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Wood anatomy and conductivity in lianas, shrubs and trees of Bignoniaceae

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ABSTRACT

This work compares potential xylem hydraulic efficiency among Bignoniaceae lianas, shrubs and trees. Five species from each growth habit were analysed to determine variance among habits based on quantitative and qualitative wood anatomical features. Potential hydraulic conductivity was calculated for each species in order to compare efficiency of water transport. Cambial variants are present in the Bignonieae tribe, as phloem wedges in lianas and phloem arcs in shrubs. Lianas present vessel dimorphism, quantitatively evidenced by the ratio of maximum by minimum vessel diameter of about 20, higher percentage of vessel area and lower percentage of fibres compared with the self-supporting species studied here. Potential hydraulic conductivity is higher in lianas due to the presence of wider vessels and it is hypothesised that the narrow vessels can function as back-up for water conduction when wider vessels are cavitated.

Keywords: Secondary xylem, climber, self-supporting, hydraulic architecture, lianescent vascular syndrome.

INTRODUCTION

Lianas, shrubs and trees are externally recognisable by their morphological attributes. Lianas can be distinguished by their narrow and long stems, typically with secondary growth, and their capacity to climb supporting hosts, usually other plants such as trees and shrubs, while ascending to the forest canopy. In general, such an ascent occurs by means of specialised structures, such as tendrils, hooks, adventitious roots, or, more commonly, twining stems (Darwin 1865; Schenck 1892; Isnard & Silk 2009; Putz 2012). Shrubs, on the other hand, can be distinguished by the presence of several woody stems departing from the plant base, while trees can be distinguished by the presence of a single woody stem that branches farther away from the base, forming its own crown (Radford *et al.* 1974). The external aspects of these habits are, more importantly, directly related to their anatomical architecture across development.

Apart from their morphological attributes, most lianas share a series of anatomical vascular features, which distinguish them from self-supporting habits. Taken together,

these special features have been coined as ‘lianescnt vascular syndrome’ (Angyalossy *et al.* 2015). The term represents a group of anatomical features that have been noted in lianas dating from the 19th century (Schenck 1893) to the present (Solereder 1908; Pfeiffer 1926; Metcalfe & Chalk 1950; Boureau 1957; Obaton 1960; Carlquist 1981, 1991; Angyalossy *et al.* 2012, 2015). These features include stems with cambial variants, secondary xylem marked by vessel dimorphism, high amounts of soft tissues (parenchyma and phloem), non-lignified parenchyma, and tall and wide rays. Although some of these features may also be present in shrubs and trees, all lianas have more than one of these characters in common (Angyalossy *et al.* 2012, 2015).

In addition, many structural anatomical features of lianas, shrubs and trees are directly related to mechanical support, flexibility, storage and hydraulic stem conductivity (Baas *et al.* 2004; Angyalossy *et al.* 2012, 2015). Lianas, for instance, share features, such as cambial variants, higher percentage of axial parenchyma, lower percentage of fibres, and rays that are both tall and wide. These are all aspects related to an increase in stem flexibility, torsion and storage (Carlquist 1991; Fisher & Ewers 1991; Rowe & Speck 2005; Isnard *et al.* 2012; Angyalossy *et al.* 2012). Lianas usually have very wide vessels combined with very narrow ones, a feature known as vessel dimorphism (Carlquist 1981), different from self-supporting plants and related to increased hydraulic efficiency and safety in water conduction, respectively (Ewers *et al.* 1991; Hacke *et al.* 2006).

Secondary xylem of self-supporting habits has anatomical features associated with both hydraulic conductivity and mechanical support, but responds to different selective pressures. For instance, shrubs and trees have a much higher percentage of fibres and narrower vessels as a result of increased demand for mechanical support and safer water transport (Baas *et al.* 2004; Rowe *et al.* 2004).

The Bignoniaceae family includes approximately 77 genera and 819 species (Olmstead *et al.* 2009; Lohmann & Taylor 2014), with 78% of these species distributed in the Neotropics and its centre of diversity in Brazil (Gentry 1991). Recent molecular phylogenetic studies (Olmstead *et al.* 2009) indicate that approximately half of the genera and species in Bignoniaceae belong to the tribe Bignonieae, a morphologically diverse group of neotropical lianas and, more rarely, shrubs (Lohmann 2004). Although lianas and trees of Bignoniaceae have similar features of secondary xylem, a number of anatomical features are exclusive to each particular habit (Gasson & Dobbins 1991; Pace & Angyalossy 2013). However, little is known about the anatomical structure of secondary xylem architecture in Bignoniaceae shrubs or how any differences among lianas, shrubs and trees might affect hydraulic efficiency in the family.

Therefore, our objectives were to (i) anatomically characterise secondary xylem of the stems of lianas, shrubs and trees of selected Bignoniaceae and, on the basis of identified anatomical differences, (ii) compare potential xylem hydraulic efficiency among the different habits.

Table 1. Collected genera, species, their authorships, habit, collector and site of collection.

Species	Habit	Collector	Site of collection
<i>Adenocalymma comosum</i> (Cham.) DC.	Liana	Pace, 53	Instituto Plantarum, Nova Odessa, São Paulo, Brazil
<i>Adenocalymma bracteatum</i> (Cham.) DC.	Liana	Lohmann, 861	Rio Negro margins, Amazonas, Brazil
<i>Cuspidaria convoluta</i> (Vell.) A.H. Gentry	Liana	Pace, 48	Instituto Plantarum, Nova Odessa, São Paulo, Brazil
<i>Fridericia platyphylla</i> (Cham.) L.G. Lohmann	Liana	Zuntini, 297	Serra do Cipó, Minas Gerais, Brazil.
<i>Fridericia chica</i> (Bonpl.) L.G. Lohmann	Liana	Pace, 50	Instituto Plantarum Nova Odessa, São Paulo, Brazil
<i>Anemopaegma robustum</i> Bureau & K. Schum.	Shrub	Gerolano, 7	Reserva Ducke, Manaus, Amazonas, Brazil
<i>Adenocalymma pedunculatum</i> (Vell.) L.G. Lohmann	Shrub	Gerolano, 1	Serra do Cipó, Minas Gerais, Brazil
<i>Adenocalymma peregrinum</i> (Miers) L.G. Lohmann	Shrub	Pace, 26	Uberlândia, Minas Gerais, Brazil
<i>Cuspidaria pulchra</i> (Cham.) L.G. Lohmann	Shrub	Pace, 24	Uberlândia, Minas Gerais, Brazil
<i>Fridericia platyphylla</i> (Cham.) L.G. Lohmann	Shrub	Pace, 22	Uberlândia, Minas Gerais, Brazil
<i>Handroanthus heptaphyllus</i> (Vell.) Toledo	Tree	Gerolano, 4	Cidade Universitária USP, São Paulo, São Paulo, Brazil
<i>Handroanthus ochraceus</i> (Cham.) Mattos	Tree	Gerolano, 2	Serra do Cipó, Minas Gerais, Brazil
<i>Jacaratanda mimosifolia</i> D. Don	Tree	Gerolano, 6	Cidade Universitária USP, São Paulo, São Paulo, Brazil
<i>Spathodea campanulata</i> P. Beauv.	Tree	Gerolano, 5	Cidade Universitária USP, São Paulo, São Paulo, Brazil
<i>Tabebuia aurea</i> (Silva Manso) Benth. & Hook.	Tree	Gerolano, 3	Cidade Universitária USP, São Paulo, São Paulo, Brazil

