



## Plant anatomy: history and future directions

# Montane forest tree species have lower hydraulic efficiency and vulnerability than lowland forest species in the Atlantic Forest

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### Abstract

The adaptations of plants to different environments are closely related to their wood anatomical traits. This study investigated the wood anatomy of a montane forest community and compared it with anatomical data from three other areas in the Atlantic Forest biome (montane and lowland forest). Samples from 23 montane forest species were processed and analyzed according to standard wood anatomy techniques. Quantitative xylem traits were measured and conductivity parameters were calculated. Wood anatomical data for 76 species from other areas were obtained from previous studies and incorporated into the analyses. Shared wood features indicated ecological trends related to the forest formations. Species of the sampled montane forest were discriminated into groups based on their qualitative and quantitative features, especially those related to ray and xylem conduction traits. Hydraulic traits were also important for distinguishing forest communities along the altitudinal gradient. The montane forest communities had lower conductivity and higher hydraulic safety than the lowland forest community. Apart from these differences, all the studied communities are in the Atlantic Forest biome and present traits related to high hydraulic efficiency and vulnerability, as expected for this tropical region.

**Key words:** anatomical traits, anatomical trends, ecological wood anatomy, plant hydraulic, xylem conductivity.

### Resumo

As adaptações das plantas aos diferentes ambientes estão intimamente relacionadas aos atributos anatômicos da madeira. Este estudo investigou a anatomia da madeira de uma comunidade de floresta montana e comparou-a com dados anatômicos de outras três áreas do bioma Mata Atlântica (floresta montana e floresta de terras baixas). Amostras de 23 espécies da floresta montana foram processadas e analisadas de acordo com técnicas padrão de anatomia da madeira. Atributos quantitativos do xilema foram medidos e parâmetros de condutividade foram calculados. Dados anatômicos da madeira de 76 espécies de outras áreas foram obtidos de estudos anteriores e incorporados às análises. As características compartilhadas da madeira indicaram tendências ecológicas relacionadas às formações florestais. Espécies amostradas da floresta montana foram

See supplementary material at <https://doi.org/10.6084/m9.figshare.25742892.v1>

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discriminadas em grupos com base em suas características qualitativas e quantitativas, especialmente aquelas relacionadas aos raios e a condução. Atributos hidráulicos foram importantes para distinguir as comunidades florestais no gradiente altitudinal. As comunidades da floresta montana tiveram menor condutividade e maior segurança hidráulica do que a comunidade da floresta de terras baixas. Apesar dessas diferenças, todas as comunidades estudadas estão na Mata Atlântica e apresentam alta eficiência hidráulica e vulnerabilidade, como esperado para esta região tropical.

**Palavras-chave:** atributos anatômicos, tendências anatômicas, anatomia ecológica da madeira, hidráulica vegetal, condutividade do xilema.

## Introduction

The Atlantic Forest biome is a biodiversity hotspot (Myers *et al.* 2000; Mittermeier *et al.* 2005), with only 12–28% of it remaining well preserved (Rezende *et al.* 2018; SOS Mata Atlântica & INPE 2023), largely composed of forest fragments embedded in open-habitat matrices because of degradation over many centuries (*e.g.*, Joly *et al.* 2014; Scarano & Ceotto 2015). The Atlantic Forest is characterized by various plant formations constituting a diverse set of associated ecosystems. Moving inland from the coastal plains to the mountain summits, there are alluvial, lowland, submontane, montane, and high montane forests, as well as other extreme environments including restingas, mangroves, altitudinal grasslands and vegetation growing on rock outcrops (Scarano 2002; IBGE 2012). This multiplicity of environments helps to explain the rich biodiversity of the Atlantic Forest and makes the region suitable for long-term ecological investigations (Scarano 2002; ForestPlots.net *et al.* 2021), including those on ecological plant anatomy (Barros *et al.* 2006; Ribeiro & Barros 2006; Marques *et al.* 2015; Campbell *et al.* 2016; Soffiatti *et al.* 2016; Macedo *et al.* 2019; Costa *et al.* 2020).

Wood anatomy can provide valuable information concerning functional traits for species and communities (Carlquist 1977, 2001, 2012; Lachenbruch & Mcculloh 2014; Beeckman 2016; Baas *et al.* 2016; Olson 2020). Anatomical traits such as wood density and vessel diameter have been used as proxies for understanding plant ecological strategies (Westoby 1998; Chave *et al.* 2009; Zanne *et al.* 2010; Apgaua *et al.* 2015). Currently, studies globally are focused on analyzing how species will respond to different climatic scenarios, and hydraulic traits of plants have been used to evaluate and understand their responses (Pandey 2021; Hollunder *et al.* 2022). Some authors have also stressed that water availability

influences the anatomical structures of wood, showing adaptations to the environment (Carlquist 1977, 2001, 2012; Baas *et al.* 1983; Dickison 1989; Woodcock *et al.* 2000; Wheeler *et al.* 2007; Apgaua *et al.* 2015, 2017; Tng *et al.* 2018; Olson 2020).

Carlquist (1977) proposed two different approaches to expressing ecological-anatomical relationships. One is based on analyzing a particular taxonomic group and aims to compare a given species along an ecological gradient; the other involves identifying anatomical characters held in common within a given plant formation in a limited area, regardless of the taxonomic groups analyzed (Barajas-Morales 1985; Noshiro & Suzuki 1995; Noshiro *et al.* 1995, 2020; Alves & Angyalossy-Alfonso 2000; Carlquist 2001; Barros *et al.* 2006; Wheeler *et al.* 2007; Apgaua *et al.* 2017; Tng *et al.* 2018). The characteristics emphasized are generally related to vessel elements, which are considered important for the performance of xylem functions under various environmental conditions and express ecological trends of water transport under a given set of environmental conditions (Callado *et al.* 1997; Alves & Angyalossy-Alfonso 2000; Barros *et al.* 2006; Soffiatti *et al.* 2016; Olson 2020).

