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Distribution of homobaric and heterobaric leafed species in the Brazilian Cerrado and seasonal semideciduous forests

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ABSTRACT

Plant species can be classified into heterobaric and homobaric, depending on whether their leaves present bundle sheath extensions (BSEs) (heterobaric) or not (homobaric). Incidences of the two leaf types seem to be related to growth environment and the light stratification. The Brazilian Cerrado and seasonal semideciduous forests are contrasting environments mainly with regard to irradiance and air humidity. However, studies comparing the distributions of homobaric and heterobaric species in these vegetation types are lacking. We investigate the presence/absence of leaf BSEs across diverse habits (herbs, shrubs and trees) in a seasonal semideciduous forest, cerrado *sensu stricto* (open physiognomy) and cerradão (forest physiognomy) to evaluate the influence of plant habit and growth environment on the distribution of homobaric and heterobaric species. Leaves from 131 species in 54 angiosperm families were analysed using standard methods of light microscopy. The distribution of the different leaf types in each environment was analysed using the χ^2 test and Fisher's exact test ($P < 0.05$). Homobaric and heterobaric leafed species occur in all environments and do not correlate with plant habit. Of the total number of species examined, 103 (78.6%) exhibited homobaric leaves and 28 (21.4%) presented heterobaric leaves. Of the total of heterobaric species, 8.8% occurred in the seasonal semideciduous forest, 50% in the cerradão and 41.2% in the cerrado *sensu stricto*. This model of distribution may be related to the higher irradiances in Cerrado. Homobaric leaves of shrubs and trees were thicker in all environments, while heterobaric leaves of herbs were thicker in the Cerrado. This study finds a tendency for the distribution pattern of homobaric or heterobaric leaves to reflect both the growth environment and the taxonomic grouping. Since leaf type is a constitutive characteristic, interspecific differences in leaf type may reflect particular physiological behaviours, linking them to success in colonising particular environments.

1. Introduction

Based on the presence or absence of bundle-sheath extensions (BSEs) in their leaves, plant species are generally classified into two groups – those having heterobaric leaves and those having homobaric leaves. In a heterobaric leaf, the parenchyma or sclerenchyma cells of the BSE extend to the epidermises on each side of the leaf (Kenzo et al., 2007), effectively isolating the leaf airspaces into numerous compartments (Terashima, 1992). In contrast, the BSEs are lacking in homobaric leaves, so the internal airspaces are essentially continuous (Kenzo et al., 2007).

The presence/absence of BSEs affects both the mechanical and the physiological properties of the two leaf types (Kenzo et al., 2007; Liakoura et al., 2009; Inoue et al., 2015). In homobaric leaves, the structural continuity of the leaf allows more efficient gas diffusion (Rhizopoulou and Psaras, 2003). On the other hand, the BSEs of heterobaric leaves may act as water (Wylie, 1943) and light conduits (Karabourniotis et al., 2000) to the inner layers of the mesophyll and may also give additional mechanical support to the leaf blade (Esau, 1977). In addition, higher rates of photosynthesis and transpiration have been recorded in heterobaric species (Inoue et al., 2015). Therefore, the architectural arrangement of a leaf's tissues may influence its physiological performance (Reich et al., 2003). This adaptation has been considered important in terms of specific growth environments and/or life-form types (Kenzo et al., 2007; Rossato et al., 2015). In addition, studies indicate that homobaric leaves are thicker than heterobaric leaves (Boeger et al., 2016) and present a higher proportion of photosynthetic areas in the mesophyll which is advantageous for capturing diffuse light (Terashima, 1992).

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Table 1
Data on the environmental features of the studied sites (CER: cerradão; CSS: cerrado *sensu stricto*; SSF: seasonal semideciduous forest. PPFD: Photosynthetic photon flux density).

Environmental characteristics	Studied sites		
	CER	CSS	SSF
Average daily PPFD (μmol m^{-2} s^{-1})	437	804	337
Maximum – average – minimum daily relative humidity of air (%)	52–41–30	49–39–28	72–69–65
Average annual precipitation (mm)	1454	1454	1428
Length of dry season (months)	5	5	5
Average altitude (m)	720	720	786
Minimun – average – maximun temperature (°C)	12.5–20.8–29	13.4–21.7–30	12–20.3–28.5
Soil type	latosols and argisols	latosols and argisols	podzolics and litholics

Recent studies have sought to correlate leaf type with environment, with life-form type and with plant habit (Kenzo et al., 2016). Thus, some researchers have shown that heterobaric species are more common in dry and/or cold deciduous forests (Terashima, 1992), while homobaric species predominate in the evergreen forests of humid and warm climates (Boeger et al., 2004; Kenzo et al., 2007). However, the distribution of light intensity, temperature and humidity is variable even inside a tropical evergreen forest (Whitmore, 1988). In this sense, Kenzo et al. (2007) proposed that the leaf type seems also to be correlated to the life form type of the species in a tropical forest, *i.e.* emergent, canopy, understorey, or canopy-gap species. Species in the upper canopy and in canopy gaps are exposed to higher irradiance and lower humidity than species in the understorey (Whitmore, 1988) favouring the abundance of heterobaric leafed species in these higher canopy layers and in gaps (Kenzo et al., 2007). Moreover, heterobaric leaves seem to be more common among tree and shrub species than among herbs in xeric environments (Liakoura et al., 2009).

