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Original article

Could leaf morphoanatomy characters help in the delimitation of *Dyckia selloa* complex?

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ABSTRACT

Among species of the genus *Dyckia* Schult. & Schult. f. there are 13 endemic species of the Brazilian states of Rio Grande do Sul and Santa Catarina informally treated as the *Dyckia selloa* complex. This study employed standard plant anatomy techniques to investigate variation in leaf morphology of species belonging to the genus *Dyckia* with a focus of establishing characters that help delimit the *Dyckia selloa* complex. The results allowed the survey of morphological and anatomical characters important for the characterization of species. Such characters include color of spines, the presence of water-storage parenchyma and mechanical hypodermis on both leaf surfaces, and the presence of tetracytic stomata on only the abaxial surface. Analyses support the current delimitation of the complex and recommend the investigation of reproductive and/or vegetative organs to better understand the relationships among these species of *Dyckia*.

Keywords: Plant morphology, plant anatomy, leaf anatomy, species complex.

Introduction

The genus *Dyckia* Schult. & Schult. f. (Bromeliaceae) comprises 179 species (Gouda *et al.* 2023, cont. updated) occurring in Argentina, Bolivia, Paraguay, Uruguay and in almost all regions of Brazil (Smith & Downs 1974; Büneker *et al.* 2021), occurring mainly on rocky outcrops, slopes and/or cliffs, and often on nutrient-poor soils with scarce water supply and high sun exposure (Smith & Downs 1974; Reitz 1983; Givnish *et al.* 2007). A total of 130 accepted species occurs in Brazil, of which 121 are endemic (Büneker *et al.* 2020, cont. updated). *Dyckia* species are terrestrial and rupicolous plants, they have rhizomes strong and functional (Strehl & Beheregaray 2006). The leaves are rigid, thick and leathery, with convex sides and fleshy mesophyll, arranged in an imbricate rosette phyllotaxy (Reitz 1983). The inflorescences are racemose, ranging from simple to paniculate (Reitz 1983). The flowers can be red, orange or yellow and are trimerous with a superior ovary (Reitz 1983). The fruit is capsular with biscidal dehiscence (Fagundes & Mariath 2010).

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The circumscription of *Dyckia* has been the subject of several morphological and taxonomic studies in recent years, with several authors using informal categories to group species with morphological similarities (e.g., *Dyckia macedoi* complex and *Dyckia saxatilis* complex by Guarçoni (2015), *Dyckia ferruginea* complex and *Dyckia selloa* complex by Büneker *et al.* (2021)). Among these species complexes, the *Dyckia selloa* complex is particularly interesting for being endemic to the Brazilian states of Rio Grande do Sul and Santa Catarina (Büneker *et al.* 2021) and for having been delimited based on in an unique combination of the morphological characteristics for the genus. According Büneker & Mariath (2022) the *Dyckia selloa* complex which was previously treated as the genus *Prionophyllum* by Koch (1873) and Mez (1896, 1935), *Dyckia* subg. *Prionophyllum* by Baker (1889), the *Prionophyllum* group by Leme *et al.* (2012) and Krapp & Eggli (2019), or the *D. maritima* complex by Strehl & Beheregaray (2006) and Büneker *et al.* (2015). According to these authors the *Dyckia selloa* complex currently consists of 13 species: *D. agudensis* Irgang & Sobral, *D. alba* S.Winkl., *D. delicata* Larocca & Sobral, *D. hebdingii* L.B.Sm., *D. maritima* Baker, *D. myriostachya* Baker, *D. nigrospinulata* Strehl, *D. pseudodelicata* Büneker & Mariath, *D. retardata* S.Winkl., *D. retroflexa* S.Winkl., *D. rigida* Strehl, *D. selloa* (K.Koch) Baker, and *D. tomentosa* Mez. Büneker & Mariath (2022) emphasize that the group is clearly distinguished from other species of the genus based on morphology, they say the main distinguishing characters are: inflorescences usually composed of first to third order (vs. simple or compound to first order), with numerous first order branches (usually more than 10 vs. less than 10 branches), generally small and inconspicuous floral bracts, numerous flowers (more than 100 vs. less than 80) with non-unguiculate (vs. usually unguiculate) petals, stigma during anthesis usually with erect to suberect (vs. twisted) stigmatic lobes, pauciovulate ovary locules (less than 18 vs. more than 30 ovules) and oblanceoloid (vs. flattened) seeds with a little developed wing. Circumscription attempts (Krapp *et al.* 2014; Schütz *et al.* 2016; Pinangé *et al.* 2016; Gomes-da-Silva *et al.* 2019) and the existence of species complexes within *Dyckia* evidence the scarcity of morphological characters available for robust delimitations and, thus, the need for studies that complement available information to provide efficient data for taxonomic decisions and circumscriptions.

