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Original Article

Can climate and soil conditions change the morpho-anatomy among individuals from different localities? A case study in *Aldama grandiflora* (Asteraceae)

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(With 3 figures)

Abstract

Vegetative aerial organs are considerably more exposed to environmental conditions and can reflect the specific adaptations of plants to their local environment. *Aldama grandiflora* species are known to be widely distributed in Brazil; therefore, individuals from different populations of this species are thought to be exposed to different abiotic and biotic conditions. Several anatomical studies conducted on Brazilian *Aldama* species have mainly focused on the qualitative anatomical characters or traits of these species, but not on their quantitative traits. In this study, we evaluated whether climate and soil conditions can change the morphometry among individuals of *A. grandiflora* collected from six sites in the Goiás State, Brazil, by assessing their anatomical characters. Further, soil sampling was performed, and climate data were collected from all the six sites. The analysis indicated few statistical differences among the populations evaluated, showing that *A. grandiflora* presented consistent leaf and stem anatomical characteristics. The small morpho-anatomical differences found among individuals of the different populations evaluated, reflected the soil conditions in which these populations were grown. Therefore, environmental factors have a significant influence on the morpho-anatomy of *Aldama grandiflora*.

Keywords: cerrado, Compositae, morphometry, phenotypic plasticity, leaf anatomy.

Condições climáticas e de solo podem alterar a morfo-anatomia entre indivíduos de diferentes localidades? Um estudo de caso em *Aldama grandiflora* (Asteraceae)

Resumo

Os órgãos vegetativos aéreos estão consideravelmente mais expostos às condições ambientais e podem refletir as adaptações específicas das plantas ao seu habitat. A espécie *Aldama grandiflora* é amplamente distribuída no Brasil e, dessa forma, indivíduos de diferentes populações podem estar expostos a diferentes condições ambientais. Vários estudos anatômicos realizados com espécies brasileiras do gênero *Aldama* têm abordado, principalmente, as características anatômicas qualitativas dessas espécies, mas não em suas características quantitativas. Neste estudo avaliamos se as condições climáticas e do solo podem alterar a morfometria entre os indivíduos de *A. grandiflora* coletados em seis populações do Estado de Goiás. Foram avaliados os caracteres anatômicos foliares e caulinares, além da amostragem do solo e coleta de dados climáticos, para os seis locais. A análise indicou algumas diferenças estatísticas entre as populações avaliadas, mostrando que *A. grandiflora* apresentou características anatômicas foliares e caulinares bastante consistentes. As pequenas diferenças morfo-anatômicas encontradas entre indivíduos das diferentes populações avaliadas, refletiram as condições do solo nos quais essas populações se desenvolveram. Assim sendo, fatores ambientais relacionados ao clima e condições do solo têm uma influência significativa sobre a morfo-anatomia de *Aldama grandiflora*.

Palavras chave: cerrado, Compositae, morfometria, plasticidade fenotípica, anatomia foliar.

1. Introduction

The family Asteraceae accounts for approximately 10% of the world's flora (Panero and Crozier, 2012) and is one of the most important among angiosperms, comprising around 1620 genera and 23600 species (Stevens, 2001). Being a large family, the niches occupied by its species vary substantially and a considerable number of anatomical differences can be explained by phenotypic plasticity (Metcalfe and Chalk, 1983), which allow adaptation to different environmental conditions (Dickison, 2000).

The tribe Heliantheae, which occurs in the Cerrado, is the second largest tribe of the family, comprising 189 genera and around 2500 species. Among its representatives is *Aldama* La Llave, a South American genus, which includes 35 Brazilian species, of which 17 are endemic (Magenta et al., 2010). The representatives of this genus are morphologically very similar, leading to problems in taxonomical delimitations among the species (Schilling and Panero, 2011).

Aldama grandiflora (Gardner) E.E. Schill. & Panero (= *Viguiera grandiflora*) is widely distributed in open areas from the Cerrado, the second largest plant formation in Brazil, and can be found in different geographic regions such as North (Amazonas state), Northeast (Bahia), Midwest (Distritio Federal, Goiás, Mato Grosso do Sul and Mato Grosso states), Southeast (Minas Gerais), and South (Paraná). This species stands out in *Aldama* genus because, in addition to its wide distribution, it has resiniferous potential (Magenta and Pirani, 2014) and high yield of essential oils (data not published), with several compounds having proven biological activity (Leite et al., 2007; Canales et al., 2008; Santos et al., 2011). This species is characterized by intraspecific structural variations and high degree of polymorphisms in color and shape of the leaf blade since its representatives are tolerant to different light intensities (Magenta and Pirani, 2014). Several taxonomic studies have been conducted on Brazilian *Aldama* species (Bombo et al., 2012, 2014; Oliveira et al., 2013; Silva et al., 2014); however, such studies have addressed only the qualitative anatomical characters of these species, but not the quantitative traits.

Environmental factors related to climate, such as water relations, photoperiod, and light intensity, as well as soil parameters such as chemical and physical characteristics, in addition to the relief and elevation of the area, significantly influence the morphology and anatomy of plants (Olsen et al., 2013). According to Scheiner (1993) and Stearns (1989), phenotypic plasticity relates to the capacity of an organism to change its physiology or morphology in response to environmental conditions. The species *A. grandiflora*, in which the organs related to survival show high phenotypic capacity (Magenta and Pirani, 2014), could have adaptive advantages when exposed to unfavorable environments (Gardoni et al., 2007) and these changes would increase the environmental stress tolerance of the plant, favoring its occupancy to new niches (Stearns, 1989; Scheiner, 1993).