MATERIALS AND METHODS

Plant sampling

Fifteen species, accounting equally for lianas, trees and shrubs (5 of each) were sampled. These taxa belong to four of the nine major clades of Bignoniaceae. Lianas and shrubs belong to the tribe Bignonieae, while the trees belong to other clades in the family: Jacarandeae, Tecomeae s.s., and the Paleotropical clade. A complete list of specimens examined, their habit, collectors and site of collection is given in Table 1. Vouchers of the specimens collected were deposited in the University of São Paulo Herbarium (SPF). We designed the collections to account for the three different habits present in the family, with five species per habit. We sampled adult stem portions of all species. For lianas and shrubs, stem segments were cut with a minimum diameter of 2 cm, following the standard proposed by Gerwing *et al.* (2006), establishing this diameter as representative of adult liana stems. Tree samples were collected at breast height (1.30 m) by removing a stem portion of approximately 8 cm³, including bark, vascular cambium and xylem, with the aid of a hammer and chisel.

Anatomical preparation and analyses of secondary xylem

Stem samples were fixed in FAA50 (formaldehyde, acetic acid and 50 % ethanol; Johansen 1940) and later transferred and preserved in 70 % alcohol. The samples, including bark and xylem, were embedded in polyethylene glycol-PEG 1500 (Rupp 1964). Transverse and longitudinal sections of the stems were cut with a thickness of 15 to 25 µm, using a sliding microtome. In order to avoid tearing apart phloem and xylem during the sectioning, expanded polystyrene dissolved in butyl acetate was brushed on the samples, and an adhesive tape was attached before a section was cut (Barbosa *et al.* 2010). The sections were stained in 1% astra blue and 1% safranin (Bukatsch 1972, as modified by Kraus & Arduin 1997). Permanent slides were made with a synthetic resin.

In order to characterise the anatomical features of the different habits, microscopic descriptions were done for the secondary xylem of each species using the IAWA List of Features for Hardwood Identification (IAWA Committee 1989) as a guide. The obtained slides were observed and photographed using a photomicroscope (Leica DML and camera DFC 310FX). We sampled quantitative features using software ImageJ, version 1.45d (National Institutes of Health, Bethesda, MD, USA; <http://rsb.info.nih.gov/ij/>). The qualitative features were: a) presence or absence of cambial variants; b) presence of growth rings; c) vessel arrangement and grouping, presence or absence of vessel dimorphism, perforation plate types, intervessel and vessel-ray pit type; d) fibre wall thickness and the presence of septate fibres; e) axial parenchyma type, and f) ray cell composition. The quantitative variables were: g) vessel diameter (µm), frequency (vessel.mm⁻²), occupied area (% of vessels), and intervessel pit chamber size; h) axial parenchyma occupied area (%) and axial parenchyma strand length (µm); i) ray width (number of cells), height (µm) and occupied area (%); and j) fibre area (%).

To measure vessel diameter and frequency we used four images of 1 mm² from random portions of the stem transverse section of each specimen. We measured the tan-

gential diameter and the frequency of all vessels in each image. To characterise the vessel diameter amplitude between the habits we divided maximum by minimum vessel diameter (ratio VD_{max}/VD_{min}) of each specimen. For the lianas, we defined two vessel diameter classes: wide ($\geq 50 \mu\text{m}$) and narrow ($< 50 \mu\text{m}$). For the inter-vessel pit chamber size and axial parenchyma strand length we made 25 measurements for each specimen. To estimate the percentage of occupied area of vessels, fibres, axial and ray parenchyma, we used six 0.1 mm^2 images from random portions of the transverse section, and in each image we measured the occupied area of vessel, fibres, axial and radial parenchyma and divided by the image area. To estimate ray height and width we measured all rays present in four images of 1 mm^2 , as seen in longitudinal tangential sections. All analyses and measurements were accomplished for the lianas in the interwedge regions, *i.e.*, portions of the stem that have regular secondary growth. All numerical data collected were used to calculate means and standard deviations for each feature of each species of Bignoniaceae studied. In the description of quantitative characters of each habit, we present the mean, minimum and maximum values.

Potential hydraulic conductivity

Potential hydraulic conductivity was used to indirectly describe water transport efficiency according to vessel diameter (including wide and narrow vessels for the lianas) and vessel frequency.

Using vessel diameter and vessel frequency of each species, we calculated the potential hydraulic conductivity (K_p , Equation 1), following Hagen–Poiseuille's law (Poorter *et al.* 2010), as

$$K_p = \left(\frac{\pi p_w}{128 \eta} \right) \times N \times D_h^4 \quad (\text{Eq. 1})$$

where K_p is the potential hydraulic conductivity ($\text{Kg.m}^{-1}.\text{Mpa}^{-1}.\text{s}^{-1}$), p_w is the density of water at 20°C (998.2 kg.m^{-3}), η is the water viscosity at 20°C ($1.002 \cdot 10^{-3} \text{ Pa.s}^{-1}$), N is the vessel frequency (m^{-2}) and D_h is vessel hydraulic diameter (m).

We calculated vessel hydraulic diameter using Equation 2 (Tyree *et al.* 1994), as

$$D_h = \left[\left(\frac{1}{n} \right) \sum_{i=1}^n d^4 \right]^{1/4} \quad (\text{Eq. 2})$$

where n is the number of vessels and d is the vessel diameter.

Statistical analyses

We used the Shapiro–Wilk test for data normality. We tested differences in the quantitative wood anatomical variables and the vulnerability index of vessels among the habits using an analysis of variance (ANOVA) and then Tukey's test at 5% probability. In order to test differences in potential hydraulic conductivity, we used the Kruskal–Wallis non-parametric test and, subsequently, Dunn's test at 5% of probability. We performed all statistical analyses using R, version 2.15.1 (<http://www.r-project.org/>).

RESULTS

Secondary xylem of lianas, shrubs, and trees

The qualitative and quantitative anatomical features of secondary xylem for all habits are summarised in Table 2 and in the sections below classified by habit ($n = 5$ per habit).

Lianas (Fig. 1–10, Table 2): *Adenocalymma comosum*, *A. bracteatum*, *Cuspidaria convoluta*, *Fridericia chica*, and *F. platyphylla*.

Cambial variant – All lianas show xylem interrupted by four phloem wedges (Fig. 1).

Growth rings – Distinct in all analysed species, having a banded axial parenchyma as the initial marker and radially flattened and thick-walled fibres as the terminal marker (Fig. 2–4). Four species also have a growth marker characterized by differences in vessel diameter between earlywood and latewood; these are *Cuspidaria convoluta* (Fig. 1), *Fridericia platyphylla* (Fig. 2), *Adenocalymma bracteatum* and *Fridericia chica*.