The Brazilian savanna (local name Cerrado) and seasonal semideciduous forests present contrasting environmental features, mainly regarding irradiance (Hoffman and Franco, 2003; Tresmondi et al., 2015). The Cerrado is a vegetation mosaic composed of different physiognomies varying from open vegetation with continuous herbaceous and sparse woody plants (cerrado *sensu stricto*) to a true forest formation (cerradão). This ecosystem is characterised by a strongly seasonal climate with distinctive rainy summers and dry winters. The soils are deep and well drained, acidic, extremely low in available nutrients and with high aluminium contents (Oliveira and Marquis, 2002). The seasonal semideciduous forest is characterised by dense vegetation with a closed canopy and a layered structure, with trees up to 30 m high. The species are conditioned to eutrophic soils (Scariot and Sevilha, 2005) and seasonal climates that determine the semideciduous nature of the forest canopy. In this sense, we hypothesise that the environmental conditions of the Cerrado could favour the higher abundance of heterobaric leafed species. However, studies comparing the distribution of homobaric and heterobaric leaves in the Cerrado and forest are lacking.

Here, we investigate the presence/absence of leaf BSEs across diverse habits (herbs, shrubs and trees) and different vegetation types (seasonal semideciduous forest, cerrado *sensu stricto* and cerradão) in order to evaluate the influence of plant habit and growth environment on the distribution of homobaric and heterobaric leafed species. In addition, we measured the blade thickness looking for a relation of this anatomical trait with the leaf type.

2. Material and methods

2.1. Areas of study and plant material

This study was conducted during 2014 and 2015 in two contiguous Cerrado physiognomies (cerradão and cerrado *sensu stricto*) located in Palmeira da Serra farm, Pratânia municipality (22° 48′ 20″ S, 48° 44′ 36″ W) and in a remnant area of seasonal semideciduous forest located in Edgardia farm, in Botucatu municipality (22° 52′ S, 48° 26′ W), both in central-west region of São Paulo State, in southeastern Brazil. Pratânia and Botucatu municipalities are 37 km distant from each other. The south marginal areas of the Cerrado and the seasonal semideciduous forest are both characterised by marked climatic seasonality (Tresmondi et al., 2015). The climate in these areas is Cfa according to Köppen classification, that is, temperate (mesothermic) climate, typical of constantly humid region (Setzer, 1966), with rains in the summer and drought in the winter, and with small hydric deficiency from April to August (Cunha and Martins, 2009).

The environmental characterization of each area is summarized in Table 1.

The cerradão (Fig. 1a) and cerrado *sensu stricto* (Fig. 1b) are contiguous areas and encompass a total of 180 ha. In the Cerrado area, 120 species of angiosperms belonging to 52 families were investigated in the cerradão and 168 species belonging to 51 families in cerrado *sensu stricto* (Ishara, 2010). The studied forest fragment (Fig. 1c) was 56 ha in size, where 61 tree species belonging to 31 angiosperm families were inventoried (Fonseca and Rodrigues, 2000).

Based on previous phytosociological surveys (Fonseca and Rodrigues, 2000; Ishara et al., 2008; Carvalho et al., 2010; Jorge et al., 2015) a square of 400 m^2 was selected inside cerradão, cerrado *sensu stricto* and forest that encompassed a representative portion of the vegetation with heterogeneity of species and habits. Ten fully-expanded and non-senescent leaves were collected from each of the plants enclosed, in the rainy season (December 2014 to February 2015). In all, 51 species were collected in the cerradão (27 trees, 17 shrubs and 7 herbs), 55 species in the cerrado *sensu stricto* (16 trees, 24 shrubs and 15 herbs) and 49 species in the seasonal semideciduous forest (22 trees, 17 shrubs and 10 herbs). A total of 131 species belonging to 54 angiosperm families were examined. Since some species occurred in two or more environments, the total number of plants studied was 155 (Table 2).

Vouchers were incorporated in the collection of Herbarium Irina Delanova de Gemtchujnicov (BOTU), IBB UNESP, Botucatu/SP.

2.2. Light microscopy

For anatomical analysis, samples excised from the median region of the leaf blade were fixed in FAA 50 (Johansen, 1940) and processed according to two protocols. Most samples were cross-sectioned (12 μm thickness) using a Ranvier microtome, stained with safranine and astra blue (Bukatsch, 1972) and mounted in glycerine jelly. Others were dehydrated in an alcohol series and embedded in methacrylate resin, cross sectioned (5 μm in thickness) using a rotatory microtome and stained with toluidine blue O 0.05% pH 4.7 (O'Brien et al., 1964). Permanent slides were mounted using synthetic resin.

The slides were examined under a light microscope (Olympus BX 41) equipped with a digital camera. The leaf thickness was cal-

Table 2
Species from the seasonal semideciduous forest (SSF), cerrado *sensu stricto* (CSS) and cerradão (CER) with different habits and leaf types (BSE = bundle sheath extension; ES = esclerenchyma; PA: parenchyma).