Plant organs such as leaves are the most exposed to environmental factors, and structural changes owing to phenotypic plasticity can be interpreted as specific adaptations to the local environment (Fahn, 1986; Dickison, 2000). The characters of stems, mainly those related to the vascular system, which are parameters that determine the efficiency and water resistance capacity (Kuniyoshi, 1993), can also exhibit variations in response to changes in relative humidity, temperature, and salinity (Yaltirik, 1970; Baas, 1982; Carlquist, 1988); therefore, they are crucial in maintaining these individuals in their habitat.

Since *A. grandiflora* is a well-represented Brazilian species owing to its widespread occurrence and geographic distribution, its individuals are submitted to different abiotic and biotic conditions that may have influence in their morpho-anatomy according to the locality in which they develop. Hence, in this study, we evaluated whether the leaf and stem anatomical characters of *A. grandiflora* can vary within the same species according to the environment in which they are grown, considering populations grown in different localities and, consequently, different soil and climatic conditions. This study aimed to evaluate whether (a) the morphometric anatomical parameters of the leaves and stems of *A. grandiflora* from six different populations sampled from two regions varied among populations and (b) variation occurred among these populations, and was it a consequence of the edaphoclimatic conditions to which these populations were exposed. Answers to these questions might provide better insight into the relations between the morpho-anatomy of the vegetative aerial organs and abiotic factors for *Aldama* species.

2. Material and Methods

Plant material and collection areas: Leaves and stems of *A. grandiflora* were sampled from adult plants from six different sites, each site representing one different population; three of them were collected from Region 1 (Brasília, Distrito Federal, and Planaltina, Goiás State) and the remaining three were collected from Region 2 (Alto Paraíso de Goiás, Goiás State; Figure 1). From each site, 10 plants were sampled, totaling 60 individuals. The two regions are more than 200 km away. All the populations from Region 1 were collected from roadside areas, from the remnants of the original vegetation, i.e., Cerrado vegetation (Figure 2A-C); fire incidences had occurred in the three areas. The three sites in Region 2 (Figure 2D-F) slightly differed from each other: Site 4 was an urban area and samples were collected from roadsides; Site 5 was a firebreak area inside the National Park Chapada dos Veadeiros; and Site 6 was a Cerrado remnant range at a roadside near the National Park. Only few plants for the last population were found, and evidence of fire was found in this area.

Information about the collection areas and geographic coordinates are shown in Table 1. Vouchers were registered and incorporated into the collection at the Luiz de Queiroz School of Agriculture, University of Sao Paulo (ESA herbarium).

Anatomical analysis: All the samples were fixed in FAA 50 (formaldehyde, acetic acid, and 50% ethanol, 1:1:18; (Johansen, 1940)), placed in a vacuum pump to remove air from the tissue, dehydrated in a graded ethanol series, and stored in 70% ethanol. For each individual, one fully expanded leaf corresponding to the medium size for the species according to Magenta (2006) was selected and, from this leaf, samples of the internervural and midrib

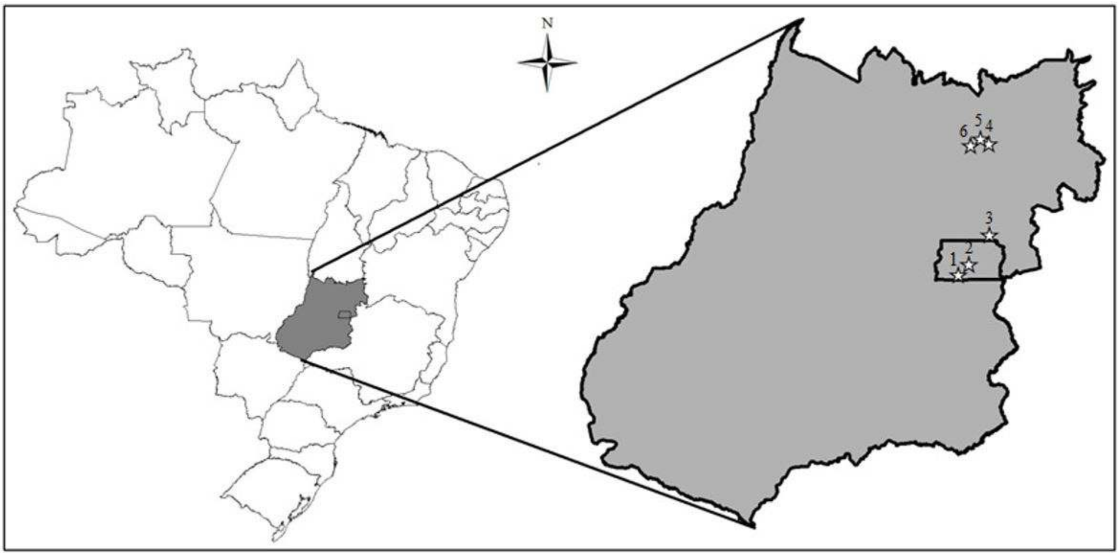


Figure 1. Localization of the sampled populations of *Aldama grandiflora* in Goiás State. 1 = population 1; 2 = population 2; 3 = population 3; 4 = population 4; 5 = population 5; 6 = population 6.

Table 1. Location, Latitude (S), Longitude (W), and voucher number of the sampled population of *Aldama grandiflora.*

Site	City, State	Latitude (S) Longitude (W)	Voucher
1	Brasília/DF	S 15°58'51.2" W 047°56'54.9"	ESA134833
2	Brasília/DF	S 15°50'05.6" W 047°48'13.1"	ESA134834
3	Planaltina/GO	S 15°25'27.3" W 047°31'03.3"	ESA134835
4	Alto Paraíso de Goiás/GO	S 14°08'34.9" W 047°31'19.8"	ESA134836
5	National Park Chapada dos Veadeiros— Alto Paraíso de Goiás/GO (Mulungu)	S 14°04'24.0" W 047°38'09.7"	ESA134837
6	Alto Paraíso de Goiás/GO (Vale da Lua)	S 14°10'06.2" W 047°46'29.8"	ESA134838

regions were obtained. Aerial stems were sampled from three different individuals for each population, and the internode nearest to the soil level, and therefore, the oldest portion of aerial stem, for each individual was examined.

