

## Leaf anatomy variation within and between three “restinga” populations of *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) in Southeast Brazil

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**ABSTRACT** – (Leaf anatomy variation within and between three “restinga” populations of *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) in Southeast Brazil). *Erythroxylum ovalifolium* is a woody shrub widespread in the “restinga”, i.e. the open scrub vegetation of the Brazilian coastal sandy plains. We examined leaf anatomy variation of this species both within populations and between populations of three “restingas” in the state of Rio de Janeiro. Sites were ca.100 km far from each other and differed in regard to rainfall and vegetation structure: a dry, open site; a wet, dense site and an intermediate one. Microhabitats within sites were: (i) exposed to full irradiance, outside vegetation islands; (ii) partially exposed to full irradiance, at the border of vegetation islands; (iii) shaded, inside vegetation islands. Leaf anatomy parameters were measured for five leaves collected in each of five plants per microhabitat, in each population; they were thickness of the leaf blade, of the palisade and spongy parenchyma, and of the adaxial and abaxial epidermis. Leaves from the dry, open site had narrower abaxial epidermis and a smaller contribution of spongy parenchyma to total leaf blade thickness than the other two sites, which we attributed to water stress. Adaxial epidermis and leaf are thicker in more exposed microhabitats (i and ii, above), irrespective of site. We proposed that between-site anatomical variation in traits related to water stress, and within-site anatomical variation in traits related to light-use are indicative of ecological plasticity and might help explain the high abundance of *E. ovalifolium* in the studied populations and along the State of Rio de Janeiro coast.

Key words - anatomic variation, *Erythroxylum ovalifolium*, mesophyll, sun-shade plants

**RESUMO** – (Variação na anatomia foliar de *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) entre e dentro de três populações de restinga no Sudeste do Brasil). *Erythroxylum ovalifolium* é um arbusto lenhoso amplamente distribuído nas restingas, i.e., a vegetação arbustiva aberta das planícies arenosas costeiras do Brasil. Examinou-se a anatomia foliar desta espécie entre populações de três restingas do Estado do Rio de Janeiro, bem como entre três distintos microhabitats no interior de cada restinga. As localidades distavam ca.100 km entre si e diferiram quanto à precipitação e estrutura da vegetação: uma localidade seca e aberta; uma localidade úmida e densamente vegetada e uma localidade intermediária quanto a estes parâmetros. Os microhabitats foram: (i) exposto a radiação total, ocorrendo fora da ilha de vegetação; (ii) parcialmente exposto a radiação total, ocorrendo na borda da ilha de vegetação; (iii) sombreado, ocorrendo dentro da ilha de vegetação. Os parâmetros anatômicos foliares foram medidos para cinco folhas coletadas em cada um de cinco indivíduos por microhabitat, nas três localidades. Foram eles a espessura da lâmina foliar, dos parênquimas esponjoso e paliçádico, e das epidermes adaxial e abaxial. Folhas da localidade seca e aberta tiveram epiderme abaxial mais estreita e menor contribuição do parênquima esponjoso para a espessura total da lâmina foliar do que nas outras duas localidades, que se atribui ao estresse hídrico. A epiderme adaxial e a lâmina foliar foram mais espessas nos microhabitats mais expostos (i e ii, acima), independente da localidade. Sugere-se que a variação anatômica entre populações, relacionada ao estresse hídrico, e a variação anatômica dentro de populações, relacionada ao uso da luz, são indicativas de plasticidade ecológica e podem explicar a alta abundância de *E. ovalifolium* nas localidades estudadas e ao longo da costa do Estado do Rio de Janeiro.