Vessels – Semi-ring porous in *Cuspidaria convoluta* (Fig. 1), *Fridericia platyphylla* (Fig. 2), *Fridericia chica* and *Adenocalymma bracteatum* and diffuse porous in *Adenocalymma comosum* (Fig. 5). Vessel dimorphism present (Fig. 2, 4–6). Vessel diameter ranges from 9 to 352 μm , having a ratio VD_{\max}/VD_{\min} of 21 (11–39) (Table 2). Wide vessels 113 μm (86–134) and narrow vessels 23 μm (11–32) in tangential diameter. Wide vessels mainly solitary or associated with the narrow vessels; narrow vessels in clusters or radial multiples, of 2–11 vessels (Fig. 2, 4–6). Vessel frequency 52 vessels/ mm^2 (30–82). Vessels occupied 30% (16–40) of the total secondary xylem area. Perforation plates simple. Intervessel pits alternate, with a medium chamber size of 7.9 μm (7.1–9.4). Vessel-ray pits of two types: similar to the intervessel pits and rounded with reduced borders. Tyloses observed in single vessels of *Fridericia chica* and *F. platyphylla*.

Axial parenchyma – Bands 1–4 cells wide in *Adenocalymma bracteatum* (Fig. 3) and *A. comosum* and more than 4 cells wide in *Cuspidaria convoluta*, *Fridericia platyphylla* (Fig. 2), and *F. chica* (Fig. 4). Scanty paratracheal to vasicentric, forming short confluences in all species, such as observed in *Cuspidaria convoluta* (Fig. 6); strand length 2–6 cells (Fig. 7) or 278 μm (142–401). Parenchyma occupied 20% (12–26) of the total secondary xylem area.

Rays – Heterocellular with procumbent, square and slightly upright cells mixed throughout the ray (Fig. 8) and non-storied (Fig. 9); varying from 2–4 cells wide, but predominantly 2–3-seriate (Fig. 7, 9, 10). Perforated ray cells encountered in *Cuspidaria convoluta*, *Fridericia chica* and *F. platyphylla*. Ray height 859 μm (577–1316). Rays occupied 19% (13–23) of the total secondary xylem area.

Fibres – Septate (Fig. 10); thin- to thick-walled. Fibres occupied 25% (18–34) of the total secondary xylem area.

Shrubs (Fig. 11–20, Table 2): *Adenocalymma pedunculatum*, *Adenocalymma peregrinum*, *Anemopaegma robustum*, *Cuspidaria pulchra*, and *Fridericia platyphylla*.

Cambial variant – All shrubs have xylem weakly interrupted by four phloem arcs (Fig. 11, 12).

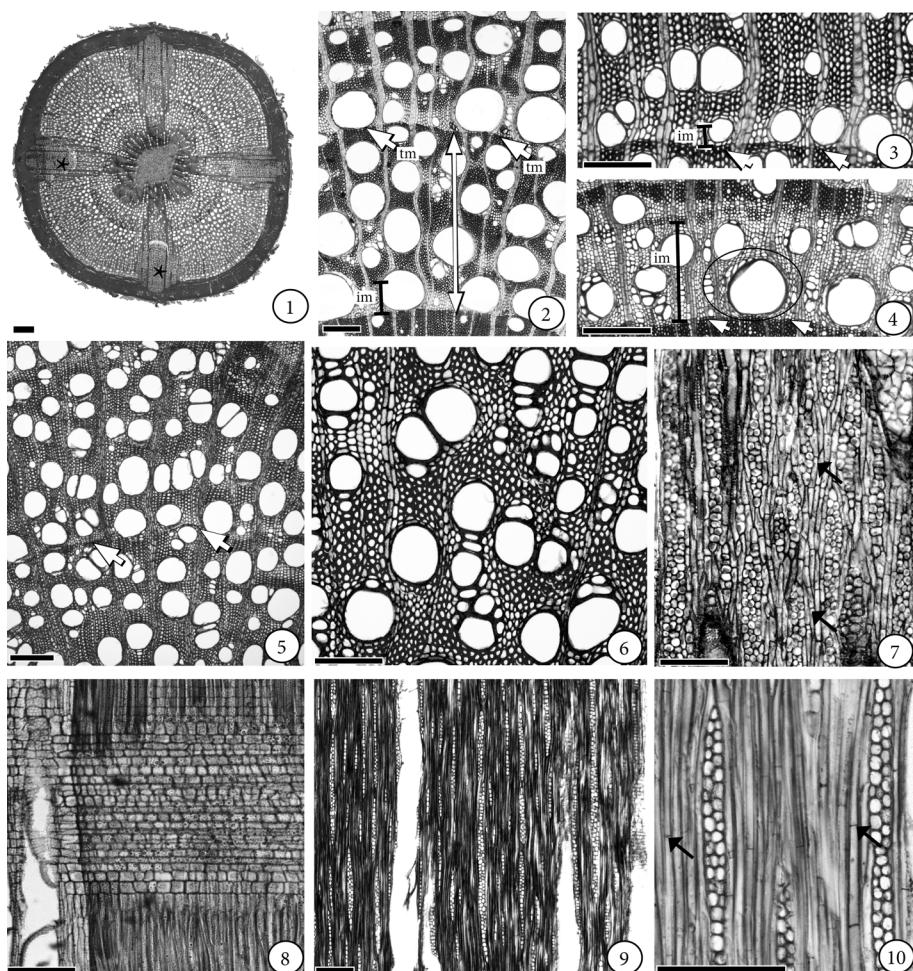


Figure 1–10. Lianas. – 1–6: TS. – 1: *Cuspidaria convoluta*; general aspect of the stem; xylem interrupted by phloem wedges (*); semi-ring porosity, with wider vessels as initial growth ring marker. – 2: *Fridericia platyphylla*; semi-ring porosity and growth ring (double arrowhead); wider vessels and axial parenchyma in bands as initial growth ring markers (im); narrower vessels and radially flattened fibres as terminal growth ring markers (tm). – 3: *Adenocalymma bracteatum* and – 4: *Fridericia chica*; axial parenchyma band as initial marker (im) and thicker-walled and radially flattened fibres as terminal growth ring marker from the previous growth ring (arrows). Note the presence of vessel dimorphism indicated by the black circle in Fig. 4. – 5: *Adenocalymma comosum*; diffuse porous, thicker-walled and radially flattened fibres as terminal growth marker (arrows). – 6: *Cuspidaria convoluta*; axial parenchyma vasicentric with short confluences; note vessel dimorphism. – 7 & 8: *Fridericia chica*. – 7: TLS; axial parenchyma strands (arrows); note rays 2–4 cells wide. – 8: RLS; heterocellular ray. – 9 & 10: *Fridericia platyphylla*. – 9: TLS; rays non-storied. – 10: TLS; septate fibres (arrows). – Scale bars: 1 = 1 mm; 2–10 = 200 µm.