The fixed samples were dehydrated in a graded ethanol series and embedded in plastic resin (Leica Historesin®) according to the manufacturer instructions. The blocks were sectioned (5-8 µm thick) using a Leica RM 2045 rotary microtome. Sections were stained with 0.05% toluidine blue O in a citrate–phosphate buffer, pH 4.5 (Sakai, 1973) and mounted in Entellan® synthetic resin (Merck, Darmstadt, Germany). Sudan IV for lipophilic substance detection (Jensen, 1962) and ruthenium red for pectic and mucilaginous substances (Johansen, 1940) were also applied to the sections.

For stomata and epidermal cell counting, in order to calculate the stomatal index (SI), the epidermis dissociation technique involving the use of 10% Jeffrey solution was applied before observing the frontal view of the leaves (Johansen, 1940). Fragments were stained with safranin and astra blue (Bukatsch, 1972) and mounted in glycerinated gelatin. The SI was calculated according to the formula: $SI = S/(E + S)$, where S is the number of stomata, and E is the number of epidermal cells (Cutter, 1986). The leaf area was determined using Area Meter Modelo Li-3000 equipment (Li-Cor Inc., USA).

For the leaves and stems, the following parameters were considered: cuticle thickness in the adaxial surface; height of epidermal cells and thickness of the outer periclinal external walls of both leaf sides; mesophyll thickness; number and distribution of secretory ducts in the ground parenchyma of the midrib; midrib height and width; internode diameter; number of cell layers and thickness of the cortex; number of ducts in the cortex; total stem area; and total area of the vascular cylinder. For each parameter, five measurements/counting were performed, and an average was obtained for each individual.

Photomicrographs were obtained using a Leica DMLB microscope and Leica DFC310Fx camera. LAS 4.0 software (Leica) was used for image analysis. For measurement and counting, Image J Software (Rasband, 2006) was used.

Soil sampling and analysis: Chemical and physical analyses of the soil samples were performed for each site. From each collection site (Table 1), ten soil samples (500 g each) from the depth of 0–20 cm were obtained using a sampler soil probe (S-60 SONDATERRA® model). These ten samples for each site were mixed to form a composite sample for each site. The analyses were performed to