Palavras-chaves - *Erythroxylum ovalifolium*, mesófilo, plantas de sol-sombra, variação anatômica

### Introduction

The diversity of plant communities comprised by the Brazilian Atlantic forest complex has prompted a number of studies on functional variation of species that

are widespread in various habitats within the complex (Scarano 2002). All these studies have indicated a large degree of intraspecific variation between distinct populations at different habitats, both in regard to anatomical and to physiological traits (Scarano *et al.* 2005a). This is particularly evident for plant species that occur in the mesic montane rain forest and also in the harsh ecosystems of the “restingas”. The “restinga” is a mosaic of habitats, ranging from reptant beach vegetation to swamps forests and open scrub vegetation organised in clumps (Henriques *et al.* 1986). Most plant species inhabiting the “restinga” originated from the

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montane forest and very few endemics are found (Rizzini 1979). However, unlike the mesic montane forest, “restingas” are subjected to extreme environmental conditions, such as high salinity, oligotrophy, flooding, drought and high irradiance (Lacerda *et al.* 1993, Franco *et al.* 1996). Plants originating from the montane forest were capable to adjust and colonise this more severe environment.

This process must have required acclimation of shade plants from the forest to the sun-exposed conditions of the “restinga”. Variation in morpho-anatomy and physiology of sun *vs.* shade leaves has been thoroughly investigated (Björkman 1981, Chazdon & Kaufmann 1993, Strauss-Debenedetti & Berlyn 1994). In the Atlantic forest complex, for instance, *in situ* quantitative information regarding leaf morpho-anatomy variation of sun-shade plants refers to individuals of a given plant species growing in different localities (Rôças *et al.* 1997, 2001) or within a small geographic area (Vieira 1995, Scarano *et al.* 2002).

Erythroxylaceae is a neotropical family occurring in Brazil, where it is represented by the genus *Erythroxylum*, which has around thirty species occurring in two biomes: “restinga” and “cerrado” (savannas of central Brazil). Araujo & Henriques (1984) identified ten different climatic regions and their corresponding vegetation types at the sandy coastal plains in the state of Rio de Janeiro, southeast Brazil. *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) occurs in all of them, except in the Itaipú area, which has been strongly modified by human interference. This woody shrub ranks among the dominant plants in the vegetations of all our study sites (Silva 1991, Araujo *et al.* 1998).

In this study, we used a more comprehensive approach and examined leaf anatomy variation of

*E. ovalifolium* both within populations and between populations of three “restingas” in the state of Rio de Janeiro. Although these sites greatly vary in rainfall regime and vegetation structure, *E. ovalifolium* is one of the dominant shrubs in all sites. We aimed to answer the following questions: a) does leaf anatomy for this species differ between sites?; b) does leaf anatomy for this species vary between distinct microhabitats in each site?; c) if so, is the leaf anatomy in each microhabitat comparable between sites?

## Material and methods

Studied sites – Our three sampling sites are located on the northern coast of the state of Rio de Janeiro. They are called thereafter Maricá (MC), Jurubatiba (JB) and Massambaba (MB) (table 1), and represent a gradient from a wet site, with a dense vegetation cover, to a dry site, with less vegetation cover (Gessler *et al.* 2005, Scarano *et al.* 2005b). Plants were collected in the “restinga” formation known as open scrub of *Clusia*, since it is dominated by *Clusia fluminensis* Planch. & Triana (in Maricá and Massambaba) or *Clusia hilariana* Schltld. (in Jurubatiba). This vegetation consists of hemispheric vegetation islands dominated by *Clusia* individuals and surrounded by bare white sand.

Although the distance between sites is less than 100 km, the sites consist of distinct phytogeographical regions according to Araujo & Henriques (1984). However, floristic similarity is as high as 47% between sites (Araujo & Henriques 1984).

Sampling, measurements and statistical analyses – For the anatomical analysis, five fully expanded leaves were collected from each of five adult individuals randomly chosen in each of the three microhabitats per sites: (i) fully exposed to light irradiation, outside vegetation islands; (ii) partially exposed to light irradiance, at the border of vegetation islands; and (iii)

Table 1. General geographic and climatic information related to the sites where *Erythroxylum ovalifolium* Peyr. was sampled for leaf anatomy analyses in the state of Rio de Janeiro, Brazil. Conservation units are APA = Area for Environmental Protection, and NP = National Park. Climate is according to Köppen (1948).