The main evolutionary mechanisms of xylem are related to adaptations to the degree of moisture availability, the rate of transpiration, water seasonality, and the need for investments in mechanical strength. It should be emphasized that such characteristics can vary considerably, even locally, and different plants may use the same habitat very differently (Carlquist 1975, 2001; Olson 2020; Olson *et al.* 2020). However, with a floristic approach it is possible to detect shared features in species occurring in the same area (Carlquist 1977, 2001; Olson 2020, 2023). Previous works on tropical rainforest tree species have demonstrated common traits for water transport among co-occurring species (Callado *et al.* 1997; Barros *et al.* 2006; Apgaua *et al.* 2015;

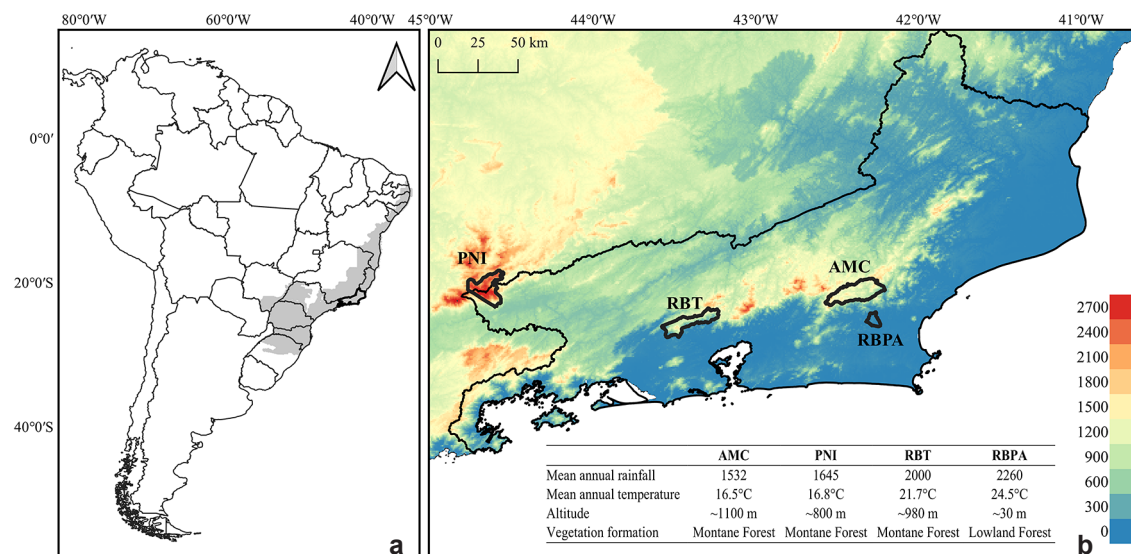
Soffiatti *et al.* 2016; Tng *et al.* 2018), but each of these cited studies was based on investigating only one site.

The present work was designed to contribute to the knowledge of the functional diversity of Atlantic Forest tree species. It also aimed to characterize their wood traits related to hydraulic efficiency strategies by describing the anatomical traits in a montane forest community and comparing the results with anatomical data from previous studies in other Atlantic Forest formations (montane and lowland forest). Montane forest trees present lower sap flow and more conservative water use than lowland trees in the Atlantic Forest, which is related to differences in water availability (Rosado *et al.* 2016). This work hypothesized that species grown in a certain forest formation respond similarly to environmental signals and should have similar anatomical wood features. Moreover, tree species in montane forest should present lower hydraulic conductivity and higher safety to embolism than when in lowland forest, which is associated with differences in vessel features and environmental conditions (Rosado *et al.* 2016; Hacke *et al.* 2022; Ewers *et al.* 2023; Olson 2023; Olson *et al.* 2023). The floristic approach used here is based on Carlquist’s

comparative method (Carlquist 1975, 1977, 1980, 2001, 2012; Carlquist & Hoekman 1985; Olson 2020, 2023).

### Material and Methods

Samples were collected in the Tinguá Biological Reserve (hereafter RBT, acronym in Portuguese), in Rio de Janeiro state, Brazil (22°22’20”S–22°45’00”S; 43°40’00”W–43°05’40”W) (Fig. 1). The RBT is a fully protected federal conservation area that represents 8% of the total protected area of Rio de Janeiro State. The landscape is generally quite steep and irregular, with scarps cut by torrential rivers. Topographically, the Tinguá Massif is an imposing and rugged geological monument reaching 1,600 m in elevation (MMA & IBAMA 2006). The regional soils include red and yellow dystrophic argisols and latosols, red eutrophic argisols, and haplic eutrophic cambisols. The regional climate is tropical humid, with dry winters and temperate summers, corresponding to type Cwb of the Köppen classification, with an average austral winter temperature of 15.7 °C, and 27.7 °C in the summer. The mean total annual rainfall is 2,000 mm, although unevenly distributed throughout the year. The driest period is between April and



**Figure 1** – a. Map of South America and the Atlantic Forest biome (gray). b. Map of Rio de Janeiro state delimiting the four conservation units containing the collection sites (heavy black line): Macaé de Cima Environmental Protection Area (AMC), Itatiaia National Park (PNI), Tinguá Biological Reserve (RBT), Poço das Antas Biological Reserve (RBPA). Altitude represented by color scale (m). Environmental features of the collection sites in the conservation units are presented in the table.

August, when monthly rainfall is below 80 mm. The RBT is an important Atlantic Forest biome remnant in Rio de Janeiro state due to its protection of mountain streams and its socio-economic value (MMA & IBAMA 2006; Iguatemy *et al.* 2017). Wood samples were collected in a permanent plot located in the Montane Dense Ombrophilous Forest formation (~980 m a.s.l.). The plot was one of the permanent plots established by the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro for a long-term biodiversity project (PPBio-MA Núcleo Sudeste). We chose to study the species with the highest Importance Values (IV) (> 0.6) based on phytosociological analyses performed by that long-term project (unpublished data). The IV represents the degree of dominance of a species in a plot, where the species with the highest IVs represent the dominant species (Curtis & McIntosh 1950). The IV is obtained for each species by summing its relative density, dominance, and frequency values. The IV has been very useful in separating different types of forests and relating them to environmental factors, including the relationships between species distributions and abiotic factors (Martins 1991).

Wood samples were collected, processed, and analyzed from 82 trees belonging to 23 species and 12 families (Tab. 1). The samples were collected using a non-destructive method (increment borer) in mature trunks at breast height without any injuries, and deposited in the Jardim Botânico do Rio de Janeiro Wood Collection (RBw). The wood samples were processed according to the usual techniques for bright field optical microscopy (Johansen 1940; Sass 1958; Machado *et al.* 1997).

