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Key anatomical attributes for occurrence of *Psychotria schlechtendaliana* (Müll.Arg.) Müll.Arg. (Rubiaceae) in different successional stages of a tropical moist forest

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ABSTRACT

We analyzed the effects of two different successional stages on leaf and wood anatomy of *Psychotria schlechtendaliana* in a tropical moist forest. Leaf and wood samples were collected from plants growing in two sites representing two successional stages: advanced and intermediate stages of forest succession (ASFS and ISFS, respectively). The leaves have typical mesomorphic anatomy. Wood exhibits growth rings slightly different. The vessels elements are solitary, arranged radial or in clusters, with diffuse porosity, simple perforation plates, septate fibers, radial parenchyma multiseriate, and heterogeneous and perforated ray cells, intervessel pits bordered, small, alternate, vestured and/or scalariform. Quantitative analyses showed significantly differences in leaf and wood anatomy. Top and base leaves on both sites differed in the thickness of cuticle, palisade parenchyma, and palisade:spongy parenchyma ratio. Plants at ISFS had leaf lamina with thinner adaxial cuticle, smaller cells on the adaxial epidermis, smaller width of palisade parenchyma, smaller palisade:spongy parenchyma ratio, and lower stomatal density than at ASFS. The wood of plants in ISFS had presented smaller diameters of the lumen of vessels and fibers, higher frequencies of vessels and rays, and fiber and rays with longer lengths than at ASFS. The differences between leaf and wood anatomy at the two sites confirm a structural adjustment in relation to forest succession for this species. The anatomical differences reflect the sunlight distribution and water availability, allowing the adjustment in photosynthetic efficiency, and safety water transport.

1. Introduction

The Brazilian Atlantic Forest is one of the most biodiverse biomes in the world with several phytophysiognomies subjected to different environmental conditions (Peixoto et al., 2002). Unfortunately, this biome has been subjected to intense anthropogenic disturbance, which has resulted in loss of much of its forest cover (Ribeiro et al., 2011). The remaining extent of Atlantic Forest is fragmented, with only 8.5% of remnants larger than 100 ha (SOS Atlantic Forest Foundation, 2014). The remaining forest remnants have experienced varying degrees of degradation and are in different stages of regeneration (Martini et al., 2007; Piotto et al., 2009). As a result, the Brazilian Atlantic Forest is being ranked among the top five world hotspots for conservation priority (Eisenlohr et al., 2015; Mittermeier et al., 2005; Myers et al., 2000).

Reducing forest cover can result in a linear loss of individuals, genera and species of understory (Andrade et al., 2015), consequently, changes the canopy structure, which severely alters the forest microclimate (Wirth et al., 2001). According to Murray-Smith et al. (2009), conservation strategies should focus on small areas representative of the diversity and endemism of the biome. Thus, the identification and study of such areas can help in conservation planning, especially in those areas that are very diverse. An example is the Serra do Conduru State Park (PESC), located in southern Bahia, which has great species

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diversity and endemism (Amorim et al., 2005; Thomas et al., 2008, 1998), and is comprised of a mosaic of forests in different stages of regeneration (Martini et al., 2007; Piotto et al., 2009).

In the PESC's region, the species of the genus *Psychotria* L. (Rubiaceae) are an important component of the forest understory, and contribute significantly to its floristic richness (Amorim et al., 2009). There are approximately 252 species of *Psychotria* in Brazil, and they include shrubs, trees, and rarely climbing (Taylor et al., 2015). Members of this genus are typically understory specialists, and exhibit conserved evolutionary traits that reflect strategies related to differences in light and moisture availability, with a structural adjustments or phenotypic plasticity capacity (Mulkey et al., 1992; Sedio et al., 2012; Sterck et al., 2013; Valladares et al., 2000). The strategies adopted for species to withstand drought can be completely different (Gullo and Salleo, 1988). Anatomy of sun-exposed and shaded leaves can to present too differences in species, as the leaf anatomy is well related to foliar physiology. (Qi et al., 2006). Tree species living in wet lowland rainforest clearly differ in drought susceptibility, suggesting that there are variable water transport strategies. The differences in some characteristics (physiological, morphological and anatomical) between and within the functional groups of trees reflect the variation in strategies of water transport and resistance to drought (Apgaua et al., 2015).

Psychotria schlechtendaliana (Müll.Arg.) Müll.Arg. is endemic to Brazil and occurs in areas of semiarid forests and Atlantic Forest in the northeast, especially in the states of Bahia, Ceará and Pernambuco (REFLORA, 2012). The studies with the species *P. schlechtendaliana* are mainly aimed at the survey and floristic composition in regions of Atlantic Forest of Northeast Brazil. (Borges and Ferreira, 2017; Coelho and Amorim, 2014; Fernandes and Queiroz, 2015; Martini et al., 2007; Nascimento et al., 2012; Piotto et al., 2009; Rodal et al., 2005). In southern Bahia, this shade-tolerant species is widely dispersed among the understory of tropical moist forests (Amorim et al., 2009; Piotto et al., 2009), at various successional stages, occurring in quite preserved areas even in much degraded areas (Andrade et al., 2015). This makes the species a suitable model for studies aimed at structural adjustments developed in different forest succession stages.