Family	Species	Plant habit	Vegetation type	Leaf type	BSE cell type	Average of leaf thickness (μm)
Acanthaceae	*Ruellia* sp.	herb	SSF	Homobaric	–	124.65
	Justicia brasiliana Roth	shrub	SSF	Homobaric	–	97.89
	Justicia sp.	shrub	SSF	Homobaric	–	100.60
Amaranthaceae	*Gomphrena macrocephala* A.St.-Hil.	herb	CSS	Homobaric	–	203.27
Anacardiaceae	*Anacardium humile* A.St.-Hil.	shrub	CSS	Heterobaric	ES	189.13
	Astronium graveolens Jacq.	tree	SSF	Homobaric	–	140.69
	Lithraea molleoides (Vell.) Engl.	tree	CER	Homobaric	–	277.75
Annonaceae	*Annona* sp.	shrub	CSS	Homobaric	–	195.70
	Annona coriacea Mart.	shrub	CSS, CER	Homobaric	–	207.37, 195.46
	Duguetia furfuracea (A.St.-Hil.) Benth. & Hook.	tree	CSS	Heterobaric	ES	93.16
Apocynaceae	*Aspidosperma polyneuron* Müll. Arg.	tree	SSF	Homobaric	–	145.27
	Aspidosperma ramiflorum Müll. Arg.	shrub	SSF	Homobaric	–	114.15
Asteraceae	*Chaptalia* sp.	herb	CSS	Homobaric	–	132.67
	Acanthospermum australe (Loefl.) Kuntze	herb	CSS	Homobaric	–	97.62
	Baccharis dracunculifolia DC.	shrub	CSS	Homobaric	–	134.31
	Emilia sonchifolia (L.) DC. Ex Wight	herb	CSS	Homobaric	–	134.84
	Gochnatia pulchra Cabrera	shrub	CSS, CER	Heterobaric	ES	120.66, 130.02
	Moquiniastrum barrosoae (Cabrera) G. Sancho	shrub	CSS, CER	Heterobaric	ES	72.38, 69.96
Bignoniaceae	*Fridericia* sp.	shrub	CSS	Homobaric	–	80.79
	Handroanthus serratifolius (Vahl.) S. Grose	tree	CSS, CER	Heterobaric	ES	160.15, 138.14
	Zeyheria montana Mart.	tree	CSS, CER	Homobaric	–	100.25, 99.84
Boraginaceae	*Heliotropium* sp.	herb	SSF	Homobaric	–	108.39
Bromeliaceae	*Ananas ananassoides* (Baker) L.B.Sm.	herb	CSS	Homobaric	–	206.87
	Bromelia balansae Mez	herb	CSS, CER	Homobaric	–	115.68, 112.92
	Bromelia sp.	shrub	CSS	Homobaric	–	271.55
Cannabaceae	*Celtis fluminensis* Carauta	shrub	SSF	Homobaric	–	91.51
Caryocaraceae	*Caryocar brasiliense* Cambess	shrub	CSS	Homobaric	–	136.61
Celastraceae	*Maytenus aquifolium* Mart.	shrub	SSF	Homobaric	–	100.54
Commelinaceae	*Commelina* sp.1	herb	SSF	Homobaric	–	139.32
	Commelina sp. 2	herb	CER	Homobaric	–	141.77
Convolvulaceae	*Evolvulus* sp.	herb	CSS	Homobaric	–	62.39
Cucurbitaceae	*Cayaponia espelina* (Silva Manso) Cogn.	herb	CSS	Heterobaric	ES	215.55
Cyperaceae	*Hypolytrum pungens* (Vahl.) Kunth	herb	CER	Heterobaric	ES	214.61
Dilleniaceae	*Davilla* sp.	shrub	CSS	Homobaric	–	189.71
Erythroxylaceae	*Erythroxylum campestre* A.St.-Hil.	shrub	CSS	Homobaric	–	144.03
	Erythroxylum deciduum A.St.-Hil.	shrub	CER	Homobaric	–	88.97
Euphorbiaceae	*Acalypha* sp.	herb	SSF	Homobaric	–	110.80
	Chamaesyce Gray	herb	CSS	Homobaric	–	129.18
	Actinostemon concepcionis (Chodat & Hassl.) Hochr.	shrub	SSF	Homobaric	–	78.36
	Croton floribundus Spreng.	tree	SSF	Heterobaric	PA	130.01
	Croton glandulosus L.	herb	CSS	Homobaric	–	139.24
Fabaceae	*Andira humilis* Mart. ex. Benth.	shrub	CSS, CER	Heterobaric	ES	185.53, 131.35
	Bauhinia sp.	shrub	CSS	Heterobaric	ES	86.94
	Bauhinia longifolia (Bong.) Steud.	shrub	SSF	Heterobaric	ES	78.59
	Bauhinia rufa (Bong.) Steud.	shrub	CSS, CER	Heterobaric	ES	98.31, 90.92
	Bowdichia virgiloides Kunth	tree	CER	Homobaric	–	105.16
	Chamaecrista flexuosa (L.) Greene	shrub	CSS	Homobaric	–	106.01
	Copaifera langsdorffii Desf.	tree	CER	Heterobaric	ES	190.81
	Dimorphandra mollis Benth.	tree	CSS, CER	Homobaric	–	128.64, 126.05
	Holocalyx balansae Micheli	tree	SSF	Homobaric	–	120.55
	Inga striata Benth.	tree	SSF	Homobaric	–	110.61
	Machaerium sp. Pers.	tree	CER	Heterobaric	PA	199.83
	Peltophorum dubium (Spreng.) Taub.	tree	SSF	Homobaric	–	81.82
	Platypodium elegans Vogel	tree	SSF, CER	Homobaric	–	211.09, 190.89
	Senna rugosa (G. Don) H.S.Irwin & Barneby	shrub	CSS, CER	Homobaric	–	95.12, 90.65
	Stryphnodendron adstringens (Mart.) Coville	tree	CSS	Homobaric	–	124.29
Lacistemataceae	*Lacistema hasslerianum* Chodat	shrub	CER	Homobaric	–	100.39
Lamiaceae	*Eriope crassipes* Benth.	herb	CSS	Heterobaric	PA	84.96
	Aegiphila sellowiana Cham.	tree	CER	Homobaric	–	180.61
	Hyptis sp.	herb	CSS	Homobaric	–	98.05
Lecythidaceae	*Cariniana estrellensis* (Raddi) Kuntze	tree	SSF	Homobaric	–	102.33
Loganiaceae	*Strychnos brasiliensis* Mart.	shrub	SSF	Homobaric	–	135.21
Lythraceae	*Cuphea calophylla* Cham. & Schltdl.	herb	SSF	Homobaric	–	71.00
Malpighiaceae	*Byrsonima pachyphylla* A. Juss.	shrub	CER	Homobaric	–	200.99
	Heteropterys sp.	shrub	CSS	Heterobaric	ES	163.57
Malvaceae	*Eriotheca grassilipes* Schott & Endl.	tree	CSS	Heterobaric	ES	177.15
	Luehea grandiflora Mart. & Zucc.	tree	CER	Heterobaric	PA	61.83
	Pseudobombax longiflorum (Mart. et Zucc.) A. Robyns	tree	CER	Heterobaric	ES	131.90
	Sida sp.	herb	CSS	Homobaric	–	95.63
	Waltheria communis A.St-Hill	shrub	CSS	Heterobaric	ES	143.55
Melastomataceae	*Miconia albicans* (Sw.) Triana	shrub	CSS, CER	Homobaric	–	161.46, 156.59
	Miconia langsdorffii Cong.	shrub	CER	Homobaric	–	150.11