The samples were sectioned (18 to 20  $\mu\text{m}$  thick) in their transverse and longitudinal planes (radial and tangential) using a Leica microtome. The sections were then bleached, dehydrated, and stained with Astra blue and Safranin (Bukatsch 1972). Permanent slides were mounted with Entellan<sup>®</sup> (Burger & Richter 1991). Macerations were used to measure the elements that compose the axial system of the wood (Franklin 1945). Dissociated cells were stained with hydroalcoholic Safranin (Strasburger 1924). A Zeiss Primostar microscope coupled with AxioCam Erc 5 camera and Zeiss Zen software were used to capture and process the images. Measurements were performed for all species using Image-Pro Plus<sup>®</sup> version 4.5 software for Windows. The wood anatomy was described and measured according to the recommendations of the IAWA Committee (1989) with modifications (measurement of ray-

vessel pit diameter was added). The vulnerability index, the mesomorphy index, the conductivity index, hydraulic diameter and theoretical hydraulic conductivity were calculated (Carlquist 1977; Zimmermann 1983; Tyree & Ewers 1991; Sperry & Sullivan 1992; Sperry & Saliendra 1994).

The formulas used were:

Vulnerability index = mean vessel diameter ( $\mu\text{m}$ ) / mean vessel frequency (vessels/ $\text{mm}^2$ ) (Carlquist 1977).

Mesomorphy index = [mean vessel diameter ( $\mu\text{m}$ )  $\times$  mean vessel element length ( $\mu\text{m}$ )] / mean vessel frequency (vessels/ $\text{mm}^2$ ) (Carlquist 1977).

Conductivity index = [mean vessel diameter ( $\mu\text{m}$ ) / 2]<sup>4</sup> / mean vessel frequency (vessels/ $\text{mm}^2$ ) (Zimmermann 1983).

Hydraulic diameter = [sum vessel diameter ( $\mu\text{m}$ )]<sup>5</sup> / [sum vessel diameter ( $\mu\text{m}$ )]<sup>4</sup> (Sperry & Sullivan 1992; Sperry & Saliendra 1994).

Theoretical hydraulic conductivity = [ $\pi \times$  [hydraulic diameter ( $\mu\text{m}$ )]<sup>4</sup> / [128  $\times$  0,000000001  $\times$  mean vessel frequency (vessels/ $\text{mm}^2$ )] (Tyree & Ewers 1991).

Wood anatomical data were obtained from previously published data of 76 other species growing in three distinct areas in the Atlantic Forest: Itatiaia National Park (PNI, acronym in Portuguese), Montane Dense Ombrophilous Forest in Serra da Mantiqueira (22°15'S–22°30'S, 44°30'W–44°45'W, ~800 m a.s.l.), annual rainfall 1,645 mm, mean annual temperature 16.8 °C (Barros *et al.* 2008); Macaé de Cima Environmental Protection Area (AMC, acronym in Portuguese), Montane Dense Ombrophilous Forest in Serra do Mar (22°21'S–22°28'S, 42°27'W–42°35'W, ~1,100 m a.s.l.) annual rainfall 1,532 mm, mean annual temperature 16.5 °C (Callado *et al.* 1997; Barros *et al.* 2001); Poço das Antas Biological Reserve (RBPA, acronym in Portuguese), Lowland Dense Ombrophilous Forest (22°30'S–22°33'S, 42°15'W–42°19'W, ~30 m a.s.l.), annual rainfall 2,260 mm, mean annual temperature 24.5 °C (Barros *et al.* 2001, 2006) (Fig. 1). The species are listed in Table S1 (available on supplementary material <<https://doi.org/10.6084/m9.figshare.25742892.v1>>) and the environmental characteristics of the areas are summarized in Figure 1. These previous studies were conducted by the same research group as the present study, with the same methods, making their data suitable for the present analysis.

Statistical analyses were performed using Statistica 7.0 software to test the similarities among RBT species and among species from all areas

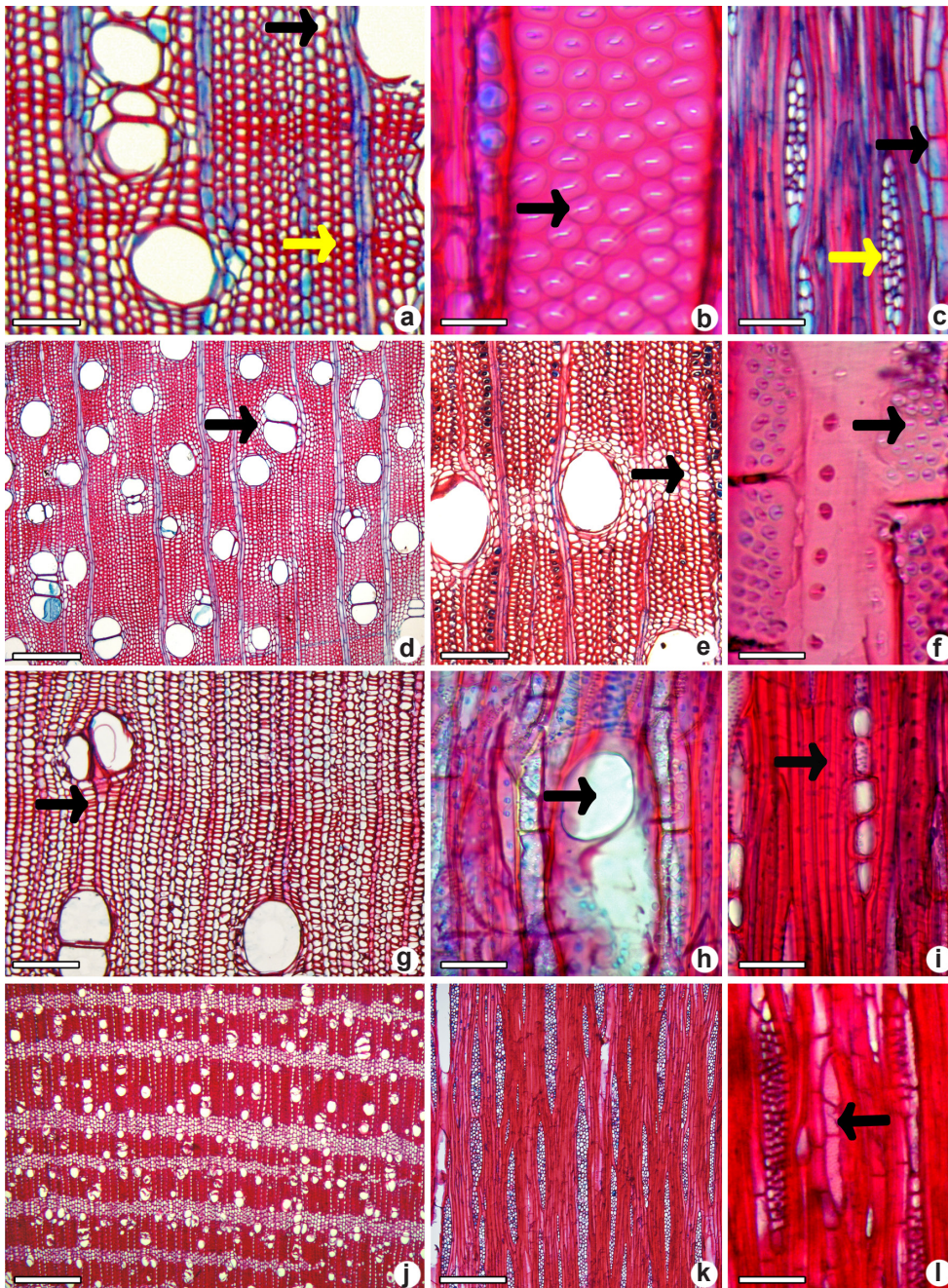
**Table 1** – Species sampled in the studied montane forest (RBT) by family, mean diameter breast height (DBH), mean tree height (H) when measured, and records in the Jardim Botânico do Rio de Janeiro Wood Collection (RBw).