Anatomical investigations can be a useful approach for understanding structural changes in the plants subjected to environmental changes in their natural habitats (*e.g.* Callado et al., 1997; Dickison, 2000; Juno et al., 2013; Mantuano et al., 2006). Analyses of intraspecific variation in leaf anatomy have shown that differences in the leaf blade thickness, palisade parenchyma, amount of fibers and the density and location of stomata are related to abiotic factors such as light intensity and water availability (Esau, 2000; Pireda, 2013; Rabelo et al., 2012; Rôças et al., 2001). The study of the structure and function of wood, especially in the hydraulics of trees, has also evolved as one of the most interesting fields of plant biology and evolution and forest ecology (Hacke, 2015). Studies of leaf and wood anatomy can be integrated to further characterize a species and identify those characteristics that are influenced by environmental variation. In this context, this work aimed to perform a comparative analysis of the anatomical structures of leaf and wood of *P. schlechtendaliana* occurring in two different successional stages within a tropical moist forest, seeking to associate the effect of microclimate on the structural adjustments of the species, and from this, to identify possible adaptive strategies that the species presents to survive in its natural habitat, but with different environmental changes.

2. Material and methods

The study was conducted in southern Bahia, Brazil, in the Serra do Conduru State Park (PESC) (14°20′ – 14°30′ S, 39°02′ – 39°08′ W, at altitudes from 130 m). The vegetation of PESC is classified as tropical moist forest (Gouvêa et al., 1976), with a uniform canopy of more than 25 m in height, a dense understory and numerous epiphytes and large lianas (Jardim, 2003). The park comprises a mosaic of forests in different stages of succession, including highly conserved mature forests (Martini et al., 2007). The climate is hot and wet, with average temperatures of 24 °C. November to March is the driest period and July to August is the coldest period. Relative humidity is often above 80%, with well-distributed annual rainfall averaging 2000 mm (Landau, 2003; Sá et al., 1982). The soil types in this area include yellow and red–yellow latosols, yellow and red–yellow podzols, as well as alluvial and sandy sections (Governo do Estado da Bahia, 1998).

Plant material was collected within PESC at two sites in two different successional stages (Martini et al., 2007). An advanced stage of forest succession (ASFS) that consists of a forest that has been regenerating from deforestation for nearly 30 years, but remains surrounded by large forest blocks with minor disturbances. The other an intermediate stage of forest succession (ISFS) that consists of a forest that has been regenerating from deforestation for approximately seven years, and is surrounded by small forest fragments in different successional stages or small grazing areas and agricultural land, which intensify the edge effect on forests. To distinguish sites we analyzed the soil and the canopy openness (CO). The CO was analyzed using hemispheric images captured with a fisheye lens (180°) attached to a digital camera (Coolpix 4300, NIKON, Japan). The camera was mounted at 1.40 m above the soil level on a tripod, each photograph having a range radius of approximately 10 m. The images were analyzed using the software Gap Light Analyzer 2.0 (Frazer et al., 1999). Both areas are located in a portion of dystroferric yellow latosol soil (IBGE, 2018). But for a better characterization of the soils, three samples were taken within a 1 m radius of the individuals at a depth of 20 cm, totaling 15 simple samples per area. Each set formed a composite sample of each area (Catani et al., 1954; Oliveira et al., 2007). The two composite soil samples were analyzed in the Soil Laboratory of the Centro de Pesquisas do Cacau (CEPEC/CEPLAC), Ilhéus, Brazil.

Five individual trees with cylindrical, straight trunks and without bifurcations or defects were identified in each site. The trees had height of about 6 m and diameter at breast height of 5 cm. Fully expanded leaves were collected from different vertical strata of each study trees, with five leaves collected at the highest stratum of the tree canopy (top leaves) and five leaves collected at the lower stratum of the tree canopy next to the forest understory (base leaves). Wood samples were collected from the same ten study trees in a non-destructive manner using a Pressler probe (Increment Borer, SUUNTO, USA) at 1.30 m above the soil, with two samples by individual. The plant material was vouchered in the reference collection of the Universidade Estadual de Santa Cruz Herbarium (HUESC nº 16,193), Universidade Estadual do Norte Fluminense Herbarium (HUENF nº 1,923) and Xylotheque Dra. Cecília Gonçalves Costa (HUENFw nº 202 until 211) of the last one institution.

2.1. Light microscopy

Fully expanded leaves were fixed in a solution of 2.5% glutaraldehyde, 2.0% formaldehyde and sodium cacodylate buffer at 0.05 M pH 7.3 (Karnovsky, 1965 modified by Da Cunha et al., 2000). Transverse sections were made from the middle-third of the leaves by free-hand cut and stained with Safranin-Astra blue (Kraus and Arduin, 1997) for the manufacture of semi-permanent slides. For a paradermic view surface of epidermis and to determine stomata density, other leaf fragments were subjected to dissociation both epidermis surfaces (Franklin, 1945). The leaf fragments were placed in an oven at 60 °C for about 24 h until they became transparent. The fragments were washed and stained with 1% aqueous Safranin. Adaxial and abaxial surfaces of the epidermis were separated and mounted on different semi-permanent slides.