Sites	APA-Maricá	NP-Restinga de Jurubatiba	APA-Massambaba
	Wet, dense	Intermediate	Dry, open
Latitude	22°52' and 22°54' S	22°00' and 22°23' S	22°56' and 23°05' S
Longitude	42°48' and 42°54' W	41°15' and 41°45' W	42°10' and 42°13' W
Climate	Hot and humid (Aw)	Hot and humid (Aw)	Transition hot and humid / semi-arid (Aw/Bsh)
Mean anual rainfall	1,230 mm	1,200 mm	800mm
Water deficit	No evaluation	2 months (July-August)	5 months (May-September)
References	Pereira <i>et al.</i> (2001)	Henriques <i>et al.</i> (1986), Liebig <i>et al.</i> (2001)	Barbière (1984), Scarano <i>et al.</i> (2001)

shaded, inside vegetation islands. Sampaio *et al.* (2002, 2005a, b) provide descriptions of light environment of the microhabitats of Maricá and Jurubatiba. It added to a total of 5 samples (adult individuals) per microhabitat and 15 samples per site. Leaf material was collected from the upper branch node on the north side, and was immediately fixed in the field in formalin-acetic acid-alcohol 70 °GL (FAA) (Berlyn & Miksche 1976). Tissues (0.5 cm<sup>2</sup> of each leaf) were gradually dehydrated in ethanol and embedded in glycol methacrylate resin (Feder & O'Brien 1968). Then, 3 µm transverse sections were made using a rotatory microtome, and were subsequently stained with 0.1% toluidine blue (Johansen 1940).

The following microchemical tests were performed: i) solubility in acetic and hydrochloric acids to detect calcium oxalate crystals (Jensen 1962); ii) 0.2% Ruthenium red to detect mucilage (Jensen 1962); iii) acid floroglucin to identify lignin (Johansen 1940); iv) Hoepfner-Vorsatz's test to detect tannins and others phenolic compounds (Reeve 1951, Sass 1951).

Twenty-five measurements per microhabitat were taken of the following leaf anatomical parameters: thickness of leaf blade, of the palisade and spongy parenchyma, and of the adaxial and abaxial epidermis. Anatomic description and measurements were done by using an Olympus BH2 microscope with the aid of the image analysis software Image-Pro Plus. The video image was acquired by using a Sony video camera attached to the same optical microscope. The comparisons between sites and between microhabitats were made using Notched Box Plot (McGill *et al.* 1978). The analyses were done with the software *Systat* 7.0.1 (SYSTAT 1992).

## Results

All leaves of *E. ovalifolium* Peyr. shared some anatomical features irrespective of site and microhabitat, such as: mucilaginous and single-layered on both epidermis surfaces, occasionally bilayered on the adaxial surface; dorsiventral mesophyll; calcium oxalate crystals in the parenchyma in bands accompanying the vascular bundle; presence of tannins in the spongy parenchyma next to the abaxial epidermis; and paracytic stomata only on the abaxial surface, characterising hypostomatic leaves (figures 1-9).

Leaves collected in Massambaba ('dry restinga') were more xeromorphic than the other two populations (figures 4-6, 13), as evidenced by the thicker palisade parenchyma. The cells of spongy parenchyma contributed less to total leaf blade thickness (figure 13A). Massambaba leaves also had low values for the abaxial epidermis thickness (figure 13B). These traits showed little or no variation in regard to microhabitat type.

The adaxial epidermis and leaf blade thickness did not differ significantly between sites. The spongy and palisade parenchymas did not differ between Maricá ('wet restinga') and Jurubatiba ('intermediate restinga').