Family	Species	DBH (cm)	H (m)	RBw
Annonaceae	<i>Duguetia microphylla</i> (R.E.Fr.) R.E.Fr.	21	-	10516-10517-10518-10519-10520-10521
Apocynaceae	<i>Geissospermum laeve</i> (Vell.) Miers	57	35	10753
Chrysobalanaceae	<i>Licania kunthiana</i> Hook.f.	20	13	10438-10439-10440-10441
Elaeocarpaceae	<i>Sloanea hirsuta</i> (Schott) Planch. ex Benth.	22	16	10770-10772
Euphorbiaceae	<i>Alchornea triplinervia</i> (Spreng.) Müll.Arg.	40	20	10442-10443-10445
Lauraceae	<i>Ocotea aciphylla</i> (Nees & Mart.) Mez	17	8	10756-10757-10758
Lauraceae	<i>Ocotea catharinensis</i> Mez	35	26	10759-10760-10762-10764
Lauraceae	<i>Ocotea domatiata</i> Mez	32	25	10526-10527-10528-10529-10530-10531
Lauraceae	<i>Ocotea indecora</i> (Schott) Mez	36	18	10446-10447-10448
Lauraceae	<i>Ocotea odorifera</i> (Vell.) Rohwer	14	10	10765-10766-10767
Leguminosae	<i>Copaifera lucens</i> Dwyer	25	17	10741-10742-10745-10746
Leguminosae	<i>Pseudoptadenia schumanniana</i> (Taub.) G.P.Lewis & M.P.Lima	30	20	10522-10523-10524-10525
Leguminosae	<i>Tachigali urbaniana</i> (Harms) L.G.Silva & H.C.Lima	42	23	10773-10774-10775
Myrtaceae	<i>Calyptanthes grandifolia</i> O.Berg	18	-	10532-10533-10534-10535-10536-10537
Myrtaceae	<i>Myrceugenia myrcioides</i> (Cambess.) O.Berg	10	7	10777
Primulaceae	<i>Myrsine gardneriana</i> A.DC.	21	-	10776
Rubiaceae	<i>Amaioua intermedia</i> Mart. ex Schult. & Schult.f.	22	16	10538-10539-10540-10541
Rubiaceae	<i>Bathysa australis</i> (A.St.-Hil.) K.Schum.	19	-	10542-10543-10544-10545-10546-10547
Rubiaceae	<i>Cordia concolor</i> (Cham.) Kuntze	25	13	10747
Sapotaceae	<i>Manilkara subsericea</i> (Mart.) Dubard	23	9	10450-10451-10452
Sapotaceae	<i>Pouteria caimito</i> (Ruiz & Pav.) Radlk.	24	17	10549-10550-10551
Vochysiaceae	<i>Qualea gestasiana</i> A.St.-Hil.	31	27	10453-10454-10455-10456-10457
Vochysiaceae	<i>Vochysia laurifolia</i> Warm.	37	28	10553-10554-10555-10556-10557-10558

(AMC, PNI, RBT, RBPA). Principal component analysis (PCA) was used to sort species from RBT and different areas, showing the variance factors. The 23 anatomical features by species and area used in the PCA are summarized in Table S2 (available on supplementary material <<https://doi.org/10.6084/m9.figshare.25742892.v1>>).

## Results

Diffuse-porous wood, alternate intervessel pits, simple perforation plates, and non-septate fibers were observed in all the species examined from the montane forest of RBT (Fig. 2; Tab. 2). Other anatomical features present in more than 50% of the species were distinct growth ring



**Figure 2** – a-l. Examples of some wood anatomical features with high occurrence in the montane forest species of RBT – a. *Ocotea catharinensis*, distinct growth ring boundary (yellow arrow) and thin- to thick-walled fibers (black arrow); b. *Ocotea catharinensis*, alternate intervessel pits (black arrow); c. *Copaifera lucens*, axial parenchyma with 5–8 cells per strands (black arrow) and ray width of three cells (yellow arrow); d. *Ocotea indecora*, diffuse-porous wood and paratracheal axial parenchyma (black arrow); e. *Qualea gestasiana*, paratracheal aliform confluent axial parenchyma (black arrow); f. *Calyptanthes grandifolia*, vessel-ray pits with distinct borders (black arrow); g. *Alchornea triplinervia*, diffuse-porous wood and distinct growth ring boundary (black arrow); h. *Myrceugenia myrcioides*, simple perforation plate (black arrow); i. *Cordia concolor*, non-septate fibers present (black arrow); j. *Cordia concolor*, frequency of vessel (5–20 vessels per square millimeter) and diffuse-porous wood; k. *Cordia concolor*, ray frequency (4–12 / mm); l. *Geissospermum laeve*, 3–4 cells per parenchyma strand (black arrow). a, d-e, g, j – transversal sections; b-c, f, h-i, k-l – longitudinal tangential sections. Scale bars: a, c, e, g, l = 100  $\mu$ m; b, f, h, i = 10  $\mu$ m; d, j, k = 200  $\mu$ m.