The parameters used for comparing the leaves from the two sites and from the different strata included: leaf blade thickness, thickness of palisade and spongy parenchyma, thickness of the adaxial and abaxial surfaces of the epidermis, thickness of adaxial and abaxial cuticles, the ratio between the thickness of the palisade and spongy parenchyma,

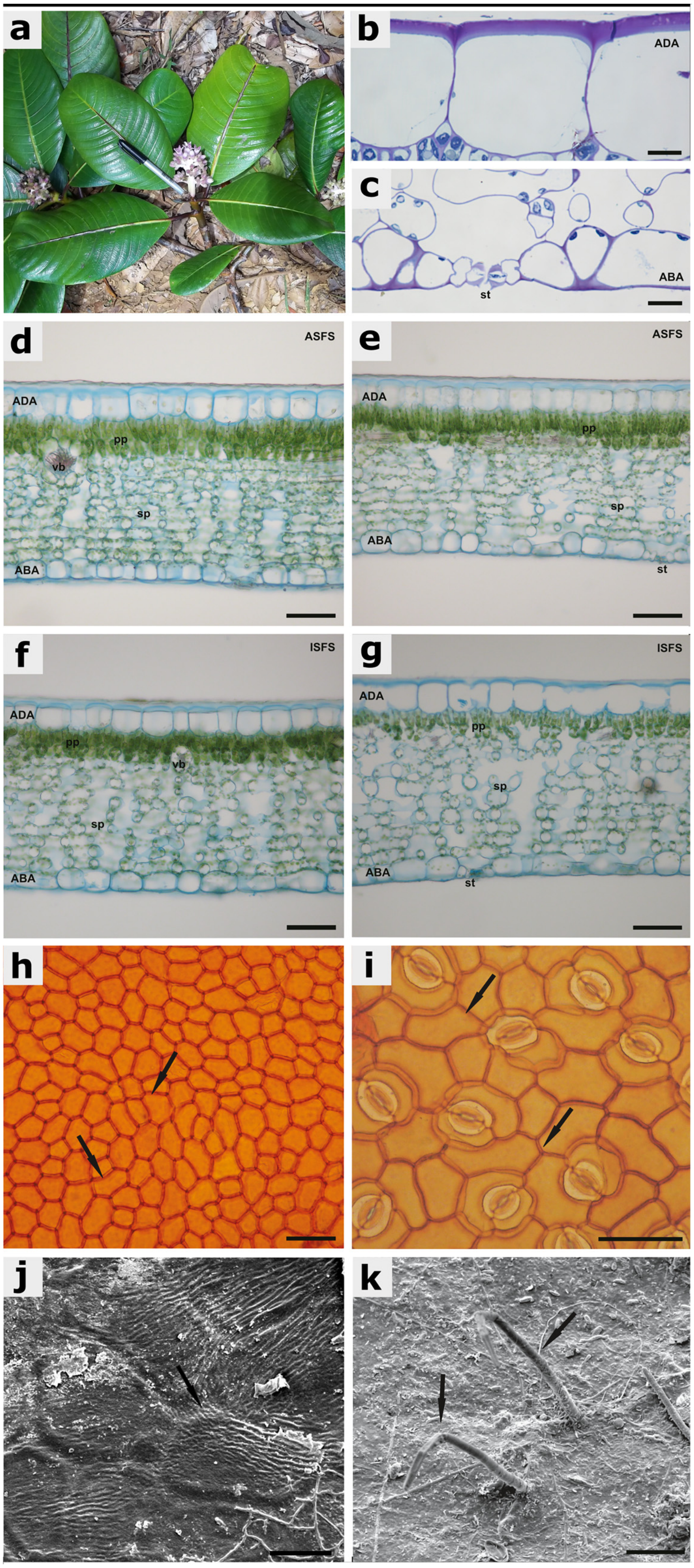

Fig. 1. Morphology and blade leaf anatomy of *Psychotria schlechtendaliana*. **a.** Leaf morphology. **b – i.** Light Microscopy. **b** and **c.** Detail of the epidermis adaxial (ADA), abaxial (ABA) and stomata (st), respectively. **d – g.** Dorsiventral mesophyll with palisade parenchyma (pp) and spongy parenchyma (sp), vascular bundle (vb), epidermis ADA and ABA, and stomata (st). **d.** Top blade leaf of advanced stages of forest succession (ASFS). **e.** Base blade leaf of ASFS. **f.** Top blade leaf of intermediate stages of forest succession (ISFS). **g.** Base blade leaf ISFS. **h.** Adaxial surface showing the straight anticline walls (arrow). **i.** Abaxial surface showing the straight anticline walls (arrow) and paracytic stomata (asterisk). **j** and **k**. Scanning Electron Microscopy. **j.** Adaxial surface with striated ornamentation (arrow). **k.** Tector trichomes on abaxial surface (arrow). Bars: **b** and **c** 20 µm; **d–h** 100 µm; **i** and **k** - j 25 µm.

and stomata density, with 75 measurements per parameter of each leaf type in each individual.