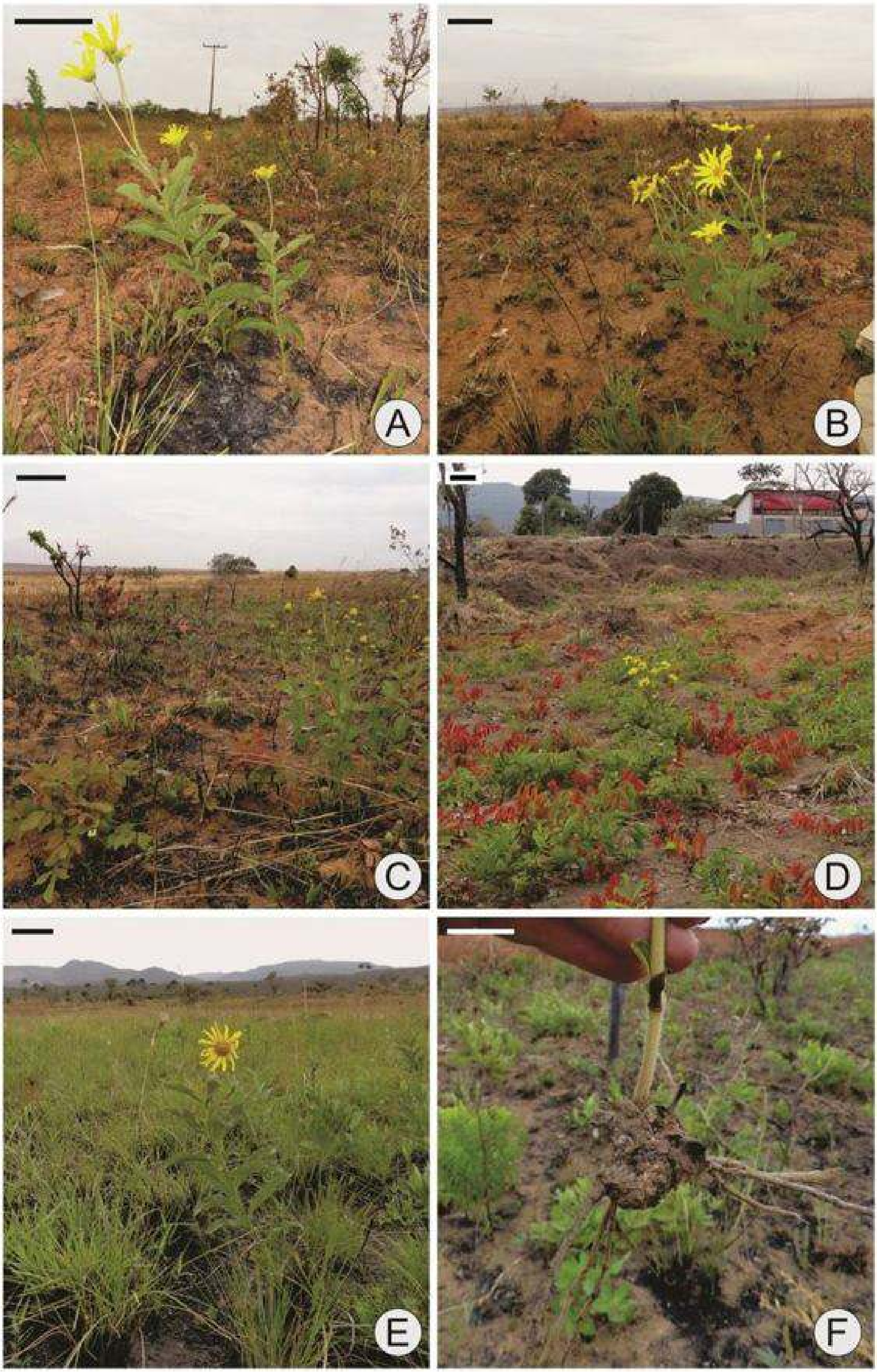


Figure 2. General view of the field in which the six *Aldama grandiflora* populations were grown. A. Site 1, Brasília/DF. B. Site 2, Brasília/DF. C. Site 3, Planaltina/GO. D. Site 4, Alto Paraíso de Goiás/GO. E. Site 5, National Park Chapada dos Veadeiros―Alto Paraíso de Goiás/GO (Mulungu). F. Site 6, Alto Paraíso de Goiás/GO (Vale da Lua). The carbonized bases of some aerial branches (arrows) and details of the underground system can be noted. Scale Bars: A-C, E = 10 cm; D = 20 cm; F = 2 cm.

evaluate the granulometry of the soil, detect and identify micronutrients, and classify the soils.

Environmental data: For the six sites, the mean annual temperature, annual precipitation, and altitude data were collected. All the environmental parameters were obtained from Worldclim website (Hijmans et al., 2005), and the values represented interpolations of observed data, which were representative of data from 1950 to 2000.

Statistical analysis: Mean and standard deviation values were obtained for each parameter for both leaf and stem organs for each population; the normality was confirmed using Kolmogorov–Smirnov & Lilliefors and Shapiro–Wilk's tests (Kolmogorov, 1933; Shapiro and Wilk, 1965; Lilliefors, 1967). The values were then submitted to analysis of similarity (ANOVA) and Tukey's test to determine the existence of potential differences among the populations. When necessary, the data were log transformed. The correlations (r) between morphometrical values and between morphometrical and edaphic-climatic variables were also analyzed. Statistical significance was set at $p < 0.05$. All analyses were performed using STATISTICA 10 software (StatSoft, Tulsa, Oklahoma, USA).

3. Results

Leaf and stem morphometry: In Figure 3, images of the leaf (Figure 3A and 3B) and stem (Figure 3C and 3D) of *Aldama grandiflora* are arranged. Among the foliar parameters evaluated, the thickness of the cuticle in the adaxial surface of the leaf blade showed significant statistical