Morphological and anatomical studies within Bromeliaceae frequently address morphological comparisons between species and ecological adaptations to xeric environments (Tomlinson 1969; Smith & Downs 1974; Benzing 2000). In addition to these, some studies have involved morphoanatomical aspects with the aim of helping to delimit taxa (e.g. Santos-Silva 2015; Carvalho *et al.* 2016; 2017; Guarçoni *et al.* 2014; 2017; Büneker & Mariath 2022). Also of significance are studies that have sought to identify characters of taxonomic value and ecological significance, such as that of Silva & Scatena (2011), who compared leaf anatomy within the subfamily Tillandsioideae and concluded that, as they are epiphytic, some of the xeromorphic characteristics may represent ancestral adaptations during speciation. Recently, leaf anatomy data have revealed xeromorphic synapomorphies for the genera *Deuterocohnia* Mez, *Dyckia* and *Encholirium* Mart. ex Schult. & Schult. f., mainly the presence of mechanical hypodermis and water-storage parenchyma, which would be related to the occupation and diversification of these genera in the dry region of South America (Santos-Silva *et al.* 2013). As highlighted, leaf anatomical studies within *Dyckia* can provide data that help to solve taxonomic problems and reveal adaptations that have contributed to the diversification of the group.

Considering that most studies carried out within the Pitcairnioideae and, more specifically, within the genus *Dyckia*, have focused on the taxonomy and phylogeny of the group (Krapp *et al.*2014; Schütz *et al.* 2016; Pinangé *et al.* 2016; Gomes-da-Silva *et al.* 2019), the investigation of morphoanatomical characters is necessary. This is especially true regarding the analysis of vegetative characters related to leaf anatomy, which can provide data helpful in the delimitation of the *Dyckia selloa* complex and the species it comprises. Thus, the present study aimed to investigate the leaf morphology of species of *Dyckia*, with a focus on establishing characters that help delimit the *Dyckia selloa* complex. More specifically, the aims were to: (1) analyze leaf external and internal morphology; (2) to test which characters are important for species delimitation, and based on this, (3) to check whether the data obtained can be useful for delimiting the *Dyckia selloa* complex.

Material and methods

Botanical Material

Five to ten individuals from throughout the geographic distribution of each species were collected for morphological and anatomical analyses. Voucher material for each analyzed species is registered in the Herbário do Instituto de Biociências (ICN) – Universidade Federal do Rio Grande do Sul (UFRGS). The following species were included: *D. alba*, *D. agudensis*, *D. delicata*, *D. domfelicianensis* Strehl (treated as a synonym of *D. hebdingii* by Büneker *et al.* 2020), *D. hebdingii*, *D. polyclada* L.B.Sm. sensu Strehl (1998) (treated as a synonym of *D. tomentosa* by Büneker *et al.* 2021), *D. selloa*, *D. tomentosa*, *D.* aff. *maritima*, *D. myriostachya*, *D. nigrospinulata*, *D. retroflexa* and *D. rigida* (Table 1). The related species *D. choristaminea* Mez and *Bromelia antiacantha* Bertol. were used as outgroup taxa for comparisons. The collected individuals for each of the species were included in the Living Collection (LC) of the Laboratório de Anatomia Vegetal – UFRGS.