Fragments of wood samples underwent chemical softening with a solution composed of distilled water, 96% ethanol, glycerin and neutral detergent (Alcorn and Ark, 1953), and then sectioned in transversal, radial-longitudinal and tangential-longitudinal planes with a sliding microtome (SM2010 R, Leica, Germany) with an average thickness of 15 µm. Sections were submitted to the following procedures:

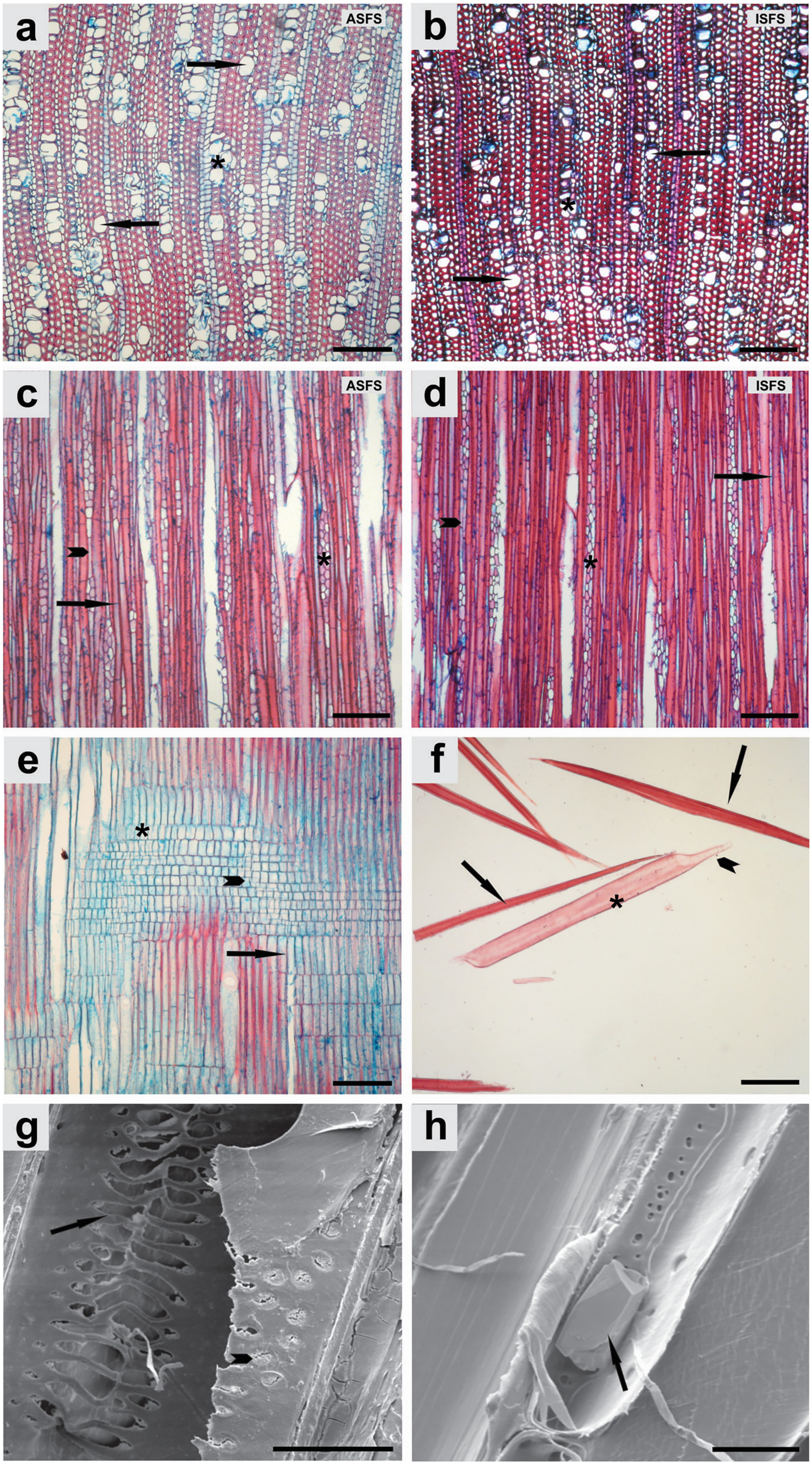

Fig. 2. Wood anatomy of *Psychotria schlechtendaliana.* **a – f.** Light Microscopy. **a** and **b.** Cross section, growth rings slightly different; diffuse porosity; solitary vessel elements (arrow), or in arranged radial (asterisk). **a.** Wood of advanced stages of forest succession (ASFS). **b.** Wood of intermediate stages of forest succession (ISFS). **c** and **d.** Tangential longitudinal section, radial parenchyma multiseriate (asterisk), fused (arrowhead) and fiber-tracheids septate (arrow). **c.** Wood of ASFS. **d.** Wood of ISFS. **e.** Radial longitudinal section, rays heterogeneous composed of procumbent cells (arrowhead), upright cells (arrow) and square cells (asterisk). **f.** Vessel elements (asterisk) with appendices in both extremities (arrow) and fiber-tracheids (arrowhead). **g** and **h.** Scanning Electron Microscopy. **g.** Vestured pits (arrowhead) and scalariform (arrow). **h.** Prismatic crystal in the radial parenchyma cells. Bars: **a** - **f** 200 μm; **g** 25 μm; h 15 μm.

clarification using sodium hypochlorite at 50% and 0.1% acidulated water; dehydration in an ascending ethanol series (Johansen, 1940); double staining with Astra blue and hydro-alcoholic Safranin; and immersion in xylene. Permanent slides were also prepared with Entellan® synthetic resin (Burger and Richter, 1991). Other fragments of the wood samples were subjected to dissociation and maceration by the Franklin method (Franklin, 1945). The macerated material was then stained with aqueous Safranin to produce semi-permanent slides.