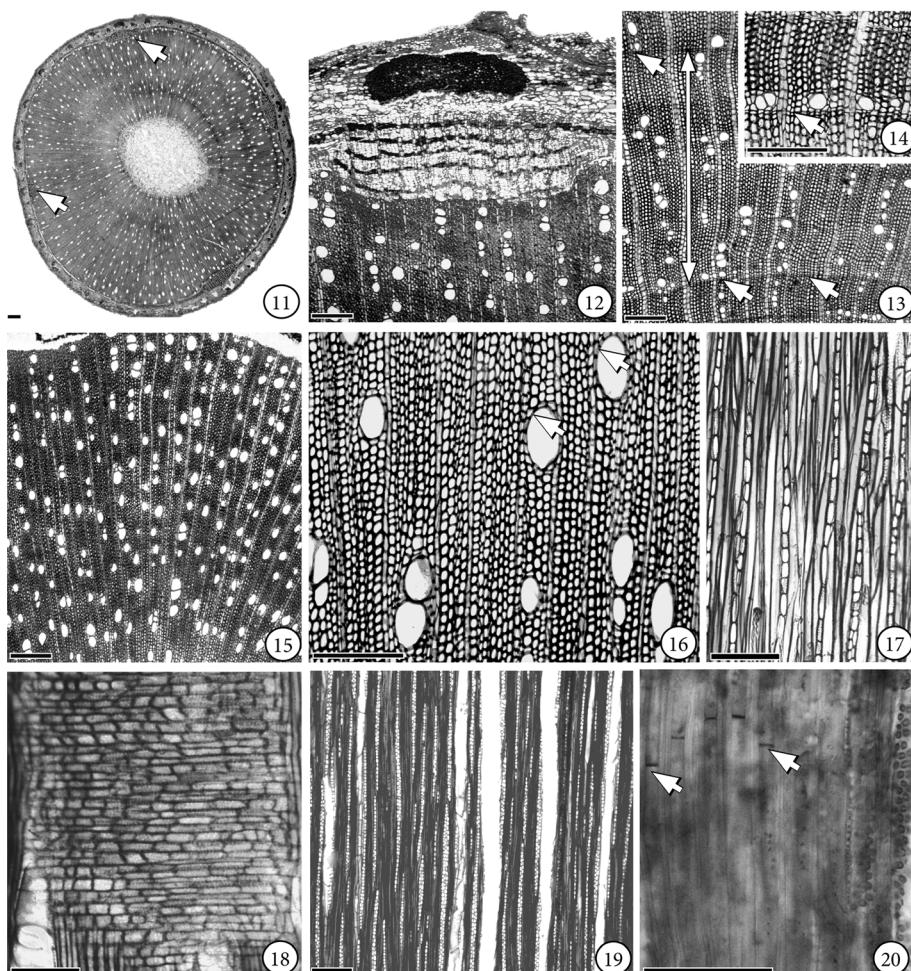


Figure 11–20. Shrubs. – 11–16: TS. – 11: *Fridericia platyphylla*; general aspect of the stem; xylem interrupted by phloem arcs (arrows) and diffuse porous. – 12: *Adenocalymma peregrinum*; phloem arc detail. – 13 & 14: *Anemopaegma robustum*; growth ring (double arrowhead); radially flattened fibres as terminal growth ring marker (arrows). – 14: Detail of radially flattened fibres. – 15: *Adenocalymma peregrinum*; growth rings absent, diffuse porous, vessels solitary and in multiples. – 16: *Fridericia platyphylla*; axial parenchyma scanty paratracheal to vascentric (arrows). – 17: *Fridericia platyphylla*; TLS; uniseriate and non-storied rays (by courtesy of Marcelo Pace). – 18: *Adenocalymma pedunculatum*; RLS; heterocellular ray. – 19: *Adenocalymma peregrinum*; TLS; multiseriate non-storied rays. – 20: *Adenocalymma pedunculatum*; TLS; septate fibres (arrows). – Scale bars: 11 = 1 mm; 12–20 = 200 µm.

Growth rings – Distinct in *Adenocalymma pedunculatum* and *Anemopaegma robustum* (Fig. 13) and marked by radially flattened and thick-walled fibres (Fig. 13, 14). Indistinct in *Adenocalymma peregrinum* (Fig. 15), *Cuspidaria pulchra* and *Fridericia platyphylla*.

Vessels – Diffuse porous with solitary and multiples of 2–4 vessels in all species (Fig. 11–13, 15, 16). Vessel dimorphism absent (Fig. 11–13, 15, 16). Vessel diameter of 41 µm in tangential diameter (10–114), having a ratio VD_{max} / VD_{min} of 7 (5–9) (Table 2). Vessel frequency 30 vessels/mm⁻² (14–44). Vessels occupied 9% (6–14) of the total secondary xylem area. Perforation plates simple. Intervessel pits alternate, with a small chamber size of 6.0 µm (5.7–6.3). Vessel-ray pits rounded with reduced borders.

Table 2. Quantitative anatomical features of the secondary xylem of lianas, shrubs and trees (mean ± standard deviation). Present (+) or absent (−). (see also the next page) →

Habit / Species	Vessel							
			Vessel diameter					
	Cambial variant	Vessel dimorphism	Diameter wide vessel (µm)	Diameter narrow vessel (µm)	Vessel/mm ⁻²	Vessel area (%)	Ratio VD_{max} / VD_{min}	Intervessel pit size (µm)
Lianas								
1: <i>Adenocalymma comosum</i>	+	+	113 (± 38)	31 (± 9.2)	32 (± 3.5)	38 (± 7.4)	13	9.4 (± 0.8)
2: <i>Adenocalymma bracteatum</i>	+	+	86 (± 20)	13 (± 11)	74 (± 22)	16 (± 5.0)	11	7.1 (± 0.7)
3: <i>Cuspidaria convoluta</i>	+	+	109 (± 29)	11 (± 9.5)	82 (± 11.5)	38 (± 8.1)	20	7.1 (± 0.5)
4: <i>Fridericia chica</i>	+	+	111 (± 49)	32 (± 9.6)	30 (± 4.6)	21 (± 5.0)	26	8.3 (± 1.0)
5: <i>Fridericia platyphylla</i>	+	+	134 (± 73)	30 (± 10)	43 (± 3.7)	40 (± 8.0)	39	7.8 (± 1.8)
Mean for all lianas	+	+	113 (± 20)	23 (± 10.4)	52 (± 21)	30 (± 11)	21 (± 11)	7.9 (± 0.9)
Shrubs								
1: <i>Adenocalymma pedunculatum</i>	+	–	49 (28.6)	44 (± 7.1)	14 (± 5.4)	9	6.0 (± 0.7)	
2: <i>Adenocalymma peregrinum</i>	+	–	32 (± 9.8)	43 (± 3.1)	11 (± 3.5)	7	5.7 (± 0.9)	
3: <i>Anemopaegma robustum</i>	+	–	41 (± 12.9)	23 (± 2.1)	7 (± 1.8)	5	–	
4: <i>Cuspidaria pulchra</i>	+	–	39 (± 17.1)	28 (± 9.9)	8 (± 7.0)	9	6.3 (± 0.8)	
5: <i>Fridericia platyphylla</i>	+	–	45 (± 15.5)	14 (± 5.8)	6 (± 0.8)	5	6.2 (± 1.3)	
Mean for all shrubs	+	–	41 (± 6.4)	30 (± 13)	9 (± 3.6)	7 (± 2)	6.0 (± 0.2)	
Trees								
1: <i>Handroanthus heptaphyllus</i>	–	–	99 (21.4)	20 (± 1.8)	13 (± 5.9)	4	3.4 (± 0.4)	
2: <i>Handroanthus ochraceus</i>	–	–	53 (± 12.9)	61 (± 4.6)	21 (± 9.3)	3	9.5 (± 0.7)	
3: <i>Jacaranda mimosifolia</i>	–	–	75 (± 18.9)	28 (± 8.2)	17 (± 8.0)	5	9.1 (± 0.7)	
4: <i>Spathodea campanulata</i>	–	–	116 (± 35.8)	10 (± 1.5)	17 (± 2.2)	4	5.0 (± 0.5)	
5: <i>Tabebuia aurea</i>	–	–	90 (± 31.8)	14 (± 5.3)	19 (± 8.1)	5	6.1 (± 0.4)	
Mean for all trees	–	–	87 (± 23.9)	26 (± 18)	17 (± 3)	4 (± 0.7)	6.6 (± 2.6)	