**Table 2** – Relative frequencies of anatomical traits in the studied montane forest (RBT). The frequencies (FR) were clipped above 50%.

Features	FR
Wood diffuse-porous	100%
Simple perforation plates	100%
Intervessel pits alternate	100%
Non-septate fibers present	100%
Vessels length (350–800 $\mu\text{m}$ )	83%
Fibers with simple to minutely bordered pits	83%
Fibers length (900–1600 $\mu\text{m}$ )	83%
Four (3–4) cells per parenchyma strand	78%
Ray width 1 to 3 cells	74%
Rays frequency (4–12 / mm)	74%
Growth ring boundaries distinct	65%
Paratracheal axial parenchyma	61%
Vessel–ray pits with distinct borders; similar to intervessel pits in size and shape throughout the ray cell	61%
Vessels frequency (5–20 vessels per square millimeter)	61%
Fibers thin- to thick-walled	61%
Tangential diameter of vessel (100–200 $\mu\text{m}$ )	52%
Eight (5–8) cells per parenchyma strand	52%

boundaries (65%), vessel frequency of 5–20/mm<sup>2</sup> (61%); vessel diameter of 100–200  $\mu\text{m}$  (52%); vessel element length of 350–800  $\mu\text{m}$  (83%); paratracheal axial parenchyma (61%), vessel-ray pits with distinct borders (similar to intervessel pits in size and shape throughout the ray cell, 61%); thin- to thick-walled fibers (61%), with simple to minutely bordered pits (83%); fiber length of 900–1,600  $\mu\text{m}$  (83%); axial parenchyma with 3–4 cells per strand (78%); axial parenchyma with 5–8 cells per strands (52%); ray frequency of 4–12/mm (74%); and ray width of 1 to 3 cells (74%) (Fig. 2; Tab. 2).

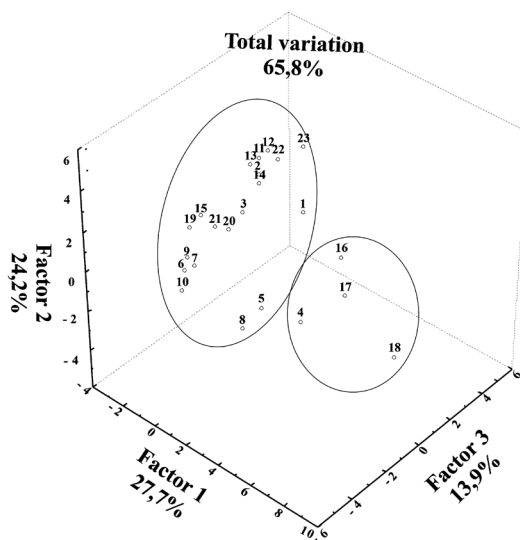
The PCA of RBT species grouped most of them by their similar qualitative and quantitative features. Three factors corresponded to approximately 66% of the total variance in the PCA (Factor 1 = 27.7%, Factor 2 = 24.2%, Factor 3 13.9%) (Fig. 3). Higher scores for Factor 1 were features related to rays. Higher scores for Factor 2 were vessel length and pit features all related to water transport, while those for Factor 3 were vessel features (Tab. 3). These features grouped

the majority of species, while influencing the separation of four: *S. hirsuta* (Elaeocarpaceae), *M. gardineriana* (Primulaceae), and *A. intermedia* and *B. australis* (both Rubiaceae) (Fig. 3).

The mean species mesomorphy index was 6,546; vulnerability index 10.6; conductivity index 1,747,620; hydraulic diameter 117  $\mu\text{m}$ ; and theoretical hydraulic conductivity  $1.43\text{E}+15 \text{ kg m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$ . Values for the species are summarized in Table 4. Except for *C. concolor* and *M. myrcioides*, all the studied species had high values for the mesomorphy index, the vulnerability index, the conductivity index, hydraulic diameter, and theoretical hydraulic conductivity.

The PCA of the 99 different species of the four Atlantic Forest areas grouped species by areas. Three factors corresponded to approximately 64% of the variance (Factor 1 = 31.4%, Factor 2 = 19.8%, Factor 3 13.0%) (Fig. 4). The AMC species were separated from those of the other montane forests areas (PNI and RBT) by a higher incidence of scalariform perforation plates (0.81 - Factor 1), while the other montane forests species

had higher incidences of simple perforation plates (-0.79 - Factor 1) [Fig. 4; Tab. 5; Tab. S2 (available on supplementary material <<https://doi.org/10.6084/m9.figshare.25742892.v1>>)]. The lowland forest (RBPA) species were separated from those of the montane forest areas by higher values for vessel diameter and the vulnerability and mesomorphy indices (-0.77, -0.78, -0.84 - Factor 2, respectively) [Fig. 4; Tab. 5; Tab. S2 (available on supplementary material <<https://doi.org/10.6084/m9.figshare.25742892.v1>>)]. Factor 3 represented 13% of the variance and also detached AMC from other areas because all species had thin- to thick-walled fibers (-0.76), while some species in the other areas had very thick-walled fibers (0.81) [Fig. 4; Tab. 5; Tab. S2 (available on supplementary material <<https://doi.org/10.6084/m9.figshare.25742892.v1>>)].