Table 1
Analyses of the soil and canopy openness for the two sites in Serra do Conduru State Park, Bahia, Brazil. Advanced Stage of Forest Succession (ASFS) and Intermediate Stage of Forest Succession (ISFS). * indicate statistically different parameters by Mann-Whitney U test; s.d. standard deviation.

Sites	pH (H_2O)	Al	Ca	Mg	Ca + Mg	K	N	P	Coarse sand	Fine sand	Silt	Clay	Silt/ Clay	Canopy Openness
		cmol$_c$/dm^3					g/cm^3	mg/dm^3	g/kg					
ASFS	4.4	1.1	0.5	0.4	0.9	0.14	2.10	2	325	123	226	326	0.69	1245 ± 0,027
ISFS	4.3	0.9	0.4	0.3	0.7	0.08	1.71	1	382	189	247	182	1.36	1750* ± 0,081

The parameters used for comparing the wood samples from the two sites were frequency, length, diameter, vessel lumina area and thickness of vessel wall elements; size of inter-vascular and radial-vascular pits; length, diameter, lumina, and wall thickness of fibers pits; and frequency, length and width of radial parenchyma, with 25 measurements per parameter in each individual. Being that for the frequency of the vessel elements 25 fields of 1 mm^2 were measured in images obtained with a 5x objective. All descriptions, cell counts and measurements of wood samples followed the rules established by the IAWA Committee (1989).

All semi-permanent and permanent wood and leaf slides were analyzed using a bright-field light microscope (Axioplan, Zeiss, USA) and/or under polarized light (Jones, 1950), and photographs were taken using a camera (PowerShot A640, Canon, USA) coupled to the microscope. The images were used for quantitative analysis, which was performed using the software Image-Pro Plus version 4.0 for Windows.

2.2. Scanning electron microscopy (SEM)

To perform observations with SEM, fragments from the middle-third of leaf blades were removed from the fixing solution, washed with 0.05 M sodium cacodylate buffer, fixed with Osmium tetroxide solution (1%) in the same buffer, dehydrated in an ascending acetone series and subjected critical point drying (CPD 030, BAL-TEC, Germany). For wood analysis, fragments of specimens were analyzed using stereoscopic microscopy after critical point drying. After critical point drying, samples of leaf blades and wood fragments were affixed to aluminum supports with carbon tape, metalized with 20 nm of gold (DSS 050, BAL-TEC, Germany) and observed using a scanning electron microscope (Quanta 250, FEI, USA).

2.3. Data analysis

The verification of normality and homogeneity of variances for the measured characteristics of leaf and wood samples was performed using Shapiro-Wilk (Shapiro and Wilk, 1965) and Levene tests (Levene, 1960), respectively. When these tests failed, values were log-transformed and, in the case of wood, tested for differences between sites using t-test. If transformation failed to achieve normality and homogeneity of variances, as happened for leaves, the non-parametric Kruskal-Wallis test was used to test for differences. The non-parametric Mann-Whitney U test was used to test for significant differences between medians of canopy openness of the two sites (Zar, 1996). All statistical analyses were performed with software Statistica 7.

3. Results

3.1. General anatomical characteristics

To complement the comparative analyses, the characteristics of the leaf and wood were described previously. The leaves of *P. schlechtendaliana* are simple, opposite deccusate and obovate blade (Fig.1a), and showed anatomical characteristics typical of mesomorphic, i.e., hypostomatic and dorsiventral mesophyll, with a single-layered epidermis (Fig. 1b, c), well-developed spongy parenchyma, palisade parenchyma composed of 1–3 layers (Fig. 1d until g), raphide crystals are present in mesophyll. The adaxial and abaxial surfaces possess straight anticlinal walls (Fig. 1h, i). Paracytic stomata in abaxial surface (Fig. 1i). The adaxial surface having striated ornamentation (Fig. 1j) and the epicuticular layer is in crusts and tector type trichomes are present on the abaxial surface (Fig. 1k).