Axial parenchyma – Scanty paratracheal to vasicentric in all shrubs (Fig. 16); 3–4 cells per strand, with 186 µm (85–308) length. Parenchyma occupied 2.4% (2–3) of the total secondary xylem area.

Rays – Uniseriate in *Fridericia platyphylla* (Fig. 17) and 1–3 cells wide in *Adenocalymma pedunculatum*, *Adenocalymma peregrinum*, *Anemopaegma robustum* and *Cuspidaria pulchra*. Heterocellular with procumbent, square and upright cells mixed throughout the ray (Fig. 18) and non-storied (Fig. 17, 19). Perforated ray cells absent. Ray height 980 µm (555–1465). Rays occupied 11% (9–14) of the total secondary xylem area.

Fibres – Septate (Fig. 20); mostly very thick-walled in *Adenocalymma pedunculatum* and thin- to thick-walled in *Adenocalymma peregrinum*, *Anemopaegma robustum*,

Table 2 continued.

←

	Axial parenchyma						Ray				Fibre			
	Vascentric	Axiform	Confluent	Marginal band	Scanty paratracheal	Axial parenchyma area (%)	Heterocellular rays	Storied rays	Ray width (cell(s))	Ray height (μm)	Ray area (%)	Fibre very thick-walled	Septate fibre	Fibre area (%)
Lianas														
1:	+	-	+	+	+	12 (± 4.4)	+	-	2-3	1316 (± 1125)	13 (± 4.5)	-	+	34 (± 4.1)
2:	+	-	+	+	+	26 (± 20)	+	-	2-3	1067 (± 720)	17 (± 7.2)	-	+	25 (± 8.9)
3:	+	-	+	+	+	21 (± 3.2)	+	-	2-3	577 (± 291)	18 (± 7.9)	-	+	23 (± 10)
4:	+	-	+	+	+	24 (± 11.5)	+	-	2-3	611 (± 208)	22 (± 3.6)	-	+	25 (± 7.4)
5:	+	-	+	+	+	18 (± 5.6)	+	-	3-4	724 (± 230)	23 (± 7.7)	-	+	18 (± 6.6)
	+	-	+	+	+/-	20.1 (± 5)	+	-	2-4	859 (± 320)	19 (± 4.1)	-	+	25 (± 5.7)
Shrubs														
1:	+	-	-	-	-	2 (± 0.9)	+	-	2-3	1041 (± 439)	9 (± 4.8)	+	+	48 (± 6.3)
2:	-	-	-	-	-	2 (± 0.6)	+	-	1-2	555 (± 165)	14 (± 1.9)	-	+	63 (± 5.9)
3:	+	-	-	-	-	3 (± 0.8)	+	-	1-2	1465 (± 500)	13 (± 5.1)	-	+	71 (± 4.8)
4:	+	-	-	-	-	3 (± 1.2)	+	-	1-2	709 (± 401)	9 (± 2.8)	-	+	78 (± 12)
5:	+	-	-	-	-	2 (± 0.7)	+	-	1	1128 (± 358)	12 (± 1.7)	-	+	75 (± 6.9)
	+/-	-	+/-	-	+	2.4 (± 0.3)	+	-	1-3	980 (± 358)	11 (± 2.4)	+/-	+	67 (± 12)
Trees														
1:	-	+	+	+	-	21 (± 12)	-	+	2	195 (± 44)	11 (± 3.5)	-	-	53 (± 17)
2:	-	+	+	+	-	26 (± 4.4)	-	+	1-2	137 (± 12)	7 (± 2.8)	+	-	37 (± 10)
3:	+	+	+	+	-	18 (± 6.3)	-	-	1	275 (± 97)	10 (± 3)	-	-	49 (± 10)
4:	-	+	+	+	-	22 (± 9)	+	-	3-4	286 (± 84)	15 (± 7.1)	-	-	41 (± 7.9)
5:	-	+	+	+	-	20 (± 4.4)	-	+	2	153 (± 25)	28 (± 7.7)	+	-	31 (± 6.7)
	+/-	+	+	+	-	21.6 (± 3)	+/-	+/-	1-4	209 (± 68)	14 (± 7.9)	+/-	-	42 (± 9)

Cuspidaria pulchra, and *Fridericia platyphylla*. Fibres occupied 67% (48–78) of the total secondary xylem area.

Trees (Fig. 21–30, Table 2): *Handroanthus heptaphyllus*, *H. ochraceus*, *Jacaranda mimosifolia*, *Spathodea campanulata*, and *Tabebuia aurea*.

Cambial variant – All trees lack cambial variants (Fig. 21).

Growth rings – Present (Fig. 22), delimited by marginal parenchyma bands of 1–4 cells wide (Fig. 22–25) and radially flattened and thick-walled fibres (Fig. 22, 23).