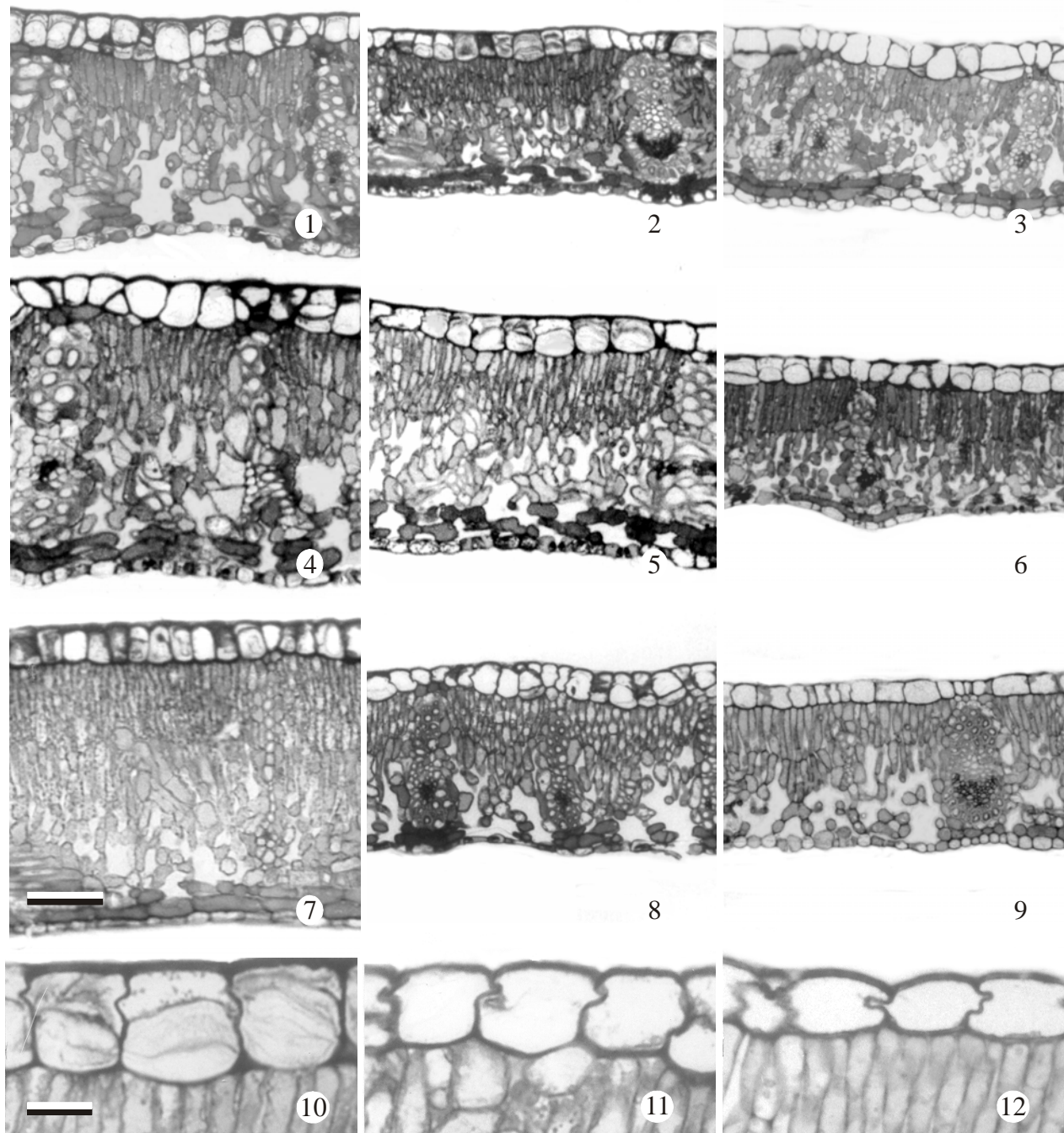
Instead, variation for these traits was related to microhabitat and, therefore, was responsive to light regime (figure 13C). Exposed leaves had a thicker mesophyll in all sites (figure 13D), due to a higher number of cell layers and cellular elongation. Nevertheless, the cells of the spongy parenchyma had a smaller intercellular space. The adaxial epidermis at sun-exposed position presented larger cell lumen, without changes in the cellular wall thickness. The external walls of the adaxial epidermal cells were flatter when exposed to light compared with the convex walls of the protected leaves (figures 10-12).

## Discussion

*Erythroxylum ovalifolium* showed no variation in some anatomical characteristics, such as: mucilaginous epidermis, dorsiventral mesophyll, presence of calcium oxalate crystals and hypostomatic leaves, despite site and microhabitat changes. Those characters were similarly found in other species of *Erythroxylum* occurring in "cerrado" vegetation (Bieras & Sajo 2004), indicating phylogenetic trends. Variation in palisade and spongy parenchyma ratio was also reported among "cerrado" species and found in the "restinga" species *Erythroxylum ovalifolium* as a variable trait among sites.