The wood anatomy of the *P. schlechtendaliana* exhibits growth rings slightly different, with moderate radial flattening of fibers and greater thickening of walls in mature wood (Fig. 2a, b). The vessel elements have diffuse porosity, outline rounded, solitary, or in arranged radial with two to five elements or in clusters of four elements (Fig. 2a, b). The fibers are septate (Fig. 2c, d) and characterized as fiber-tracheids. Axial parenchyma is rare or absent. The radial parenchyma has width 1–3 cells, heterogeneous being composed of procumbent cells in the central region and mostly two to four rows of upright and/or square cells at the margins (Fig. 2e) and with perforated ray cells. The perforation plates and side plates of vessel elements are simple and there are appendices in both extremities (Fig. 2f). The intervessel pits are bordered, small, alternate, vestured and/or scalariform (Fig. 2g); vessel-ray pits are similar to intervessel pits. Prismatic crystals are present in the secondary xylem, mainly in radial parenchyma cells (Fig. 2h).

3.2. Comparative anatomy

The two forest sites, ASFS and ISFS, have soil with different characteristics and significant differences in canopy openness (Table1). The difference in canopy openness (ASFS 12,45% and ISFS 17,50%) results in differences in the distribution of sunlight reaching the forest understory in each site. While the soil tends to be more clayey in ASFS and probably better in water retention compared to IFSF (Table 1).

Significant differences between sites involved mainly the weight of the leaf blade, adaxial cuticle and epidermis, palisade parenchyma, spongy parenchyma, palisade: spongy ratio and stomata density. Being that plants at ISFS had leaf lamina with thinner adaxial cuticle, smaller cells on the adaxial epidermis, smaller width of palisade parenchyma, smaller palisade:spongy parenchyma ratio, and lower stomatal density than at ASFS. In all the anatomical characteristics between top and base leaves, significant differences were observed, but mainly in the thickness of cuticle, palisade parenchyma and palisade:spongy parenchyma ratio that were greater in top leaves at both sites (Table 2, Fig. 1d until g).

For wood anatomy, significant differences between the two sites were also observed and involved frequency values, tangential and radial diameters, and vessel lumina area; all fibers characteristics; intervessel pits; and frequency and height of radial parenchyma. The plants at ISFS had presented smaller diameters of the lumen of vessels and fibers, higher frequencies of vessels and rays, and fiber and rays with longer lengths than at ASFS (Table 3, Fig. 2a until d).

4. Discussion

Our results show that the leaf and wood anatomy of *P. schlechtendaliana* underwent structural adjustments due to different conditions of successional stages and consequently with changes in the understory and canopy structure of the two study sites.

Studies of environmental features that influence structural adjustments and leads to morphoanatomical changes are very important for

Table 2
Quantitative anatomical parameters of the leaf of *Psychotria schlechtendaliana* in the two sites in Serra do Conduru State Park, Bahia, Brazil. Advanced Stage of Forest Succession (ASFS) and Intermediate Stage of Forest Succession (ISFS). Different letters indicate significant differences ($p < 0.05$) by Kruskal-Wallis test, followed by multiple comparisons. Uppercase letters refer to sites (comparisons in columns) and lowercase for top and base leaf (comparison lines).

Parameters		**Mean ± standard deviation**	
		Top leaves	**Base leaves**
Whole transverse section			
	ASFS	298.47 ± 31.79 Aa	288.82 ± 31.88 Ab
	ISFS	275.93 ± 38.1 Bb	287.44 ± 27.74 Aa
Cuticle			
Adaxial (µm)	ASFS	9.54 ± 2.37 Aa	8.33 ± 1.77 Ab
	ISFS	7.96 ± 2.15 Ba	6.9 ± 1.87 Bb
Abaxial (µm)	ASFS	3.17 ± 0.76 Aa	2.77 ± 0.59 Bb
	ISFS	3.22 ± 0.97 Aa	2.78 ± 0.72 Bb
Epidermis			
Adaxial surface (µm)	ASFS	61.02 ± 5.58 Aa	60.01 ± 5.66 Aa
	ISFS	57.23 ± 7.93 Bb	57.93 ± 6.29 Ba
Abaxial surface (µm)	ASFS	41 ± 6 Ab	42.45 ± 5.75 Aa
	ISFS	42 ± 7 Ab	43.76 ± 7.03 Aa
Parenchyma			
Palisade (µm)	ASFS	70.91 ± 11.32 Aa	65.6 ± 10.86 Ab
	ISFS	61.52 ± 11.38 Ba	57.62 ± 7.3 Bb
Spongy (µm)	ASFS	227.57 ± 28.9 Aa	223.22 ± 30.7 Bb
	ISFS	214.4 ± 31.63 Bb	229.82 ± 27.07 Aa
Palisade:spongy ratio			
	ASFS	0.32 ± 0.06 Aa	0.30 ± 0.07 Ab
	ISFS	0.3 ± 0.05 Ba	0.25 ± 0.45 Bb
	ASFS	203.94 ± 44.85 Aa	196.32 ± 46.08 Aa
	ISFS	186.03 ± 45.55 Ba	169.4 ± 40.61 Ba

Table 3
Quantitative anatomical parameters of the wood of *Psychotria schlechtendaliana* in two sites in Serra do Conduru State Park, Bahia, Brazil. Advanced Stage of Forest Succession (ASFS) and Intermediate Stage of Forest Succession (ISFS). * Indicates significant differences between sites ($p < 0.05$) by t-test.