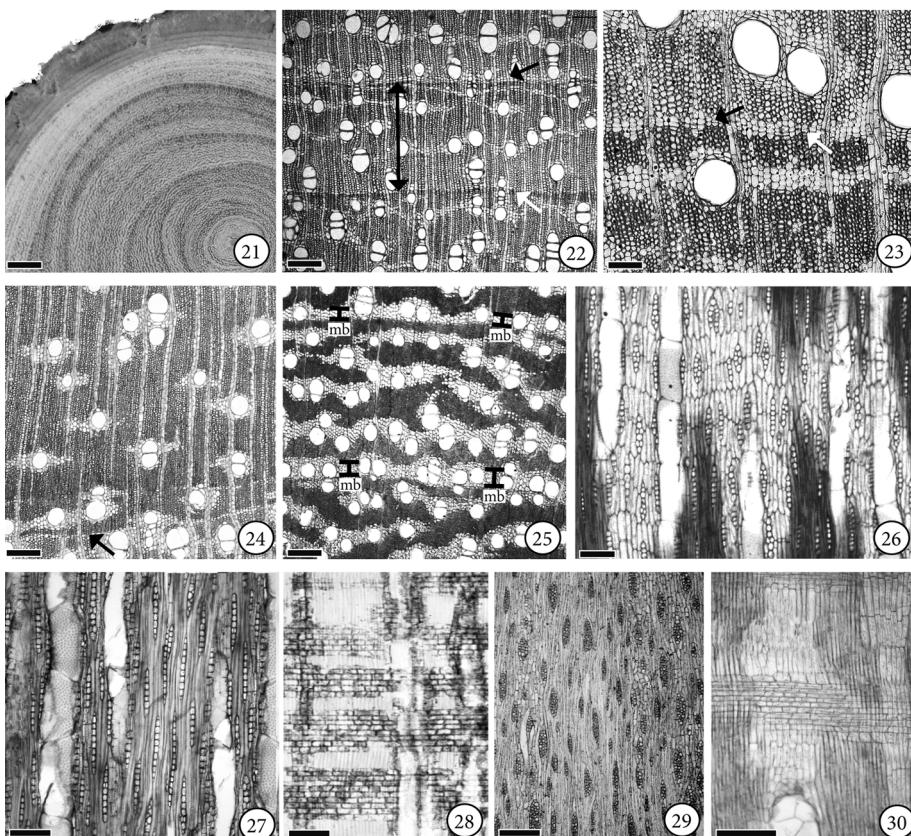


Figure 21–30. Trees. – 21–25: TS. – 21: *Handroanthus heptaphyllus*; general aspect of the stem; cambial variant absent. – 22: *Jacaranda mimosifolia* and – 23: *Spathodea campanulata*; growth ring present (double arrowhead, Fig. 22); marginal banded axial parenchyma (black arrow); thicker-walled, radially flattened fibres (white arrow). – 24: *Tabebuia aurea*; axial parenchyma aliform, forming short confluentes and in marginal band (arrow). – 25 & 26: *Handroanthus ochraceus*. – 25: Axial parenchyma aliform forming long confluentes; marginal bands (mb). – 26: TLS; axial parenchyma storied, in strands of 2–4 cells; storied rays. – 27 & 28: *Jacaranda mimosifolia*. – 27: TLS; rays uniseriate non-storied. – 28: RLS; homocellular rays. – 29 & 30: *Spathodea campanulata*. – 29: TLS; multiseriate non-storied rays. – 30: RLS; heterocellular rays. – Scale bars: 21 = 2 mm; 22–30 = 200 µm.

Vessels – Diffuse porous (Fig. 22–25); solitary or in multiples of 2–4 vessels (Fig. 22–25). Vessel diameter of 87 µm in tangential diameter (22–193), having a ratio VD_{max}/VD_{min} of 4 (3–5) (Table 2). Vessel frequency 26 vessels.mm⁻² (10–61). Vessels occupied 17% (13–21) of the total secondary xylem area. Perforation plates simple. Intervessel pits alternate and small, 6.6 µm (3.4–9.5). Vessel-ray pits similar to the intervessel pits.

Axial parenchyma – Aliform (Fig. 24), forming short (Fig. 22, 24) to long confluences (Fig. 25) and in marginal bands of 1–4 cells wide in all species (Fig. 22–25); 2–4 cells per strand (Fig. 26); strands 243 µm (180–446) length, storied in *Handroanthus heptaphyllus*, *H. ochraceus* (Fig. 26) and *Tabebuia aurea* and non-storied in *Jacaranda mimosifolia* and *Spathodea campanulata*. Parenchyma occupied 21.6% (18–26) of the total secondary xylem area.

Rays – Storied and homocellular in *Handroanthus ochraceus* (Fig. 26) and *Tabebuia aurea* and irregularly storied in *Handroanthus heptaphyllus*; 1–2 cells wide in these three species. Non-storied, homocellular and uniserial in *Jacaranda mimosifolia* (Fig. 27, 28). Non-storied and heterocellular, with one or, occasionally, two rows of square marginal cells in *Spathodea campanulata* (Fig. 29, 30); 3–4 cells wide (Fig. 29). Perforated ray cells absent. Ray height 209 µm (137–286). Rays occupied 14% (7–28) of the total secondary xylem area.

Fibres – Non-septate, mostly very thick-walled in *Handroanthus ochraceus* (Fig. 25) and thin- to thick-walled in *Tabebuia aurea*, *Spathodea campanulata*, *Handroanthus heptaphyllus*, and *Jacaranda mimosifolia* (Fig. 22, 23). Fibres occupied 42% (31–53) of the total secondary xylem area.

Comparison of the quantitative anatomical features among habits

All results from the statistical analyses comparing the quantitative data among the different habits are summarised in Table 3.

Vessel diameter – Lianas have the widest range of vessel diameters, from 9 to 352 µm, and the mean ratio VD_{max}/VD_{min} is significantly higher in lianas (21) when compared with the trees (4.4) and the shrubs (7). Vessel diameters of shrubs and trees have more restricted distribution, ranging from 10 to 114 µm in shrubs and from 20 to 193 µm in trees.

Vessel frequency – No statistical difference was detected for vessel frequency among the habits (Table 3).

Vessel area – The area occupied by vessels was significantly higher in lianas when compared to self-supporting species (Table 3).

Intervessel pit chamber size – No statistical difference was detected for intervessel pit chamber size among the habits (Table 3).

Axial parenchyma area – Similar between lianas and trees and significantly higher than in shrubs (Table 3).

Axial parenchyma strand length – No statistical difference was detected for axial parenchyma strand length among the habits (Table 3).

Ray height – Similar between lianas and shrubs and significantly taller than that observed in trees (Table 3).

Ray area – No statistical difference was detected for ray area among the habits (Table 3).

Fibre area – Similar between shrubs and trees and significantly lower in the lianas. More than half of the total secondary xylem area is composed of fibres in the shrubs (67%), while it is 50% in trees and 25% in lianas (Table 3).

Table 3. Statistical comparison of the anatomical features among lianas, shrubs and trees.

Equal letters indicate statistical similarities among the habits to each variable according to Tukey's test at 5% ($N = 15$). F_{df} value and p-value refer to the analysis of variance (ANOVA). Significant values are in bold.