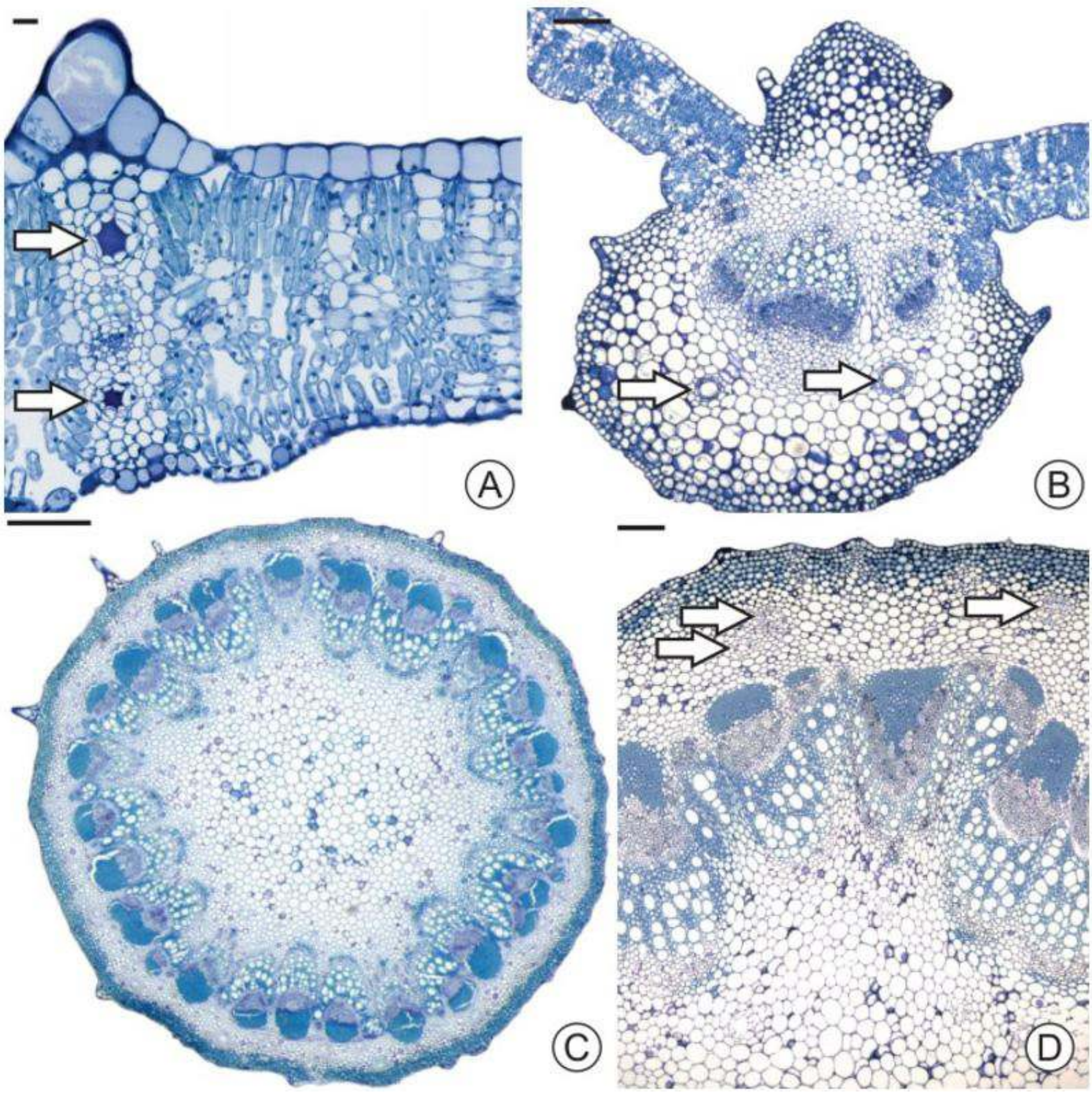


Figure 3. Photomicrographs of leaves (A-B) and stems (C-D) of *Aldama grandiflora*. A-B. Cross sections of leaf blade (A) and midrib (B). C-D. Cross sections of the aerial stem. C. Overview. D. Detail of the secretory ducts in the cortical parenchyma. Arrows indicate secretory ducts. Bars: A-B, D = 100µm and C = 500µm.

difference between the two regions and similarity between sites collected from the same region (Table 2). For the other parameters, the range was not restricted to the study sites, i.e., individuals from populations from the two regions had the same condition for a given parameter. In addition to cuticle thickness, the parameter that significantly differed between the populations was SI of both the leaf sides; the height of the midrib, which was positively correlated with the thickness of the outer periclinal external walls of the adaxial surface of the leaf (r = 0.8357, p = 0.38); the number of ducts in the ground parenchyma of the midrib, which was positively correlated with the midrib width (r = 0.6482, p = 0.164) and the leaf area (r = 0.5573, p = 0.251); and the thickness of the mesophyll. The leaf area, which did not differ between the populations, was negatively correlated with the SI of the abaxial surface (r = -0.8699, p = 0.24). Midrib width also was positively correlated with leaf area (r = 0.8046, p = 0.054), midrib height (r = 0.7322, p = 0.098), adaxial epidermis height (r = 0.6118, p = 0.197), and adaxial and abaxial outer periclinal external wall thickness (r = 0.9034, p = 0.014 and r = 0.6343, p = 0.176, respectively).

The stem features were more similar (Table 3), and no statistically significant difference was noted among the sites for any of the parameters evaluated. However, the internode diameter values, cortex thickness, stem area, and vascular cylinder area were lower for individuals from site 6. Moreover, these parameters were strongly correlated, such as internode diameter and vascular cylinder area (r = 0.9216, p = 0.009), cortex thickness and stem internode area (r = 0.46, p = 0.8199), and total stem and vascular cylinder area (r = 0.9199, p = 0.01).

Secretory duct arrangements: The duct arrangements found in the ground parenchyma of the midrib were based on Filartiga et al. (2016) who described 23 different types of duct distributions. Different duct arrangements were found in *A. grandiflora* (Table 4), and the distribution in the different populations is shown in Table 5. The patterns varied within the same population and even within the same individual, depending on the section analyzed. Furthermore, two of the patterns found in *A. grandiflora* from populations 5 and 6 were not described before in any other *Aldama* species, even by Filartiga et al. (2016), who conducted a detailed study about secretory ducts in Brazilian *Aldama* species.

Soil analysis: For the micronutrients (Table 6), the largest variations were observed for the levels of copper, iron, and manganese for site 6, which showed higher values than those for the other sites. The pH values of water and potassium chloride (KCl), as well as phosphorus, calcium, manganese, and aluminum saturation (m) values, showed no variation among the six sites. The potassium content was slightly higher at site 6. The aluminum content and potential acidity (H + Al) were higher at sites 5 and 6 than in the other localities. Site 5 also showed the highest values of cation exchange capacity (CEC), followed by that for site 6. Regarding the CEC saturation by bases (V), site 5 showed the lowest values.

In relation to the soil particle size, site 6 showed the highest clay content and was classified as clayey soil. The soils from the other populations were classified as sandy medium (sites 1, 3, and 5) or clayey medium (sites 2, 4) soils.

Some of the anatomical parameters were correlated with the constituents evaluated for soil conditions. Among these parameters, the midrib height was positively correlated with iron content (r = 0.8374, p = 0.038), mesophyll thickness was negatively correlated with water pH (r = -0.8178, p = 0.047) and positively correlated with aluminum content (r = 0.8811, p = 0.02), and cuticle thickness was negatively correlated with water pH (r = -0.8677, p = 0.025). Regarding stem parameters, a strong negative correlation was noted between the cortical thickness and levels of boron (r = -0.9069, p = 0.013), copper (r = -0.9519, p = 0.03), iron (r = -0.8149, p = 0.048), manganese (r = -0.9431, p = 0.005), and potassium (r = -0.8964; p = 0.016), and a positive correlation was noted with the total amount of sand (r = 0.9474, p = 0.004). The total internode area and vascular cylinder area were also negatively correlated with iron content (r = -0.8740, p = 0.023; r = -0.8473, p = 0.033) and clay amount in the soil (r = -0.9704, p = 0.001; r = -0.8863, p = 0.019).

Environmental data: According to the data obtained from Worldclim (Table 7), Region 2 (sites 4, 5, and 6) had the highest mean annual temperature as well as the highest precipitation values. Elevation was higher in two of the three sites from Region 2. The mean annual temperature was positively correlated with the SI of leaf abaxial surface (r = 0.8607, p = 0.028) and, along with the annual precipitation, was positively correlated with the cuticle thickness (r = 0.8305, p = 0.41, r = 0.8880, p = 0.018).