Table 2 (*Continued*)

Family	Species	Plant habit	Vegetation type	Leaf type	BSE cell type	Average of leaf thickness (μm)
	Miconia fallax DC.	tree	CSS	Homobaric	–	142.59
Meliaceae	*Trichilia casaretti* C.DC.	tree	SSF	Homobaric	–	110.49
	Trichilia catigua A.Juss.	tree	SSF	Homobaric	–	138.34
	Trichilia elegans A.Juss.	shrub	SSF	Homobaric	–	105.28
	Trichilia pallida Sw.	tree	SSF	Homobaric	–	124.13
Myrtaceae	*Calyptranthes lucida* Mart. ex. DC.	shrub	CER	Homobaric	–	160.61
	Eugenia aurata O. Berg	shrub	CER	Homobaric	–	162.17
	Eugenia blastantha (O.Berg) D. Legrand	tree	SSF	Homobaric	–	140.78
	Eugenia florida DC.	tree	SSF, CER	Homobaric	–	150.98, 160.56
	Eugenia sp.	tree	CSS	Homobaric	–	206.78
	Myrcia sp. 1	tree	CER	Homobaric	–	195.73
	Myrcia sp. 2	tree	CER	Homobaric	–	194.69
	Myrcia bela Cambess.	tree	CSS, CER	Homobaric	–	131.91, 125.61
	Myrcia lingua (O.Berg) Mattos	tree	CSS, CER	Homobaric	–	220.23, 212.13
	Myrcia multiflora (Lam.) DC.	tree	CSS	Homobaric	–	212.52
	Myrcia selloi (Spreng.) N.Silveira	tree	CER	Homobaric	–	195.81
	Myrciaria floribunda Miq.	shrub	SSF	Homobaric	–	92.71
	Neomitranthes glomerata (D.Legrand) D. Legrand	tree	SSF	Homobaric	–	141.11
Ochnaceae	*Ouratea spectabilis* (Mart.) Engl.	tree	CSS, CER	Homobaric	–	88.23, 85.42
Opiliaceae	*Agonandra engleri* Hoehne	tree	SSF	Homobaric	–	118.81
Orchidaceae	*Oeceoclades maculata* (Lindl.) Lindl.	herb	SSF, CER	Homobaric	–	280.28, 250.61
Oxalidaceae	*Oxalis* sp.	herb	SSF	Homobaric	–	100.03
Passifloraceae	*Piriqueta* Aubl.	herb	CSS	Homobaric	–	107.66
Peraceae	*Pera glabrata* (Schott) Poepp. ex Baill.	tree	CSS, CER	Homobaric	–	143.09, 141.08
Phytolaccaceae	*Gallesia integrifolia* (Spreng.) Harms	tree	SSF	Homobaric	–	157.53
Piperaceae	*Piper amalago* L.	shrub	SSF	Homobaric	–	140.76
	Piper arboreum Aubl.	shrub	SSF	Homobaric	–	184.66
Poaceae	*Panicum* sp. 1	herb	SSF, CER	Heterobaric	ES	94.10, 100.69
	Panicum sp. 2	herb	CER	Homobaric	–	102.54
Portulacaceae	*Talinum paniculatum* (Jacq.) Gaertn.	herb	SSF	Homobaric	–	98.99
	Portulaca oleracea L.	herb	SSF	Homobaric	–	250.50
Primulaceae	*Myrsine guianensis* (Aubl.) Kuntze	tree	CSS, CER	Homobaric	–	224.58, 215.24
	Myrsine umbellata Mart.	tree	SSF, CER	Homobaric	–	163.46, 170.09
Proteaceae	*Roupala montana* Aubl.	tree	CER	Heterobaric	ES	231.81
Rhamnaceae	*Rhamnidium elaeocarpum* Reissek	tree	SSF	Homobaric	–	95.79
Rubiaceae	*Cordiera sessilis* (Vell.) Kuntze	shrub	CER	Homobaric	–	170.51
	Borreria schumannii (Standl. ex Bacigalupo) E.L.Cabral & Sobrado	herb	CSS	Homobaric	–	98.37
	Psychotria carthagenensis Jacq.	shrub	SSF	Homobaric	–	117.60
	Psychotria sp.	herb	CER	Homobaric	–	99.13
	Tocoyena formosa (Cham. & Schltdl.) K.Schum.	shrub	CSS	Homobaric	–	78.55
Rutaceae	*Metrodorea nigra* A.St.-Hil.	tree	SSF	Homobaric	–	187.82
	Zanthoxylum fagara (L.) Sarg.	tree	CSS	Homobaric	–	140.12
Salicaceae	*Casearia sylvestris* Sw.	tree	SSF, CSS, CER	Homobaric	–	120.44, 190.35, 130.78
Sapotaceae	*Chrysophyllum gonocarpum* (Mart. & Eichler ex Miq.) Engl.	tree	SSF	Homobaric	–	117.80
Siparunaceae	*Siparuna guianensis* Aubl.	shrub	CER	Homobaric	–	102.81
Solanaceae	*Cestrum mariquitensis* Kunth.	shrub	SSF	Homobaric	–	76.31
	Solanum sp. 1	herb	CSS	Homobaric	–	128.14
	Solanum sp. 2	herb	CSS	Homobaric	–	128.00
	Solanum lycocarpum A.St.-Hil	shrub	CER	Heterobaric	ES	121.46
Thymelaeaceae	*Daphnopsis utilis* Warms	tree	CER	Homobaric	–	160.54
Verbenaceae	*Lantana camara* L.	shrub	CSS, CER	Heterobaric	PA	172.88, 170.03
Violaceae	*Hybanthus atropurpureus* (A.St.-Hil) Taub.	shrub	SSF	Homobaric	–	86.11
	Hybanthus sp.	shrub	SSF	Homobaric	–	70.17
Vitaceae	*Cissus* sp.	shrub	CSS	Homobaric	–	106.55
Vochysiaceae	*Qualea dichotoma* (Mart.) Warm.	tree	CER	Heterobaric	PA	110.09
	Qualea grandiflora Mart.	tree	CER	Heterobaric	ES	114.06
	Vochysia tucanorum Mart.	tree	CER	Heterobaric	PA	112.48

