the proximity of *P. nuda, P. brasiliensis* and *P. vellosiana* and the proximity of *P. ruelliifolia* and *P. carthagenensis*. These differences between the two analyses may be related to other characteristics, such as the composition of the rays, and the size of fiber pits.

Although *P. hoffmannseggiana, P. pubigera, P. deflexa* and *P. leiocarpa* are of shrubby habit, they remained as a well-defined group after cluster and grouping analyses without the features that are directly influenced by the habit, such as vessel frequency, length and diameter.

Although *Psychotria brasiliensis* is a synonym of *P. nuda* (Taylor 2007), we chose to maintain the separated species according to differences observed in wood structure, especially the vessel frequency and length, ray composition and length and presence of disjunctive ray parenchyma in the cell wall present only in *P. brasiliensis*. The fact that these two species are sympatric, and were sampled under similar environmental conditions, strongly suggests two distinct groups of individuals. Based on this finding, and also on other morphological differences (SJS Neto unpubl.res.), work is being done to revalidate the binomial *P. brasiliensis*.

Conclusion

Despite the occurrence of many common structural characteristics, the nine species of *Psychotria* can be segregated by wood anatomy. The diagnostic parameters are the presence or absence of crystals; crystal types (prismatic or raphide); cellular composition of rays; presence or absence of disjunctive ray parenchyma in the cell wall; and size and format of vestured pits.

Wood anatomical characters, such as vessel frequency, length and diameter, can be susceptible to environmental influence. In addition, these characters have a direct relationship with the different habits of plants. Thus, the use of these as diagnostic characters must be done with caution even when separate species are being analyzed.

The comparative analyses of both qualitative and quantitative wood anatomical characteristics of the nine species of *Psychotria* and the two *Coussarea* outgroups were successful in segregating and grouping the species.

It should be noted that the conclusion that the wood characteristics are more related to the specificities of each studied taxon than to environmental variation is likely due to the sampling of only one study site (Ilha Grande) in the present work.

Nonetheless, wood anatomy proved to be effective in segregating the species of *Psychotria*, however, phenotypic variation resulting from environmental response deserves greater study because the extent to which these characteristics can vary in each species remains unknown.

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