4. Discussion

We aimed to determine whether the different localities, and thus the different soil and climate conditions to which the six populations were exposed, were sufficient to modify the anatomical structure of the individuals. The leaf and stem parameters evaluated showed an anatomical constancy for the *A. grandiflora* individuals among the six sampled sites. For the leaf, seven of the thirteen parameters did not differ statistically among the sites; the stem features were even more consistent, and none differed statistically among the areas.

Among the parameters that showed statistical differences, the variations did not reflect the region from where the populations were sampled. The only parameter that differed between the two regions was cuticle thickness of the adaxial leaf surface, which was slightly thicker for individuals from Region 2. Cuticle plays an important role in reducing water loss, waterproofing, and sunlight reflection (Haberlandt, 1990; Larcher, 2000); its thickness and composition can be influenced by environmental factors (Esau, 1976; Dickison, 2000). In this study, thicker cuticles were correlated to places with higher mean temperature, which might have influenced this result.

Table 2. Leaf anatomical parameters (mean ± standard deviation) and Tukey test results for the six populations of *Aldama grandiflora* evaluated.

Parameters/Sites	Region 1						Region 2					
	1		2		3		4		5		6	
Leaf area (cm²)	36.32 ± 10.56	a	23.79 ± 6.81	a	29.11 ± 7.67	a	23.49 ± 10.59	a	30.30 ± 15.58	a	26.46 ± 11.10	a
Stomatal index of adaxial surface	16.13 ± 2.10	ab	17.64 ± 2.42	a	16.04 ± 2.11	ab	16.90 ± 1.22	ab	17.75 ± 1.74	a	14.76 ± 1.57	b
Stomatal index of abaxial surface	17.06 ± 2.06	b	19.25 ± 2.66	ab	20.15 ± 2.44	ab	18.96 ± 3.14	ab	20.97 ± 2.46	a	21.34 ± 2.59	a
Midrib height (µm)	1421.21 ± 191.38	ab	1205.27 ± 141.51	b	1331.70 ± 157.03	ab	1381.69 ± 195.86	ab	1368.00 ± 172.78	ab	1525.32 ± 162.97	a
Midrib width (µm)	1385.47 ± 160.36	a	1071.46 ± 109.21	b	1164.40 ± 170.67	ab	1135.32 ± 174.45	b	1178.25 ± 190.68	ab	1276.38 ± 165.84	ab
Number of ducts in the midrib	5.74 ± 1.23	a	4.82 ± 0.94	a	4.72 ± 1.27	ab	3.36 ± 0.77	b	4.52 ± 1.14	ab	5.36 ± 1.35	a
Mesophyll thickness (µm)	301.93 ± 43.35	ab	263.81 ± 24.22	b	288.73 ± 28.81	b	300.82 ± 52.68	ab	348.22 ± 34.73	a	313.96 ± 28.88	ab
Height of adaxial epidermis (µm)	38.70 ± 4.62	a	36.04 ± 3.73	a	37.81 ± 4.18	a	36.15 ± 3.91	a	40.06 ± 5.12	a	39.60 ± 6.75	a
Height of abaxial epidermis (µm)	23.16 ± 4.58	a	22.68 ± 3.69	a	20.17 ± 3.52	a	19.74 ± 4.51	a	23.08 ± 4.10	a	21.56 ± 4.81	a
Thickness of adaxial outer periclinal external walls (µm)	6.83 ± 1.05	a	5.66 ± 0.57	a	5.92 ± 0.52	a	6.09 ± 1.21	a	6.50 ± 1.13	a	6.67 ± 1.04	a
Thickness of abaxial outer periclinal external walls (µm)	7.29 ± 1.81	a	5.96 ± 1.00	a	7.29 ± 1.19	a	6.65 ± 1.94	a	7.52 ± 1.80	a	7.25 ± 1.54	a
Cuticle thickness in adaxial surface (µm)	1.92 ± 0.17	b	2.03 ± 0.28	b	2.05 ± 0.19	b	3.32 ± 0.40	a	3.80 ± 1.16	a	3.39 ± 0.57	a

Different letters in the same row represent significant differences among populations ($p < 0.05$).

Table 3. Stem anatomical parameters (mean ± standard deviation) and Tukey test results for the six populations of *Aldama grandiflora* evaluated.

Parameters/Sites	Region 1						Region 2					
	1		2		3		4		5		6	
Internode diameter (cm)	0.47 ± 0.11	a	0.50 ± 0.10	a	0.52 ± 0.10	a	0.45 ± 0.07	a	0.59 ± 0.17	a	0.42 ± 0.03	a
Number of cortical layers	14.60 ± 3.14	a	15.33 ± 2.58	a	14.20 ± 1.40	a	15.33 ± 2.81	a	11.20 ± 0.35	a	12.07 ± 1.50	a
Cortical thickness (mm)	0.33 ± 0.12	a	0.32 ± 0.06	a	0.35 ± 0.05	a	0.33 ± 0.03	a	0.32 ± 0.06	a	0.25 ± 0.05	a
Number of ducts in the cortex	27.73 ± 1.60	a	28.93 ± 1.22	a	34.60 ± 3.56	a	34.20 ± 3.70	a	24.93 ± 4.60	a	26.40 ± 5.60	a
Total stem area (mm²)	22.30 ± 9.68	a	20.20 ± 9.68	a	22.73 ± 8.20	a	18.13 ± 5.78	a	22.39 ± 9.35	a	15.50 ± 1.74	a
Total area of vascular cylinder (mm²)	16.10 ± 6.07	a	15.83 ± 8.11	a	16.07 ± 6.82	a	14.13 ± 5.20	a	18.07 ± 7.79	a	12.17 ± 0.85	a

Different letters in the same row represent significant differences among populations ($p < 0.05$).