culated in cross sections of 5 individuals of each species (n = 5) using the software Olympus Cell B.

For analyses of the leaf surfaces, fresh samples were analysed under a stereomicroscope Leica M205C.

2.3. Statistical analyses

The frequency of homobaric and heterobaric leafed species among the vegetation types and the plant habits was compared using the $\chi 2$ test and the Fisher exact test with 5% significance level when the frequency was lower than five (Snedecor and Cochran, 1974), using SAS 9.2 software. The leaf thickness in homobaric and heterobaric leaves in each environment and habitat was subjected to Levene's test to determine the homogeneity of the treatment variances. Data were subjected to an analysis of variance (ANOVA), and the means were compared by Tukey's test with a 5% probability ($P \leq 0.05$).

3. Results

Both homobaric (Fig. 2a) and heterobaric (Fig. 2b,c) leafed species occurred in the seasonal semideciduous forest, in the cerradão and in the cerrado *sensu stricto* (Table 2). The homobaric species predominated in all vegetation types (Table 3). Of the 131 species studied, 103 (78.6%) exhibited homobaric leaves.

The proportion of species with each leaf type differed among the study areas (χ^2 test, $P \leq 0.05$, df = 2, $N = 155$; Table 3). In the seasonal semideciduous forest, 6.1% of the species presented heterobaric leaves, while in the cerradão and the cerrado *sensu stricto* the heterobaric species represented were 33.3 and 25.5%, respec-

Table 3
Number of species with heterobaric and homobaric leaves in the seasonal semideciduous forest, cerradão and cerrado *sensu stricto* (χ^2 test, $P \leq 0.05$, df = 2, $N = 155$).

Vegetation type	Total of sampled species	Species with heterobaric leaves	Species with homobaric leaves
Seasonal semideciduous forest	49	3	46
Cerradão	51	17	34
Cerrado *sensu stricto*	55	14	41

Table 4
Number of heterobaric and homobaric leafed species grouped according to their different habits.

a) in the seasonal semideciduous forest (Fisher exact test, $P \leq 0.05$, df = 2, N = 49).

Plant habit	Total of sampled species	Species with heterobaric leaves	Species with homobaric leaves
Herbs	10	1	9
Shrubs	17	1	16
Trees	22	1	21

b) in the cerrado *sensu stricto* (Fisher exact test, $P \leq 0.05$, df = 2, $N = 55$).

Plant habit	Total of sampled species	Species with heterobaric leaves	Species with homobaric leaves
Herbs	15	2	13
Shrubs	24	9	15
Trees	16	3	13

c) in the cerradão (Fisher exact test, $P \leq 0.05$, df = 2, $N = 51$).