Feature	Habit	Mean ($\pm SD$)	Tukey (5%)	$F_{2,12}$	p-value
Ratio VD_{max}/VD_{min}	Liana	21.0 (± 11)	A	7.92	0.006
	Shrub	7.0 (± 2.2)	B		
	Tree	4.4 (± 0.7)	B		
Vessels. mm^{-2}	Liana	52 (± 3.7)	A	2.44	0.12
	Shrub	30 (± 13)	A		
	Tree	26 (± 18)	A		
Vessel area (%)	Liana	30 (± 11.3)	A	11.4	0.001
	Shrub	9 (± 3.6)	B		
	Tree	17 (± 3.0)	B		
Intervessel pit size (μm)	Liana	7.9 (± 0.9)	A	1.4	0.27
	Shrub	6.0 (± 0.2)	A		
	Tree	6.6 (± 2.6)	A		
Axial parenchyma area (%)	Liana	20.1 (± 5.0)	A	45.3	<0.001
	Shrub	2.4 (± 0.3)	B		
	Tree	21.6 (± 3.0)	A		
Axial parenchyma strand (μm)	Liana	278 (± 39.2)	A	2.16	0.16
	Shrub	186 (± 55.0)	A		
	Tree	243 (± 91.9)	A		
Ray height (μm)	Liana	859 (± 320)	A	10.8	0.02
	Shrub	980 (± 358)	A		
	Tree	209 (± 68)	B		
Ray area (%)	Liana	19 (± 4.1)	A	2.35	0.13
	Shrub	11 (± 2.4)	A		
	Tree	14 (± 7.9)	A		
Fibre area (%)	Liana	25 (± 5.7)	B	25.9	<0.001
	Shrub	67 (± 12.0)	A		
	Tree	42 (± 9.0)	A		

Differences in potential conductivity among habits

Potential hydraulic conductivity – The mean potential hydraulic conductivity in lianas (209 ± 195) is significantly higher than that in shrubs (7.0 ± 9.3) and trees (44 ± 22) (Table 4).

Table 4. List of potential hydraulic conductivity (K_p , 10^{-6} $\text{Kg} \cdot \text{m}^{-1} \cdot \text{Mpa}^{-1} \cdot \text{s}^{-1}$) for each of the species and habits.

H_{df} and p-values refer to the non-parametric analysis of variance (Kruskal-Wallis) used for potential hydraulic conductivity. Equal letters indicate statistical similarity among the habits under Dunn's test at 5%.

Habit	Species	K_p ($H_2 = 10.02$, p < 0.05)
Liana	<i>Adenocalymma comosum</i>	309.4
Liana	<i>Adenocalymma bracteatum</i>	40.8
Liana	<i>Cuspidaria convoluta</i>	82.7
Liana	<i>Fridericia chica</i>	108.4
Liana	<i>Fridericia platyphylla</i>	504.9
Mean for all lianas		209.2 ± 194.9 (A)
Shrub	<i>Adenocalymma pedunculatum</i>	23.6
Shrub	<i>Adenocalymma peregrinum</i>	1.9
Shrub	<i>Anemopaegma robustum</i>	2.7
Shrub	<i>Cuspidaria pulchra</i>	3.9
Shrub	<i>Fridericia platyphylla</i>	2.6
Mean for all shrubs		6.9 ± 9.3 (B)
Tree	<i>Handroanthus heptaphyllus</i>	62.2
Tree	<i>Handroanthus ochraceus</i>	16.7
Tree	<i>Jacaranda mimosifolia</i>	29.7
Tree	<i>Spathodea campanulata</i>	68.9
Tree	<i>Tabebuia aurea</i>	40.3
Mean for all trees		43.6 ± 21.8 (B)

DISCUSSION

External and internal morphological aspects of the studied species are a key to distinguishing among different habits, and these differences also lead to functionally different hydraulics between lianas and self-supporting species. In our study, stem cambial variants and, in secondary xylem, vessel diameter and vessel area, axial parenchyma type and parenchyma area, ray height and fibre area were the main anatomical features that varied among habits. Potential hydraulic conductivity also varied among the habits.

Comparison of the anatomical features according to habit

Cambial variant – Unlike the trees of Bignoniaceae, which lack cambial variants (Gasson & Dobbins 1991; Pace & Angyalossy 2013), lianas and shrubs of the tribe Bignonieae herein analysed have xylem interrupted by phloem, in lianas, by four phloem wedges, and in shrubs, by four phloem arcs. The less pronounced cambial variants in shrubs have been reported for shrubs in the tribe Bignonieae earlier (Pace *et al.* 2009; Pace & Angyalossy 2013) and have been related to paedomorphosis in the development of the cambial variant. The tribe Bignonieae constitutes a monophyletic group in which the cambial variant is synapomorphic (Lohmann 2006; Lohmann &

Taylor 2014), and it is well known that lianas of this group, like the species studied here, have cambial variants (Schenck 1893; Gentry 1980; Dos Santos 1995; Lohmann 2006; Pace *et al.* 2009). Shrubs in this tribe have sometimes been described as also having cambial variant (Pace *et al.* 2009, 2015) and sometimes as lacking cambial variants altogether (Gentry 1980). In this study, the cambial variant was detected in shrubs and lianas, although less pronounced in the shrubs. We attribute the presence of the cambial variant in the stems of shrubs in this tribe to their derivation from lianoid ancestors (Lohmann 2003).

Porosity and Growth rings – Semi-ring porosity and growth rings delimited by marginal parenchyma, wide vessels and radially flattened fibres are both present in the studied lianas. These characters have also been well documented by Lima *et al.* (2010) and complemented by observations of Pace and Angyalossy (2013) and Pace *et al.* (2015). In Bignoniaceae, ring porosity and semi-ring porosity are thought to have evolved from diffuse porous species that originally inhabited mesic environments, but then migrated to more seasonal environments, either marked by a conspicuous winter (*e.g.*, *Bignonia capreolata* in high latitudes of the northern hemisphere) or a conspicuous dry season (*e.g.*, *Catophractes* in African savannas; Pace & Angyalossy 2013).

Vessels – Vessel dimensions vary among the habits, with the widest range observed in the lianas, as also noted by previous authors (Carlquist 1985; Ewers & Fisher 1989; Gutiérrez *et al.* 2009; Pace & Angyalossy 2013). Moreover, in lianas the maximum vessel diameter is about 20 times higher than the minimum vessel diameter, which, quantitatively, is a clear illustration of vessel dimorphism. Comparing this ratio through the habits, the ratio in lianas is about five times that in the trees and three times that in the shrubs. Vessel dimorphism is one of the main features of the lianescent vascular syndrome (Angyalossy *et al.* 2015; Pace *et al.* 2015), and has been associated with a combination of an increasing of efficiency in water conduction provided by the wide vessels and safety in transport provided by the narrow vessels that function as back-up for water conduction when wider vessels are cavitated (Ewers 1985; Ewers *et al.* 1991; Gutiérrez *et al.* 2009). This will be further explored in this discussion.