The morphometrical parameters evaluated in this study were not correlated to the altitude of the sites from where the populations were collected, likely because the difference between the amplitude of altitude among the sites was less pronounced than that of sites investigated by Tiwari et al. (2013), who evaluated the anatomical differences in an altitude gradient of more than 1700 m and found that anatomical properties of needles in *Pinus roxburghii* Sarg. exhibited variation from lower to higher elevation.

Table 4. Secretory duct distribution patterns in the ground parenchyma of the midrib in *Aldama grandiflora* (Adapted from Filartiga et al., 2016).

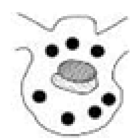
Pattern	Arrangement	Pattern	Arrangement
III		XIV	
IV		XV	
V		XVI	
VIII		XVIII	
IX		XX	
X		New 1	
XIII		New 2	

Table 5. Distribution patterns of secretory ducts in the ground parenchyma of the midrib of the six different populations of *Aldama grandiflora*, based on Filartiga et al. (2016).

Population	Distribution patterns
1	IV; VIII; XIV; XV; XX
2	XIV; XV; XVI; XIII; XVIII
3	VIII; XIII; XIV; XV; XVI
4	III; IV; VIII; IX; XIII
5	III; XIII; XIV; XV; New 1
6	IV; V; XIV; XV; XVI; XIII; New 2

The studied species, *A. grandiflora*, occurs exclusively in open areas of Cerrado (Magenta et al., 2010); it is subjected to typical Cerrado environmental conditions such as high solar radiation, low availability of nutrients in the soil like calcium and magnesium, and high aluminum levels, in addition to low water availability, mainly in the upper soil layers (Ratter et al., 1977). These conditions might influence the anatomy of plant species. The SI, which was one of the parameters that differed among the sites and showed a positive correlation with the annual mean temperature, showed the highest values for individuals from site 5, which were collected from a locality having one of the highest temperatures and altitude. High values of stomatal density and SI can be considered as an adaptation that can lead to an increase of the CO_2 uptake (Dickison, 2000) and promote larger output of water vapor and internal cooling of leaves (Lima Junior et al., 2006), which is an important response to higher temperatures. High values of SI are also associated with higher elevations, and thus to a higher light incidence and lower content of available O_2 and CO_2 (Apel, 1989; Furukawa, 1997; Gardoni et al., 2007).

Taller epidermal cells with thicker walls can disperse more light, thereby protecting the photosynthetic tissues and avoiding leaf overheating and or protect photosynthetic tissues from excessive irradiance (Roth, 1984; Feller, 1996; Evert, 2006). Anticlinal or periclinal cell walls with more thickening are often found in species from regions subjected to water deficit (Solereder, 1908; Metcalfe and Chalk, 1979) and are related to the reduction of water loss by transpiration, decreasing heating within plant organs, maintaining its architecture, and reflecting higher luminosity (Dickison, 2000; Leite and Scatena, 2001). All the sampled individuals showed tall epidermal cells on the adaxial surface of the leaves as well as pectin-thickened external periclinal walls in both the leaf surfaces. Considering the studies of Marques et al. (2000), who reported that *Miconia stenostachya* showed an increase in thickness of cuticle and epidermis of leaves in the sun exposed cerrado and stated it may be related to an increase in leaf reflectance, we can suggest that taller epidermal cells with thicker walls are important characteristics for *A. grandiflora* to live in the open areas of Cerrado.

Leaves characters such as the type and position of secretory structures have been useful for species differentiation (Castro et al., 1997; Fahn, 2000; Oliveira et al., 2013), including in Asteraceae (Solereder, 1908; Metcalfe and Chalk, 1950; Wagenitz, 1976; Castro et al., 1997; Adedeji and Jewoola, 2008; Fritz and Saukel, 2011). However, some studies (Kakrani et al., 1991; Sheue et al., 2003; Filartiga et al., 2016) showed that the number and position of secretory ducts can vary, rendering it difficult to use this character as the only parameter of differentiation among species. As previously reported for other Brazilian *Aldama* species (Filartiga et al., 2016), in *A. grandiflora*, variation

Table 6. Chemical and physical soil analysis for the six populations of *Aldama grandiflora* evaluated.

Nutrients/Sites	Region 1			Region 2		
	1	2	3	4	5	6
B (mg·dm^{-3})	< 0.12	< 0.12	< 0.12	< 0.12	0.13	0.14
Cu (mg·dm^{-3})	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	0.9
Fe (mg·dm^{-3})	35	25	31	52	35	73
Mn (mg·dm^{-3})	4.2	0.8	0.9	2.1	0.6	37.1
Zn (mg·dm^{-3})	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
pH H_2O	5.3	5.4	5.3	5.2	5.2	5.2
pH KCl	4	4.1	4.1	4	4.1	4
P (mg.kg^{-1})	1	1	1	< 1	2	1
K (mmolc.kg^{-1})	1.1	1.1	1	1.1	2	2.7
Ca (mmolc.kg^{-1})	< 2	< 2	3	< 2	< 2	< 2
Mg (mmolc.kg^{-1})	< 1	< 1	1	1	1	2
Al (mmolc.kg^{-1})	15	15	14	17	41	24
H + Al (mmolc.kg^{-1})	31	52	27	34	151	77
SB (mmolc.kg^{-1})	2.3	2.2	4.5	3	3.4	5.4
CEC (mmolc.kg^{-1})	33.3	54.3	31.1	36.7	154	82.5
V (%)	7	4	14	8	2	7
m (%)	87	87	75	85	92	82
Total sand (g.kg^{-1})	709	611	772	630	485	268
Silt (g.kg^{-1})	89	81	76	65	327	370
Clay (g.kg^{-1})	202	208	151	305	188	362
Texture classes	Sandy medium	Clayey medium	Sandy medium	Clayey medium	Sandy medium	Clayey

SB: sum of exchangeable bases; CEC: cation exchange capacity; V: CEC base saturation; m: saturation of aluminum.