**Figure 3** – Principal component analysis of the species from the studied montane forest (RBT). Graphical representation of the species described by three factors – 1 = *Duguetia microphylla*; 2 = *Geissospermum laeve*; 3 = *Licania kunthiana*; 4 = *Sloanea hirsuta*; 5 = *Alchornea triplinervia*; 6 = *Ocotea aciphylla*; 7 = *Ocotea catharinensis*; 8 = *Ocotea domatiata*; 9 = *Ocotea indecora*; 10 = *Ocotea odorifera*; 11 = *Copaifera lucens*; 12 = *Pseudopiptadenia schumanniana*; 13 = *Tachigali urbaniana*; 14 = *Calyptanthes grandifolia*; 15 = *Myrceugenia myrcioides*; 16 = *Myrsine gardneriana*; 17 = *Amaioua intermedia*; 18 = *Bathysa australis*; 19 = *Cordia concolor*; 20 = *Manilkara subsericea*; 21 = *Pouteria caimito*; 22 = *Qualea gestasiana*; 23 = *Vochysia laurifolia*.

## Discussion

Species that grow in a certain forest physiognomy respond similarly to environmental signals, and thus should have similar wood anatomical features (Carlquist 1977). Evaluations of the relationships between wood anatomy and ecology in floristic approaches are essential to better understand aspects of functional anatomy (Carlquist 1977, 2001; Alves & Angyalossy-Alfonso 2000; Olson *et al.* 2020, 2023; Olson 2023). The present analysis documented differences between communities in the forest formations and the influence of altitude on hydraulic structure. Montane forest species in the Atlantic Forest have similar anatomical wood traits with less efficient water conductivity and higher vulnerability to embolism than lowland forest species. Species from AMC (~1100 m a.s.l.) had the narrowest vessel diameter of the analyzed areas, while those from PNI and RBT (~800 m and ~980 m, respectively) had similar values, and RBPA the widest. The response of communities to environmental conditions, such as temperature, precipitation, atmospheric pressure, solar radiation and UV-B radiation, change with altitude, so influences on wood anatomical traits are expected (van der Graaff & Baas 1974; Noshiro & Suzuki 1995; Noshiro *et al.* 1995, 2010, 2020; Körner 2007; Bosio *et al.* 2010).

Analysis of the wood anatomical features of the four studied communities in the Atlantic Forest along an altitudinal gradient (AMC, PNI, RBT and RBPA) revealed anatomical and functional features that differentiate them (perforation plates, vessel features, vulnerability and mesomorphy) (Callado *et al.* 1997; Barros *et al.* 2006, 2008). For lowland forest species (RBPA), the highest values of vessel diameter, vessel length and mesomorphy are likely related to the high pluviosity in the area, and explain adjustments in xylem anatomy to high conductivity (Dickison 1989; Wheeler & Baas 1991; Alves & Angyalossy-Alfonso 2000; Barros *et al.* 2006; Wheeler *et al.* 2007; Körner 2007; Soffiatti *et al.* 2016; Olson 2020). Besides that, species of AMC, the study area with highest altitude, showed different features separating them by statistical analysis. Thus, a functional trade-off was documented here between hydraulic safety and efficiency, whereby montane species that experience drier conditions have reduced conductive efficiency and enhanced safety for better fitness, when compared with lowland species.



**Table 3** – Highest scores from the PCA performed for the studied montane forest (RBT). Scores were clipped above  $\pm 0.44$ .

Features	Factor 1	Factor 2	Factor 3
Rays width ( $\mu\text{m}$ )	<u>0.78</u>	-0.14	0.35
Ray height > 1 mm	<u>0.74</u>	-0.22	0.15
Perforated ray cells	<u>0.74</u>	0.16	-0.26
Larger rays commonly 4- to 10-seriate	<u>0.73</u>	-0.14	0.10
Sheath cells	<u>0.69</u>	-0.27	0.10
Rays per millimeter ( $\leq 4$ / mm)	<u>0.69</u>	-0.27	0.10
Mean fiber lengths (1600 $\mu\text{m}$ )	0.62	-0.53	0.05
Vessels per square millimeter (40–100 vessels per square millimeter)	0.57	0.14	-0.42
Diameter of fiber	0.50	-0.53	0.30
Mean vessel element length (800 $\mu\text{m}$ )	0.49	<u>-0.57</u>	0.08
Vessel–ray pits with distinct borders; similar to intervessel pits in size and shape throughout the ray cell	0.44	<u>0.77</u>	0.22
Tangential diameter of vessel (50–100 $\mu\text{m}$ )	0.44	-0.35	-0.19
Vessels lenght	0.39	<u>-0.72</u>	-0.09
Vessels per square millimeter	0.30	0.23	-0.76
Rays with multiseriate portion(s) as wide as uniseriate portions	0.15	0.38	-0.58
Tangential diameter of vessel (50 $\mu\text{m}$ )	0.12	0.45	<u>-0.70</u>
Axial parenchyma aliform	-0.20	0.39	<u>0.58</u>
Vessel–ray pits with much reduced borders to apparently simple: pits rounded or angular	-0.40	<u>-0.79</u>	-0.26
Vessel–ray pits with much reduced borders to apparently simple: pits horizontal (scalariform, gash-like) to vertical (palisade)	-0.41	<u>-0.74</u>	-0.25
Radial diameter of vessel	-0.42	-0.11	<u>0.78</u>
Body ray cells procumbent with one row of upright and /or square marginal cells	-0.43	-0.53	0.00
Oil and /or mucilage cells associated with ray parenchyma	-0.44	-0.56	-0.35
Tangential diameter of vessel	-0.45	-0.06	<u>0.78</u>
Mean vessel element length (350–800 $\mu\text{m}$ )	-0.49	<u>0.57</u>	-0.08
Tyloses common	-0.52	<u>-0.65</u>	-0.32
Intervessels pits (size)	<u>-0.53</u>	<u>-0.70</u>	0.00
Ray-vessel pits (size)	<u>-0.54</u>	<u>-0.76</u>	-0.22
Mean fiber lengths (900–1600 $\mu\text{m}$ )	<u>-0.62</u>	0.53	-0.05
Rays per millimeter (4–12 / mm)	<u>-0.63</u>	0.42	-0.18

Measurements of sap flow in Atlantic Forest species following an altitudinal gradient have shown higher sap flow in lowland forest than in montane forest and hydraulic adjustments in response to water availability (Rosado *et al.* 2016). These findings are congruent with our results and

reinforce the importance of hydraulic conductivity indices and data calculated on xylem quantitative features, as performed here, because they help to understand plant hydraulic functioning by plant structure (Ewers *et al.* 2023; Olson *et al.* 2023). Although previous studies have demonstrated an

**Table 4** – Hydraulic indexes, hydraulic diameter ( $\mu\text{m}$ ) and theoretical hydraulic conductivity ( $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$ ) of the species of the studied montane forest (RBT).