Parameters	Mean ± standard deviation	
Vessel elements	ASFS	ISFS
Frequency (vessels. mm^{-2})	67.24 ± 17.89	78.1 ± 16.21 *
Length (µm)	1085.74 ± 246.44	1093.5 ± 281.83
Diameter tangential (µm)	46.05 ± 7.13 *	37.5 ± 6.62
Diameter Radial	45.5 ± 8.1*	32.4 ± 5.98
Vessel lumina area (µm^2)	1879.01 ± 522.71 *	1111.1 ± 323.1
Wall thickness (µm)	3.15 ± 0.68	3.0 ± 0.59
Intervessel pits (µm)	3.5 ± 0.61	3.83 ± 0.66 *
Vessel-ray pits (µm)	4.6 ± 0.82	4.55 ± 0.64
Fibres		
Diameter (µm)	32.68 ± 5.38 *	28.16 ± 4.55
Lumina (µm)	19.30 ± 5.44 *	15.88 ± 4.53
Length (µm)	1464.46 ± 248.31	1559.97 ± 191 *
Wall thickness (µm)	6.69 ± 1.63 *	6.14 ± 1.61
Pits (µm)	5.9 ± 1.86 *	4.28 ± 1.32
Radial parenchyma		
Frequency (rays. mm^{-1})	5.51 ± 1.54	6.92 ± 2.6 *
Length (µm)	622.74 ± 184.29	686.78 ± 201.63 *
Width (µm)	38.13 ± 8.06	37.43 ± 6.73

understanding species coexistence and the diversity of plant communities (Bittebiere et al., 2012). Species of the family Rubiaceae, such as those of the genus *Genipa* L. and *Psychotria* may have great ability to show phenotypic differences and high capacity to acclimate to sun and shade environments, which can provide a competitive advantage in dynamic and disturbed ecosystems (Lima et al., 2010; Sterck et al., 2013; Valladares et al., 2000). This study emphasizes an ecological-anatomical analysis using comparative studies of quantitative characteristics of leaves and wood of *P. schlechtendaliana*, complemented by qualitative diagnosis of the anatomy of these structures.

The findings regarding qualitative anatomy of the leaves and wood of *P. schlechtendaliana* are consistent with those reported for other species of the genus, and several species of Rubiaceae. For examples: leaf blade hypostomatic with dorsiventral mesophyll, presence of raphide crystals, striated cuticle, uniseriate epidermis, tector type trichome and paracytic stomata, and wood with layers of growth, rare axial parenchyma, vestured pits, vessel elements with simple, small and narrow perforation plates, perforated ray cells and the presence of prismatic crystals (Alexandrino et al., 2011; Assis and Giuliette, 1999; Campbell et al., 2016; Jansen et al., 2002, 2001; Kocsis et al., 2004; Marques et al., 2015; Metcalfe and Chalk, 1950; Moraes et al., 2011; Solereder, 1908; Vieira and Gomes, 1995). These characteristics were also observed in *P. schlechtendaliana*, this was the first description of blade leaf and wood anatomy for the species.

The presence of *P. schlechtendaliana* in the two sites is an evidence of its capacity to change under different environmental characteristics throughout the forest succession. The use of hemispherical images has been used extensively in the analysis of forest canopy structure and infers the availability and distribution of sunlight in the understory, among other microclimatic factors (Pinheiro et al., 2013; Suganuma et al., 2008). Different successional stages reflect changes in vegetation structure and species composition of forests (Solórzano et al., 2012). Therefore, the differences in canopy openness of ASFS and ISFS can explain the quantitative differences we found for *P. schlechtendaliana*. Since the two study sites are under the same rainfall regime, are at the same altitude and experience the same amount of sunlight, canopy openness and understory density are the primary factors influencing variation between the successional stages and it can be possibly related to microclimate differences. The measurement of changes in canopy openness during succession is a good indicator of microclimatic changes in the understory (Pinheiro et al., 2013). Thus, the canopy openness and soil influences the development of plants within a forest; this is particularly true for shade-tolerant individuals, as was found for *P. schlechtendaliana.* Also, the higher canopy openness increased atmospheric demand in ISFS understory, and soil with less clay and more sand tends to have lower water availability, suggesting that this site is prone to drought.

Changes in environmental factors, such as light availability, temperature and water, influence growth and organization of the leaf blade, changing the photosynthetic efficiency. They also influence the thickness and composition of the cuticle, the degree of development of the palisade parenchyma, the volume of intercellular spaces, the frequency of stomata and the density of vascular bundles (Dickison and Weitzman, 1996). In general, sun leaves have thicker cuticles with a greater proportion of wax than those that develop in moist, shaded environments (Esau, 2000), which was observed among the top leaves of *P. schlechtendaliana* from both sites. However, a thicker adaxial leaf cuticle was observed at ASFS. Mesophyll anatomy also varies with light availability (Baliza et al., 2012; Justo et al., 2005; Rabelo et al., 2013, 2012). Variation in leaf structure represents acclimation mechanisms related to biochemical changes in light capture and carbon fixation (Vogelmann et al., 1996). The presence of a more developed palisade parenchyma, for example, enhances photosynthesis, as it allows better penetration of parallel light than diffuse light (Vogelmann and Martin, 1993). Thus, cellular anatomy influences the path of light in the mesophyll (Brodersen and Vogelmann, 2010).