Plant habit	Total of sampled species	Species with heterobaric leaves	Species with homobaric leaves
Herbs	7	2	5
Shrubs	17	6	11
Trees	27	9	18

tively. Of the 34 heterobaric leafed species, 3 (9%) occurred in the seasonal semideciduous forest, 17 (50%) in the cerradão, and 14 (41%) in the cerrado *sensu stricto*.

Differences in the distributions of homobaric and heterobaric species with the three habits were not significant in any of the areas studied (Fisher exact test, $P \geq 0.05$, df = 2, $N = 49$ for seasonal semideciduous forest, Table 4a; Fisher exact test, $P \geq 0.05$, df = 2, $N = 55$ for cerrado *sensu stricto*, Table 4b; and Fisher exact test, $P \geq 0.05$, df = 2, $N = 51$ for cerradão, Table 4c). However, there may be a tendency for a greater abundance of heterobaric species among trees and shrubs in the Cerrado. In the cerrado *sensu stricto*, 64.3% of the heterobaric leafed species were shrubby (Table 4b) and in the cerradão, 35% of them were shrubs and 52.9% trees (Table 4c). In the seasonal semideciduous forest, the percentages of species with heterobaric leaves were about the same among herbs, shrubs and trees (Table 4a).

Concerning the cell composition, heterobaric leaves can present parenchymatous or sclerenchymatous BSE. In seasonal semideciduous forest, 66.7% of the heterobaric leafed species presented sclerenchymatous BSE and 33.3% had parenchymatous BSE. In cerradão, 70.6% of the sampled heterobaric leafed species presented sclerenchymatous BSE and 29.4% had parenchymatous BSE. In the cerrado *sensu stricto*, 85.8% of the heterobaric leafed species exhibited sclerenchymatous BSE and 23.5% presented parenchymatous BSE. Concerning the distribution of sclerenchymatous and parenchymatous BSE among the different studied families, we observed that three plant families presented only species with parenchymatous BSE; 10 families exhibited species with sclerenchymatous BSE and four families presented both parenchymatous and sclerenchymatous BSE (Table 2).

The comparison of homobaric and heterobaric leaves showed that homobaric leaves of shrubs and trees were thicker in all environments, while heterobaric leaves of herbs are thicker in the Cerrado (Table 5).

Table 5
Average thickness (μm) of homobaric and heterobaric leaves by environment and plant habit (CER = cerradão, CSS = cerrado *sensu stricto*, SSF = seasonal semideciduous forest). Values followed by the same letter do not differ significantly according to Tukey's test ($P < 0.0001$, $n = 5$); lower case letters in the column and capital letters in the line.

Leaf type	Environment		
	CER	CSS	SSF
	Herb		
Heterobaric	157.66 aA	150.26 aB	94.10 bC
Homobaric	141.40 bA	130.14 bB	130.93 aB
	Shrub		
Heterobaric	117.40 bB	138.04 bA	78.59 bC
Homobaric	137.87 aB	142.01 aA	110.96 aC
	Tree		
Heterobaric	143.44 bA	143.49 bA	130.02 bB
Homobaric	166.79 aA	153.49 aB	134.86 aC
C. V. = 34%			

4. Discussion

The occurrence of homobaric and heterobaric leafed species did not depend on the habit types or on the vegetation formations. Homobaric and heterobaric leaves occurred in herbaceous, shrubby and tree species growing in the forest and also in the savanna ecosystems (both the cerradão and the cerrado *sensu stricto*).

A predominance of homobaric leafed species is commonly associated with forest trees of warm, humid regions (Wylie, 1952; McClendon, 1992; Kashimura et al., 2000; Boeger et al., 2004; Kenzo et al., 2007). However, our results show that homobaric leafed species also predominate in forest and savanna systems, having contrasting environments mainly with regard to irradiance (Hoffman and Franco, 2003; Tresmondi et al., 2015). According to Kenzo et al. (2007), shade and wet conditions are more suited to homobaric leaves than to heterobaric leaves. The predominance of homobaric leafed species in the cerrado *sensu stricto* is unexpected since this ecosystem is characterised by intense sunlight that generates a drier microclimate (Coutinho, 1978, 2002) compared with either the seasonal semideciduous forest or the cerradão