Two major trends emerged from the data analysed. First, leaf anatomical differences between populations were more conspicuous for the Massambaba ('dry restinga') population, which is yearly subjected to longer periods of soil water shortage, higher values of air water deficit and lower mean annual rainfall (Barbière 1984). Abaxial epidermis was narrower in this population than in the populations of the 'wet' and 'intermediate' "restingas". Additionally, the contribution of spongy parenchyma to total leaf blade thickness was smaller in the dry "restinga" than in the other two sites. These traits did not vary within these populations in response to microhabitat type. This has been previously found for abaxial epidermis (Rôças *et al.* 1997, 2001) to this environment, which suggests that variation in this trait is less related to light than to tissue water status. These patterns characterise the Massambaba population as a more xeromorphic one, which is probably a response to underground and atmospheric water shortage. Xeromorphism in this site has also been found for several other woody species in this site, when compared to wetter "restingas", as regards ecophysiological traits (Scarano *et al.* 2001, 2005, Duarte *et al.* 2005, Gessler *et al.* 2005).





Figures 1-12. Leaf anatomy of *Erythroxylum ovalifolium* Peyr. in three different localities and three distinct microhabitats. 1-9. Cross sections of leaf blades from: 1. Jurubatiba (intermediate), fully exposed to sunlight. 2. Jurubatiba, semi-exposed. 3. Jurubatiba, shaded. 4. Massambaba (dry, open), exposed to sunlight. 5. Massambaba, semi-exposed. 6. Massambaba, shaded. 7. Maricá (wet, dense), fully exposed to sunlight. 8. Maricá, semi-exposed. 9. Maricá, shaded. 10-12. Adaxial epidermis of *Erythroxylum ovalifolium* in three different microhabitats of Massambaba locality. Cross sections of upper leaf blades from: 10. Fully exposed to sunlight. 11. Semi-exposed. 12. Shaded. Bars = 100  $\mu\text{m}$  (1-9); 20  $\mu\text{m}$  (10-12).

Second, leaf anatomical differences within populations were found for the two other traits measured, which were not responsive to site differences: adaxial epidermis and leaf blade thickness. These traits have been reported to vary in response to changes in light regimes (Boardman 1977, Björkman 1981, Strauss-Debenedetti & Berlin 1994, Rôças *et al.* 1997, Lee *et al.* 2000). The open “restingas”, organized in vegetation islands, have a clear gradient inside-border-outside island, which represents a gradient from shadier to more exposed conditions. The micro-environments along this gradient have been reported to differently affect seed

germination, clonal growth and whole-plant physiological behaviour in the “restingas” (Franco *et al.* 1996, Sampaio *et al.* 2002, 2005a). Our results show that this also applies to leaf anatomy. Moreover, phenolic compounds and vascular bundles were observed more frequently on exposed leaves, although quantitative methods were not performed. This observation for *E. ovalifolium* is consensual with the theory that the development of defences increases with increasing environmental stresses (Coley *et al.* 1985) and that great amounts of sclerenchyma and phenolic compounds offer mechanical support (Esau 1977) and protection against herbivory