Table 7. Elevation (m), annual temperature average (°C), and annual precipitation (mm) in the six localities evaluated.

Parameter/ Sites	Region 1			Region 2		
	1	2	3	4	5	6
Elevation	1169	1079	1155	1243	1206	1079
Mean annual temperature	20.5	21.0	21.1	21.5	21.7	22.3
Annual precipitation	1593	1627	1429	1805	1839	1780

Data obtained from Worldclim (Hijmans et al., 2005).

in the number and position of secretory ducts was found in the midrib ground parenchyma, and also was observed among individuals from different populations, individuals of the same population, and within the same individual.

In the species analyzed herein, the total number of secretory ducts found in the midrib parenchyma was correlated positively with the midrib width and the total leaf area, i.e., wider midribs indicate a greater number of secretory ducts. This correlation was not observed by Filartiga et al. (2016), who reported such a tendency only in the leaves of *Aldama corumbensis*, among the 17 species analyzed. However, the position of secretory ducts has been associated with the presence of vascular bundles (Gregio and Moscheta, 2006; Bombo et al., 2012) and, in the case of *A. grandiflora*, the midrib can present 3 to 5 collateral vascular bundles (Bombo et al., 2016), which certainly influences the midrib width and the number of secretory ducts in the ground parenchyma.

In relation to the duct distribution, Filartiga et al. (2016) identified 23 different patterns, based on the number and position of ducts in the ground parenchyma of the midrib. Of the 23 already reported distributions in *Aldama* species (Filartiga et al., 2016), 12 types, as well as two new patterns, were identified in *A. grandiflora* in this study. Thus, the results obtained in *A. grandiflora* for the number and distribution of secretory ducts in the midrib corroborate the results for the *Aldama* group, and reaffirm that considering only the number and position of secretory structures are not sufficient for the taxonomic delimitation of a species.

The nutrient requirement of herbaceous layers, which is an essential component in savanna vegetation, is extremely low, ensuring the high resilience of the Cerrado ecosystem, especially after disturbances such as fire (Batmanian and Haridasan, 1985; Villela and Haridasan, 1994). The sites investigated in this study, except site 4, were exposed to recent fire incidences, confirmed by the base of the remaining carbonized branches (Figure 2F) and charcoal remains on the ground (Figure 2A-C, E-F). In all these sites, *A. grandiflora* plants showed the highest sprouting ability after fire among all the other plants (field observation). Fire events in Cerrado, mainly in open areas where *A. grandiflora* usually occurs, control the dynamics of populations of shrubs and trees species (Hoffmann, 1998), and induce flowering and renewal of the herbaceous stratum (Simon and Pennington, 2012).

The soil conditions in the evaluated sites showed higher amounts of iron in the soils from Region 2, especially in sites 4 and 6. This micronutrient is essential because it promotes plant development and plays an important role in chlorophyll synthesis as well as in respiration process and N_2 fixation (Alexandre et al., 2012). Some of the leaf parameters were slightly higher for the individuals sampled from these sites, such as mesophyll and midrib thickness, and this could be related to the soil iron availability in the soil.

In addition to the high amounts of iron in the sampled soils, the soil pH, which can influence the availability and deficiency of nutrients as well as the toxicity of these nutrients to plant species (Haridasan, 2008), was acidic (between 5.2 and 5.4). This values are characteristic of soils from Cerrado *sensu stricto* and open physiognomies of Cerrado (Lopes and Cox, 1977; Furley and Ratter, 1988; Haridasan, 1992). Although iron is known to be toxic to many plants, its toxicity occurs only under soil conditions that are more acidic (Haridasan, 2008) and, for *A. grandiflora*, the conditions did not seem to be harsh, since it showed the highest sprouting ability in the evaluated sites after a disturbance.

Soil fertility was low at site 4, especially with regard to the nutrients phosphorous and potassium; this site also showed lower water retention capacity, which was associated with the high total sand content in the upper soil layer. The relatively smaller size of individuals in this population (field observation) as well as lower values of foliar area and internode diameter might be associated with these soil features.

Ecological adaptation can often be associated with an unfavorable mineral nutrition (Ratter et al., 1977; Dickison, 2000), and Gardoni et al. (2007) showed that phenotypic variation found in *Marcetia taxifolia* (A. St.-Hil.) DC. (Melastomataceae) was related to the edaphic or geological conditions, since the variations did not reflect the geographical and/or climatic conditions. For *Aldama grandiflora*, some of the leaf and stem parameters showed significant variation among the sites, and exhibited positive or negative correlation with soil features, such as, the midrib size and mesophyll thickness that were positive correlated with levels of iron and aluminum, respectively, and total area of vascular cylinder and stem were correlated with iron levels. Although literature shows that aluminum and iron toxicity at low pH can directly influence plant development (Lopes and Cox, 1977; Haridasan, 2008), leading to the development of scleromorphic characters in cerrado plants (Goodland, 1971), the levels of iron and aluminum in the soil did not harm the development of *Aldama grandiflora* plants. Furthermore, *A. grandiflora* showed an anatomical stem development very similar to other species of the group already described (Bombo et al., 2012, 2014; Oliveira et al., 2013; Silva et al., 2014; Bombo et al., 2016), indicating a consistency within the genus and that this species is well adapted to these soil conditions.

In this study, *A. grandiflora* showed considerably consistency in leaf and stem anatomical characteristics since only slight morphometric differences were found among the populations analyzed. The variations observed were mainly correlated to the soil parameters, and the climatic conditions evaluated in this study had little influence on the morpho-anatomical features. Further studies including *Aldama grandiflora* populations from further locations could provide a wider understanding about how anatomical features in this species, and related ones, respond to uttermost envirormental conditions.

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