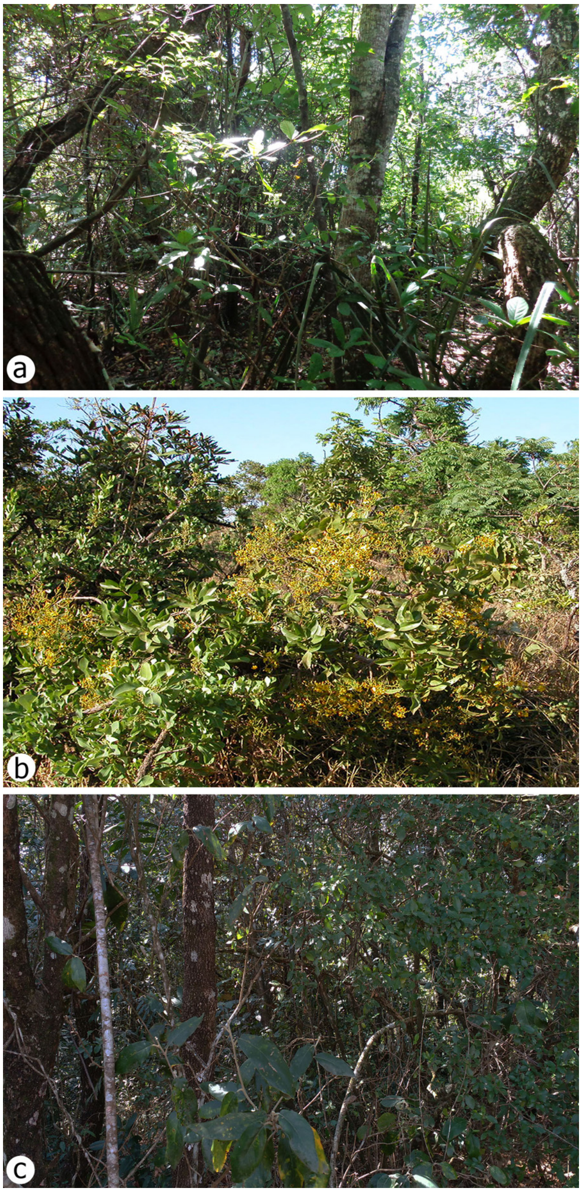

Fig. 1. Aspects of the study areas. A. Cerradão. B. Cerrado *sensu stricto*. C. Seasonal semideciduous forest.

(Tresmondi et al., 2015). On the other hand, this is not entirely surprisingly, given that shrubs and trees from the Cerrado usually have deep roots and so likely experience satisfactory access to water (Machado et al., 1997).

Nevertheless, comparing the distributions of heterobaric species in the three vegetation types studied, we note that this leaf type is more common in the Cerrado than in the seasonal semideciduous forest. This may be related to the microclimate around the Cerrado plants, i.e. higher irradiance and lower humidity (Tresmondi et al., 2015). Following Kenzo et al. (2007), the compartmentalisation of the leaf mesophyll by BSEs is more common in environments with characteristics that expose the plants to greater levels of desiccation. So, in the Cerrado, the BSEs could be related to the efficient use of water, since they are responsible for the more rapid response of stomata to drought signals such as the reduction of water potential in the mesophyll (Terashima, 1992). In addition, the more efficient water transport in heterobaric leaves reduces water usage associated with photosynthesis more than in homobaric leaves (Inoue et al., 2015). Other roles have been attributed to BSEs, such as the protection of leaves against water loss after leaf injury (Aldea et al., 2005) and the conduction of visible light to the inner layers of mesophyll in thick leaves (Karabourniotis et al., 2000; Nikolopoulos et al., 2002; Liakoura et al., 2009).

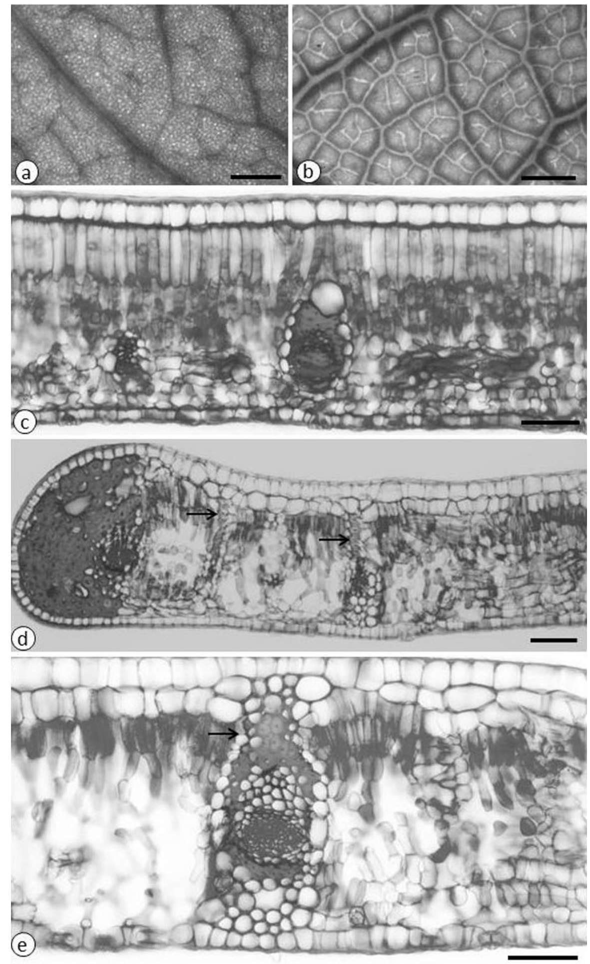

Fig. 2. Light micrographs of representative homobaric and heterobaric leaves. A. The abaxial surface of homobaric leaf of *Emilia sonchifolia*. B. The abaxial surface of heterobaric leaf of *Bauhinia rufa*. C. Cross section of homobaric leaf of *Cordiera sessilis* (Rubiaceae). D, E. Cross sections of heterobaric leaves of *Eriotheca gras-silipes* (Malvaceae). Arrows indicate bundle sheath extensions. Bars: A, B = 500 μm. C, D = 150 μm. E = 100 μm.