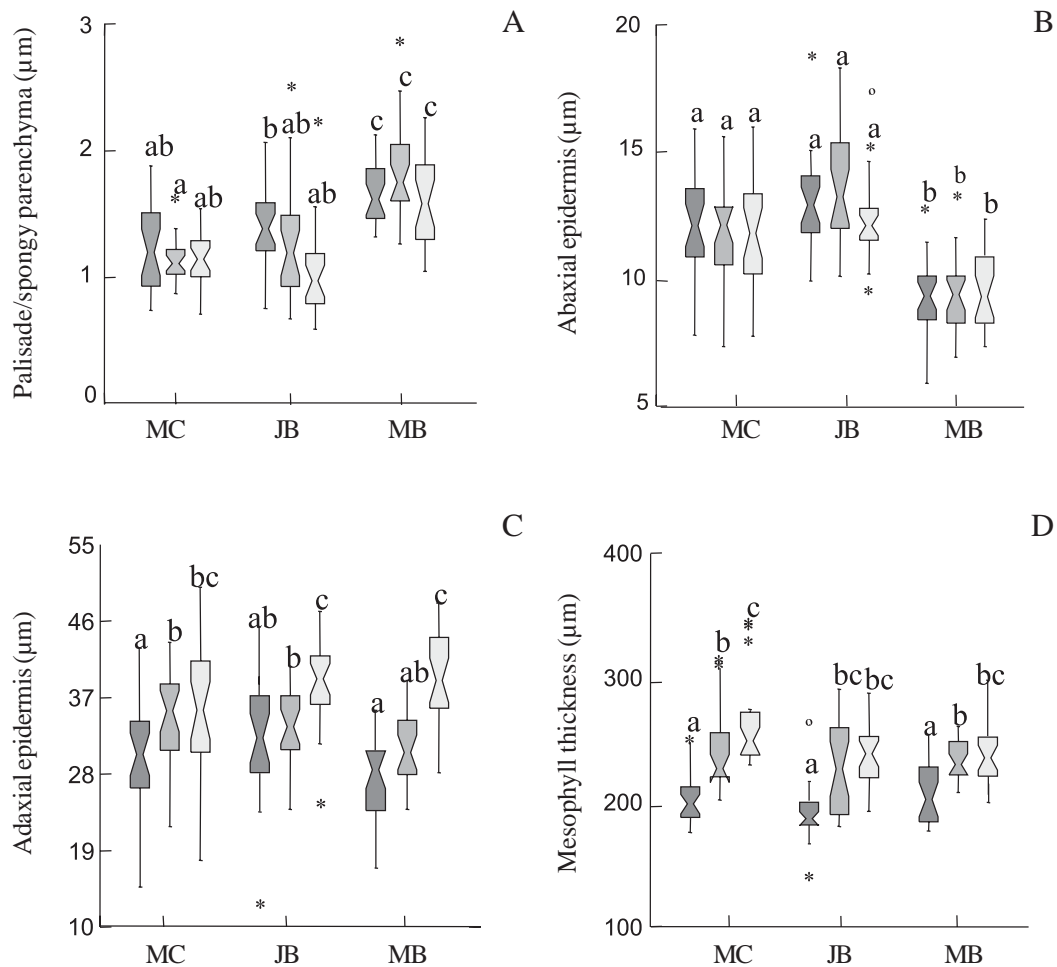


Figure 13. Notched box plot of leaf anatomy traits of *E. ovalifolium*. (A) ratio thickness of palisade to spongy parenchyma; (B) thickness of the abaxial epidermis (µm); (C) thickness of the adaxial epidermis (µm) and (D) leaf blade thickness (µm). Boxes present the (MC) Maricá, (JB) Jurubatiba and (MB) Massambaba populations growing under three luminous intensities and comprise 50% of the data. They are notched at the median and return to full width at the lower and upper 95% confidence intervals values. Interquartile ranges define inner and outer fences. Asterisks are “outside values” and circles are “far outside values”. The medians of the samples are statistically different ( $\alpha = 0.95$ ) when the confidence intervals do not overlap. Different letters indicates statistical differences. (☒ = Shared; ☒ = Semi-exposed; ☒ = Exposed).

(Feeny 1976, Rhoades 1979), respectively.

Thus, this species shows trait variation in response to distinct humidity regimes and to different light regimes. For instance, exposed plants from all sites showed thicker chlorophyllian tissues and a more compact cell layer leading to a reduction of the water diffusion surface, enhancement of light absorption and accumulation of photosynthetic products (Levitt 1980, Vogelmann & Martin 1993). Although controlled experiments should be required to assess the extent of such variation and its implications to acclimation and fitness, the high abundance of this plant throughout the study sites (Silva 1991, Araujo *et al.* 1998) suggests that this variation is a measure of this plant's ecological plasticity.

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