Axial parenchyma – Lianas and trees have a higher area of their secondary xylem occupied by axial parenchyma than the shrubs. Previous studies have also reported a decrease in axial parenchyma abundance in shrubs of the tribe Bignonieae (Pace & Angyalossy 2013). Comparing the species belonging to *Adenocalymma* and *Cuspidaria*, as analysed herein, in addition to the different specimens of *Fridericia platyphylla* growing either as lianas or as shrubs, we noticed that lianas have from 6 to 13 times more axial parenchyma by area compared to the shrubs. This difference is related to the type of axial parenchyma present in both habits. The presence of vasicentric axial parenchyma forming confluences and in marginal bands, as seen in the lianas of Bignoniaceae studied here and elsewhere (Dos Santos 1995; Pace & Angyalossy 2013), increases the total area of secondary xylem occupied by this cell type. On the other hand, the shrubs studied here have scanty to vasicentric axial parenchyma, occupying a much lower area of secondary xylem. In general, lianas are known to have more abundant axial parenchyma than self-supporting plants (Carlquist 1985;

Angyalossy *et al.* 2012, 2015), which phenomenon has been related to an increased flexibility allowing stems to climb (Rowe *et al.* 2004; Wagner *et al.* 2012), to store water and carbohydrates and repair injury (Carlquist 1985; Ewers & Fisher 1989). Additionally, in our study, the total area occupied by axial parenchyma in lianas was shown to be very similar to that of trees. This probably results from the type of parenchyma of trees and lianas in Bignoniaceae, which is formed by vasicentric and/or aliform confluent parenchyma and also marginal bands. Different from many other tropical species, Alves and Angyalossy-Alfonso (2002) demonstrated that trees of Bignoniaceae have a higher percentage of axial parenchyma of the same type observed here. The authors attribute this to an accessory system supporting hydraulic conduction (cf. Czaninski 1968; Braun 1984).

Rays – Ray height and composition varied among the habits in a manner similar to that described by Gasson & Dobbins (1991), Dos Santos (1995) and Pace & Angyalossy (2013). Heterocellular rays are present in all habits, especially in lianas and shrubs of tribe Bignonieae where different cell shapes are mixed. Homocellular rays are common in trees of the family. Lianas and shrubs in this study have a ray height exceeding 1 mm and thus about eight times higher than that of trees. Perforated ray cells are exclusively present in the lianas, a feature strongly related to the lianescent habit, but absent elsewhere in the family (Pace & Angyalossy 2013).

Fibres – The total area of secondary xylem occupied by fibres is lower in lianas (25% of the total area) than in the self-supporting species (67% and 42% in shrubs and trees, respectively), as has been found in other lianoid taxa (Angyalossy *et al.* 2012; Crivellaro *et al.* 2012). For instance, if we compare the lianescent specimen of *Fridericia platyphylla* with the shrubby specimen of the same species, the percentage of fibres is three times higher in the shrubby specimen. A similar difference was observed by Crivellaro *et al.* (2012), who reported that the percentage of fibres in lianas was two times lower than that of shrubs from Cyprus. The higher abundance of fibres in self-supporting species, when compared to the lianas, is directly related to their erect habit, which requires mechanical support (Baas *et al.* 2004; Rowe *et al.* 2004).

Apart from having fewer fibres, lianas also have septate fibres (Fahn *et al.* 1986; Pace & Angyalossy 2013), a feature also seen in the studied shrubs. Septate fibres are associated with starch storage (Harrar 1946), and they are frequently found in species with scanty axial parenchyma (Carlquist 2001; Wheeler *et al.* 2007; Pace & Angyalossy 2013). However, in some Bignoniaceae woods analysed, it was observed that plants with abundant axial parenchyma may also have septate fibres (Pace & Angyalossy 2013), and in eudicots, as a whole, septate fibres are also common in trees and shrubs of certain families.

The presence of cambial variant, high rays and septate fibres, both in the lianas and shrubs herein analysed, may be attributed to the fact that the studied species belong to the same tribe, independent of habit. Shrubs in Bignonieae are known to be derived from lianas (Lohmann 2003); therefore, it is likely that features of lianas present in these shrubs result from phylogenetic inertia (*sensu* Burt 2001), a case in which features from ancestors are retained in the descendants, albeit without any obvious adaptive

advantage, as a sign of common descent. However, such anatomical differences as the large area of vessel occupancy, vessel dimorphism and reduced area occupied by fibres are also noted in lianoid species.

Comparison of potential conductivity among habits

Independently from the habit, the higher the hydraulic conductivity the lower the water transport safety, which may become prone to vessel embolism (Tyree & Zimmermann 2002; Baas *et al.* 2004). The potential hydraulic conductivity is higher in the lianas than in the self-supporting species studied here. This higher hydraulic conductivity in lianas is associated with the presence of wide vessels, which increased water conduction efficiency (Ewers *et al.* 1991; Hacke *et al.* 2006; Van der Sande *et al.* 2013).

However, experimental data comparing lianas and trees that took into consideration the hydraulic potential within which a plant loses 50% of its conducting capacity (P_{50}) have demonstrated that lianas have a lower resistance to embolism (P_{50}), meaning that lianas are less safe when submitted to drought stress (Hacke *et al.* 2006; Van der Sande *et al.* 2013). Despite drought-induced embolism being indirectly related to vessel diameter, it is directly related to the intervessel pit structure, the chamber size, intervessel pit areas per vessel, and thickness and porosity of the pit membrane (Tyree & Sperry 1989; Baas *et al.* 2004; Lens *et al.* 2011; Li *et al.* 2016). However, the pit data related to chamber size evaluated in the present study were shown to be similar among the different habits.

On the other hand, as a hydraulic strategy regarding lower safety, the narrow vessels in lianas are generally grouped around wide vessels, guaranteeing water conduction safety (Hacke *et al.* 2006) and acting as a back-up when wide vessels get embolised (Carlquist 1988; Ewers *et al.* 1991; Brodersen *et al.* 2013).

The lianas studied here seem to have a synergistic investment in hydraulic efficiency, by the presence of wide vessels, and hydraulic safety, by the presence of very narrow vessels in high frequency, a contrasting pattern when lianas are compared to their self-supporting counterparts.

CONCLUSIONS

Secondary xylem anatomy varies qualitatively and quantitatively among lianas, shrubs and trees of Bignoniaceae and can be seen as a reflection of functional adaptations related to mechanical support and hydraulic efficiency within the vascular system. 1) Lianas have cambial variants, a low area of the secondary xylem occupied by fibres, high rays, more vessels per area, and a maximum vessel diameter of about 20 times wider than the minimum vessel diameter, providing quantitative evidence of vessel dimorphism. 2) Shrubs and trees have more fibres per area and lower rays (in trees), with a lower potential conductivity compared with the lianas. 3) Shrubs of Bignonieae share anatomical features with lianas of this tribe as a result of phylogenetic inertia.

Future experimental studies, encompassing broader sampling correlated with their xylem anatomy, may be able to shed further light on the structural variations among the different habits and their functional influence on hydraulic conductivity.

ACKNOWLEDGEMENTS

The authors thank A.C.F. Barbosa for help with anatomical procedures; L.G. Lohmann, A.R. Zuntini, G.C.T. Ceccantini and M.R. Pace for collections and identifications in Brazil; M.R. Pace, A. Nogueira, C.L. Bastos and an anonymous reviewer for helpful suggestions; the São Paulo Research Foundation (FAPESP, 2013/10679-0) and the National Council of Technological and Scientific Development (CNPq, grant 481034/2007-2) for financial support and the entire staff of the University of São Paulo Botany Department (Brazil).

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Accepted: 20 December 2016