Family	Species	Mesomorphy index	Vulnerability index	Conductivity index	Hydraulic diameter	Theoretical conductivity
Annonaceae	<i>Duguetia microphylla</i>	1,829	4.2	116,420	90	9.10E+13
Apocynaceae	<i>Geissospermum laeve</i>	2,872	7.7	578,697	118	3.48E+14
Chrysobalanaceae	<i>Licania kunthiana</i>	9,397	15.0	1,857,405	164	2.10E+15
Elaeocarpaceae	<i>Sloanea hirsuta</i>	4,046	5.2	178,767	93	1.18E+14
Euphorbiaceae	<i>Alchornea triplinervia</i>	16,371	16.0	2,037,282	167	2.40E+15
Lauraceae	<i>Ocotea aciphylla</i>	3,821	5.7	390,143	105	1.88E+14
Lauraceae	<i>Ocotea catharinensis</i>	7,923	8.8	411,927	122	4.48E+14
Lauraceae	<i>Ocotea domatiata</i>	7,694	8.3	873,539	140	6.44E+14
Lauraceae	<i>Ocotea elegans</i>	2,440	4.4	218,119	114	1.99E+14
Lauraceae	<i>Ocotea odorifera</i>	1,237	1.8	32,963	63	1.24E+13
Leguminosae	<i>Copaifera lucens</i>	8,176	22.7	1,704,658	129	1.47E+15
Leguminosae	<i>Pseudopiptadenia schumanniana</i>	5,152	11.5	957,683	123	5.78E+14
Leguminosae	<i>Tachigali urbaniana</i>	6,314	15.5	2,007,320	141	1.24E+15
Myrtaceae	<i>Calyptanthes grandifolia</i>	9,180	10.9	781,701	132	7.80E+14
Myrtaceae	<i>Myrceugenia myrcioides</i>	274	0.4	1,706	47	1.16E+12
Primulaceae	<i>Myrsine gardneriana</i>	2,873	4.1	81,031	74	4.42E+13
Rubiaceae	<i>Amaioua intermedia</i>	2,571	3.1	79,362	94	7.67E+13
Rubiaceae	<i>Bathysa australis</i>	1,701	1.6	34,575	96	4.98E+13
Rubiaceae	<i>Cordia concolor</i>	366	0.7	3,777	49	6.41E+04
Sapotaceae	<i>Manilkara subsericea</i>	3,013	5.1	125,283	97	1.35E+14
Sapotaceae	<i>Pouteria caimito</i>	5,253	10.8	435,772	137	8.33E+14
Vochysiaceae	<i>Qualea gestasiana</i>	15,685	22.9	4,214,409	163	2.75E+15
Vochysiaceae	<i>Vochysia laurifolia</i>	32,362	56.6	23,072,728	223	1.83E+16
Mean	--	6,546	10.6	1,747,620	117	1.43E+15

increase in conservative water use in woody species of the Atlantic Forest from lowland to montane forest (Rosado *et al.* 2016), further measurements and analysis of conductivity, transpiration, and carbon assimilation, can elucidate the differences in water-use efficiency of these communities and their responses to climate change.

The indices and calculations used in the present study represent a quantitative approach to understanding functional and ecological wood anatomy, mostly hydraulic strategies (Carlquist 1977; Zimmermann 1983; Tyree & Ewers 1991; Sperry & Sullivan 1992; Sperry & Saliendra

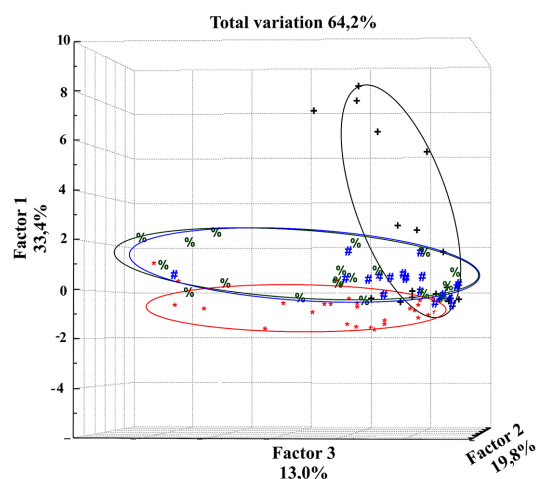
1994; Hacke *et al.* 2022; Ewers *et al.* 2023). Carlquist (1977) proposed the mesomorphy and vulnerability indices, which integrate the features of vessel diameter, vessel element length, and vessel frequency to provide a broad understanding of ecological strategies. High values of mesomorphy and vulnerability imply great hydraulic efficiency, and low values imply great safety against embolism (Carlquist 1977; Ewers *et al.* 2023). Anatomical studies have also used hydraulic diameter, the conductivity index, and theoretical hydraulic conductivity, based on the Hagen-Poiseuille Law, as more representative

conductivity parameters. The increase in vessel diameter increases conductivity to the fourth power; species with wide vessels are more efficient at conduction but more susceptible to embolism. Besides, vessel frequency is a feature linked to hydraulic safety by redundancy and embolism resistance (Zimmermann 1983; Tyree & Ewers 1991; Sperry & Sullivan 1992; Sperry & Saliendra 1994; Hacke *et al.* 2022; Ewers *et al.* 2023). Thus, mesomorphic species tend to have wide vessels in low frequency and high mesomorphy and vulnerability indices, while xeromorphic species tend to have narrow vessels in high frequency and low mesomorphy and vulnerability indices (Carlquist 1977, 2001).