Although we did not observe a direct relation between the leaf type and habit, heterobaric leaves do seem to be more common among shrubs and trees, mainly in the Cerrado. Our results corroborate the findings of McClendon (1992) and Kenzo et al. (2007) who report the frequency of leaves with BSEs is higher among plants in the higher strati of the canopy and also those found in gaps. Clearly, these plants will be more subject to desiccation by exposure to higher VPDs, higher leaf temperatures, higher irradiation levels and stronger winds (Aoki et al., 1978). In this situation, the presence of BSEs could represent an advantage due to their action in the rapid stomatal responses to desiccation and also in the structural protection they offer reducing leaf compression under conditions of low water status as has been suggested by Terashima (1992) and Kenzo et al. (2007). Future research should involve experimental verification that the hypothesised physiological benefits of the heterobaric feature translate to actual physiological benefits in these species.

Sclerenchymatous BSE were more abundant in the three studied environments in comparison with parenchymatous BSE, wherein the higher proportion of species with sclerenchymatous BSE was observed in the Cerrado. This is a xeromorphic aspect that has been associated with the environmental conditions of the Cerrado (Bieras and Sajo, 2009; Rossato et al., 2015) and enable the species to cope with abiotic filters such as drought, high light intensities and oligotrophic soils (Rossato et al., 2015) in addition to provide mechanical support and protection against leaf collapse after water deficit (Wylie, 1943; Terashima, 1992; Kenzo et al., 2007) and herbivores (Sack and Scoffoni, 2013).

The highest thickness of the homobaric leaves as in shrub and trees in Cerrado and seasonal forest is a common trait to other studies (Terashima, 1992; Kenzo et al., 2007; Liakoura et al., 2009; Pieruschka et al., 2010; Lynch et al., 2012; Boeger et al., 2016) and has been associated to a well-developed spongy parenchyma (Boeger et al., 2016). According to Vogelman et al. (1996), a thicker spongy parenchyma is advantageous for capturing diffuse light, mainly under limited light conditions. The occurrence of thicker heterobaric leaves in herbs from cerrado *sensu stricto* and cerradão is noticeable in this study. This fact is probably associated with the presence of xeromorphic features in the herb species in these environments. Succulent plants, such as Bromeliaceae species common in Cerrado (Table 1), are characterised by a well-developed water-storage tissue (Esau, 1977). In addition, the developed sclerenchyma and more strongly differentiated palisade parenchyma collaborate to the increased thickness of the leaves (Esau, 1977). In contrast to other studies, we opted for not exclude these species (with very thick leaves) from our results.

Among the species, genera and families which we sampled we note that, at family level, both homobaric and heterobaric leafed species can sometimes occur in the same families e.g. in Anacardiaceae, Annonaceae, Asteraceae, Euphorbiaceae, Fabaceae, Malpighiaceae, Malvaceae. Nevertheless, the majority of species from any particular family presented only one of these leaf types and this consistency seemed to be independent of habit and vegetation type. Thus, species belonging to the Acanthaceae, Bromeliaceae, Erythroxylaceae, Melastomataceae, Meliaceae, Myrtaceae, Rubiaceae, Rutaceae and Violaceae exhibited only homobaric leaves while, in contrast, species belonging to the Vochysiaceae presented only heterobaric leaves.

At the genus level, the majority of species from any particular genus also present the same leaf type. Thus, the *Bauhinia* species (Fabaceae), and *Qualea* species (Vochysiaceae) exhibited only heterobaric leaves, while the *Justicia* species (Acanthaceae), *Miconia* species (Melastomataceae), *Trichilia* species (Meliaceae), *Myrsine* species (Primulaceae), and *Eugenia* and *Myrcia* species (Myrtaceae) exhibited only homobaric leaves. The exception was *Solanum* (Solanaceae) where among the species appearing in our study, some exhibited homobaric and others heterobaric leaf types (Table 2).

Considering the cell composition of the BSE, except in Bignoniaceae, Fabaceae, Malvaceae and Vochysiaceae that present both parenchymatous and sclerenchymatous BSE, most of the plant families here studied exhibited heterobaric leaves with only one cell type, parenchymatous or sclerenchymatous.

It is fair to conclude that, in most cases, leaf type and cell composition of the BSE is related to taxonomic group (Kenzo et al., 2007) and the trait seems to be phylogenetically conserved. To better evaluate these phylogenetic hypotheses, larger numbers of representative species inside these families are under analysis.

Our findings seem to be consistent with hypotheses that seek to explain the distribution and dominance of some families in a given biome. According to Franco (2002), species from the Amazonian and Atlantic basins have contributed to form the Cerrado flora. In this sense, Veloso (1964) affirmed that the semideciduous seasonal forests and the Cerrado originated from the same floristic trunk, and the dominance of some families in a given area results from the higher ability of these plants to survive under the given edaphic and climatic conditions. It is notable that the forests and Cerrado are found in juxtaposition and in the same general climate (Aoki and Santos, 1979), with differences of microclimate and soil seeming to influence the Cerrado distribution more strongly (Alvim and Araujo, 1952; Alvim, 1954; Arens, 1958; Aoki and Santos, 1979).

In summary, this study finds a tendency for the distribution pattern of homobaric or heterobaric leaf types to reflect both the growth environment and also the taxonomic grouping. Since leaf type is a constitutive characteristic – it does not change with leaf development or among individuals of a same species – interspecific differences in leaf type may reflect particular physiological behaviours, linking them to success in colonising particular environments.

Further analysis on the anatomical organization of heterobaric and homobaric leaves as well as the micromorphology of the leaf surfaces in conjunction with the photosynthetic performance of the species are in course.

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