Comparative anatomical analyses of species in environments at different successional stages showed that characteristics, such as thickness of the cuticle of the adaxial epidermis and the palisade and spongy parenchyma, were greater in individual plants located in areas in a more advanced stage of succession compared to those in an area of intermediate stage of succession (Boeger and Wisniewski, 2003). The same was true for forests with few disturbances when compared to those that were disturbed (Rabelo et al., 2012). This is like to what was observed for the thickness of the adaxial cuticle, adaxial epidermis and palisade parenchyma of the *P. schlechtendaliana* leaves at ASFS.

Some studies have confirmed that environmental cues such as changes in atmospheric CO_2 concentration, light intensity, temperature, soil water and nutritional status also influence and determine the variation in the stomatal density and patterns (e.g. Barbieri et al., 2012; Beerling and Chaloner, 1993; Bergmann, 2004; Woorwark and Kelly, 1995). In our study *P. schlechtendaliana* individuals in the ISFS have low stomatal density to maintain low water flow and reduce water loss as consequence of the environment cues on this site. It is clear that investment in the tissue differentiation led structural adjustments, which is in accordance with the specific characteristics of each species and the phytophysiognomies and distribution of all other individuals in the forest.

The different environmental conditions at the two study sites at PESC also resulted in anatomical differences in wood of *P. schlechtendaliana*, corroborating the study of Barros et al. (2006) on other plant species of tropical moist forests. Variation in vessel elements, such as frequency, length and tangential diameter, and the diameter and thickness of the fibers, are closely related to environmental conditions, since they function to provide safe and efficient transport of water and solutes (Campbell et al., 2016; Carlquist, 2001; Dickison, 2000; Fichtler and Worbes, 2012; Ribeiro and Barros, 2006).

During periods of low water availability, vessel elements become shorter, narrower and greater in frequency (Angyalossy and Alves, 2005; Dickison, 2000; Luchi, 2004). These characteristics are consistent with those presented by individuals from ISFS that, even though situated in a forest with no defined dry season, has a more open canopy. In other words, the open canopy resulted in higher temperatures, lower air, and soil humidity in a manner consistent with to dry periods. The development of plastic characteristics ensures water flow and a positive water balance (Baas et al., 1983; Baas and Carlquist, 1985). At ASFS, *P. schlechtendaliana* had wider vessel elements, which are considered more efficient at transporting water than narrower elements, although they provide less hydraulic safety (Bosio et al., 2010).

According to Luchi (2004), the presence of fibers with larger diameters and larger lumina at ASFS is consistent with feature among individuals from humid environments where they serve to provide support to the secondary xylem, especially the cells of vessel elements with larger diameters. Regarding the frequency and height of the radial parenchyma, larger rays may also be associated with both humid environments (Alves and Angyalossy-Alfonso, 2002) and environments with lower water availability (Lima et al., 2009; Luchi, 2004). The cells of conductive structures provide hydraulic efficiency and safety, and their variation affects photosynthetic rates and leaf gas exchange (Chen et al., 2009).

Anatomical attributes of leaves and wood are very important for studies of ecological structural variation since they are correlated with microclimate changes, especially regarding the water and light influences to which plants are subjected to (Carlquist, 1977), something that was observed in this study. Other studies confirm that leaf and wood plasticity can be related to morphological, anatomical and physiological characters, and can help to better understand the photosynthetic standards and hydraulic processes throughout the plant (Fu et al., 2012; Kotowska et al., 2015; Markesteijn et al., 2011; Santiago et al., 2004).

Our results indicate that *P. schlechtendaliana* had quantitative differences in leaf blade features and wood produced by investing in specific anatomical changes for each organ. So each of the organs analyzed exhibited specific anatomical features for maintaining a survival of the individual to the peculiar conditions of different successional stages. The anatomical differences for this species that explained the variation between sites are those related to sunlight distribution and availably water, especially those involving the cuticle, stomata, photosynthetic parenchyma, vessel elements, fibers and radial parenchyma. Anatomical differences found between individuals of *P. schlechtendaliana* at the two PESC study sites confirm that structural adjustment in this species is a response to different successional stages. Therefore, structural responses commonly found in seasonal forests may also vary according to successional stage in rainforests (Kalacska et al., 2004). These results show that the ability to structural adjustments of the *P. schlechtendaliana* allows the occurrence in environments with different forest cover, contributing to understanding species diversity even in more deforested landscapes (Andrade et al., 2015).

5. Conclusions

The differences found between leaf blade and wood anatomy of individuals at the two sites confirm structural adjustments in relation to forest succession gradients, showing the interference of the sunlight distribution and of the water availability in the soil-plant-atmosphere continuum. The main anatomical attributes developed allows an adjustment in photosynthetic efficiency and safety water transport in *P. schlechtendaliana* according to variations of each site. Therefore, these findings may be very useful for understanding the biological basis of adaptation to environment and the structural adjustments developed by this species.

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Conflicts of interest

None.

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