Since wood anatomical traits are extremely important for the adaptation of plant communities to different conditions of water availability, by adjusting hydraulic safety and efficiency (Carlquist 1977, 2012; Poorter *et al.* 2008; Zanne *et al.* 2010; Baas & Wheeler 2011; Apgaua *et al.* 2015, 2017; Olson 2020; Pandey 2021), comparisons of these traits in different climates and environments can elucidate responses to future climatic scenarios. Many authors have related wide vessels to high water availability, high conductivity and low safety from embolism (Carlquist 1977, 2001; Sperry

2003; Zanne *et al.* 2010; Olson 2020; Olson *et al.* 2020, 2023; Hacke *et al.* 2022; Ewers *et al.* 2023), as seen in the present results. We therefore hypothesize that the alterations in water availability predicted by future climate change scenarios will affect the hydraulic functioning of montane and lowland forest communities in the Atlantic Forest (Baas & Wheeler 2011; Tng *et al.* 2018; Olson 2020; Pandey 2021; Hollunder *et al.* 2022). Climate change can increase the frequency of extreme events, including drought, likely inducing hydraulic failure that causes productivity loss and plant mortality even in water-unlimited environments (McDowell *et al.* 2008; Allen *et al.* 2010; Choat *et al.* 2012). The xylem trait data provided here can be incorporated into a meta-analysis to model vegetation response and forecast responses to changing environmental scenarios (*e.g.*, Wiemann 1998, 1999, 2001; McDowell *et al.* 2008; Baas & Wheeler 2011; Choat *et al.* 2012; Anderegg 2015; Pandey 2021). Choat *et al.* (2012), based on published and unpublished data on vulnerability to drought-induced embolism for many woody species (226) and sites (81), reported that all forest biomes, regardless of their current rainfall, are equally vulnerable to hydraulic failure due to drought, indicating a global convergence in the vulnerability of forests to drought. The availability of xylem trait data has allowed, and will continue to allow, an understanding of how climate affects forests and the forecasting of climate change impacts (Pandey 2021).

Even though montane forest species in the Atlantic Forest have less efficient species water conductivity and lower vulnerability to embolism than lowland forest species, some common features of RBT species are related to high hydraulic efficiency, typical in tropical forests with high water availability (Carlquist 1977, 2001, 2012; Dickison 1989; Wheeler & Baas 1991, 2019; Alves & Angyalossy-Alfonso 2000; Barros *et al.* 2006; Wheeler *et al.* 2007; Soffiatti *et al.* 2016; Olson 2020) such as diffuse-porous wood, simple perforation plates, vessel diameter of 100–200  $\mu\text{m}$ , vessel element lengths of 350–800  $\mu\text{m}$ , and vessel frequency of 5–20/mm<sup>2</sup>. The RBT community is mesomorphic with high conductivity and high hydraulic efficiency, although with vulnerability to high water-column tensions (Bosio *et al.* 2010; Carlquist 2012; Hacke *et al.* 2017, 2022). In contrast, woody plants in dry regions have adaptations that provide greater hydraulic safety, such as high vessel frequencies of narrow and



**Figure 4** – Principal component analysis of the species from the four different Atlantic Forest areas along an altitudinal gradient. Graphical representation of the species described by three factors. Black + = Macaé de Cima Environmental Protection Area (AMC), Red \* = Poço das Antas Biological Reserve (RBPA), Green % = Tinguá Biological Reserve (RBT), and Blue # = Itatiaia National Park (PNI).

**Table 5** – Highest scores from the PCA performed for four Atlantic Forest areas. Scores were clipped above  $\pm 0.52$ .

Features	Factor 1	Factor 2	Factor 3
Scalariform perforation plates > 10mm	<u>0.81</u>	-0.31	-0.08
Vessel length	<u>0.79</u>	-0.34	-0.01
Fiber length	0.69	-0.43	0.32
Parenchyma rare	0.67	-0.02	0.03
Ray height > 1mm	0.56	-0.10	0.16
Fibers very thick-walled	0.07	0.38	<u>0.81</u>
Fibers thin- to thick-walled	0.01	-0.43	<u>-0.76</u>
Mesomorphy index	-0.37	<u>-0.84</u>	0.26
Vessel tangential diameter	-0.47	<u>-0.77</u>	0.19
Vulnerability index	-0.48	<u>-0.78</u>	0.26
Growth rings present	-0.52	0.09	-0.26
Parenchyma paratracheal	-0.58	-0.18	-0.07
Simple perforations plates	<u>-0.79</u>	0.27	0.08

short vessel elements (Carlquist 1977, 2001, 2012; Hacke *et al.* 2022). For example, some Cerrado (Brazilian savanna) species have lower vessel diameter and higher vessel frequency than those of the present study (Melo Júnior *et al.* 2017; Simioni *et al.* 2021). There are trade-offs of hydraulic safety versus efficiency, and vessel diameter versus frequency (Zanne *et al.* 2010; Carlquist 2012; Hacke *et al.* 2017). There are also some differences between sympatric species. RBT species exhibited differences in ray (height, frequency, width) and water transport (vessel length and pit features) features. A systematic approach allows the interpretation of these features. For example, *S. hirsuta* (Elaeocarpaceae), *M. gardineriana* (Primulaceae), and *A. intermedia* and *B. australis* (both Rubiaceae) belong to families with high, wide, and low-frequency rays (Carlquist 2001), while other species lack these features. Another example is *C. lucens*, *P. schumanniana*, and *T. urbaniana* of the family Leguminosae, which presents short vessels, alternate vested intervessel pits, and vessel-ray pits with distinct borders, similar to intervessel pits in size and shape throughout the ray cell (Carlquist 2001; Baretta-Kuipers 1981), sometimes differing from species belonging to other families.

In summary, new wood anatomical data was provided here about a montane forest community in the Atlantic Forest, which was compared to

data from other communities in the same biome, revealing that montane forest species have a safer and less efficient hydraulic system than lowland forest species. Regardless of the differences among these communities, they are in the Atlantic Forest and present elevated hydraulic efficiency and vulnerability to embolism, as expected for a tropical region. This study also suggests that species will possess characteristics related to their area of occurrence, growing and responding to environmental signals. It also demonstrated the importance of recognizing the functional traits of wood to better understand them and infer the responses of woody plants to future climate changes.

### Acknowledgements

We are grateful to the Programa de Pesquisa em Biodiversidade na Mata Atlântica (PPBio-MA), Núcleo Sudeste, and the Reserva Biológica do Tinguá, for logistic support; and to Erik Wild, for English language review.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES) - Finance Code 001. We also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), for financial support.

## Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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