Light Microscopy

Fresh leaves were collected from the leaf node right before the inflorescence for observation under light microscopy and stereomicroscopy. Leaves were sectioned transversally, at the median region, and immersed in a fixative solution containing 1% glutaraldehyde and 4% formaldehyde (McDowell & Trump 1976) in a 0.1M sodium phosphate buffer solution pH 7.2 and kept under vacuum for 24 hours. The material was subsequently dehydrated in an ascending ethanol series (Johansen 1940), and after subjected to an alcohol:chloroform series (3:1, 1:1, 3:1), and embedded in hydroxyethylmethacrylate resin (Gerrits & Smid 1983). Sections were made using a Leica 2265 rotary microtome, equipped with a high-profile disposable blade, to obtain 3–5 µm in thick sections. The material was stained with 0.1% Toluidine Blue O in 0.1M sodium phosphate buffer pH 4.4 (Feder & O'Brien 1968). Images were recorded using a Leica DMR HC microscope equipped with a Zeiss a Zeiss AxioCam digital camera and the free software Carl Zeiss ZEN LITE 2012.

Histochemistry

Fresh leaves were free hand transversally sectioned at the median region of the leaf and used for the histochemical characterization of the cell wall composition as well as the presence of the compounds being stored in the tissues. A Lucifer yellow CH (LYCH) apoplastic tracer (Oparka & Read 1994) was used to confirm the presence of water-storage parenchyma; Ruthenium red (Jensen 1962) for pectic acids; acidified phloroglucinol generic test (Johansen 1940) for lignins; Mäule test (Jensen 1962) for syringyl lignin; chlorosulfite test (Jensen 1962) for guaiacyl lignin – coniferilic acid; Sudan black B (Johansen 1940) for lipids; and phenol (Johansen 1940) for silica identification (Table S1).

Scanning Electron Microscopy (SEM)

For SEM analysis, fixed leaves were washed with 0.1M sodium phosphate buffer pH 7.2 and immersed in a 2.2-dimethoxypropane (DMP) solution. The samples were critical point dried (BAL-TEC CPD 030) (Gerstberger & Leins 1978), placed onto stubs and covered with a 10-15nm gold film using a BAL-TEC SPD 050 equipment. The material was analyzed with a JEOL JSM 6060 scanning electron microscope, under 10kV, at the Centro de Microscopia e Microanálise of UFRGS.

Morphological Characters and Multivariate Analysis

Morphological and morphometric data were obtained from morphological observations and measurements performed on fresh and processed material using a caliper and/or stereomicroscope and/or microscope. Characters

Table 1. Morphological and anatomical species sampled, with voucher information, number of registration in the Living Collection (LC) (LAVeg-UFRGS) and geographical origin (country: state: city).

Species	Voucher number (ICN[1])	LC number[2]	Geographical Origin
D. selloa complex			
Dyckia agudensis Irgang & Sobral	188075	1374	BR: RS: Agudo
Dyckia alba S.Winkler	188076	1326/1327/1328	BR: RS: Caçapava do Sul
Dyckia delicata Larocca & Sobral	188077	1321/1322	BR: RS: Barros Cassal
Dyckia domfelicianensis Strehl	188078	1369/1370/1371	BR: RS: Dom Feliciano
Dyckia hebdingii L.B. Smith	188079	1336/1337/1338/1339	BR: RS: Barra do Ribeiro
Dyckia maritima Baker	188080	1332/1333	BR: RS: Santo Antônio da Patrulha
Dyckia myriostachya Baker	188081	1323/1324/1325	BR: RS: Candelária
Dyckia nigrospinulata Strehl	188082	1315/1316	BR: RS: Santa Maria do Herval
Dyckia polyclada L.B. Smith	188083	1375	BR: RS: Santa Maria
Dyckia retroflexa S.Winkler	188087	1317/1318	BR: RS: Morro Reuter
Dyckia rigida Strehl	188084	1334/1335	BR: RS: Riozinho
Dyckia selloa Baker	188085	1372/1373	BR: RS: Caçapava do Sul
Dyckia tomentosa Mez	188086	1319/1320	BR: RS: Tabaí – pedra Rosa
Outgroup			
Dyckia choristaminea Mez	199483	1347/1348/1349	BR: RS: Barra do Ribeiro
Bromelia antiacantha Bertol.	185360	1035	BR: RS: Porto Alegre

Notes: 1= Herbário do Instituto de Biociências – Universidade Federal do Rio Grande do Sul (UFRGS); 2= Living Collection – Laboratório de Anatomia Vegetal - Universidade Federal do Rio Grande do Sul (UFRGS); BR= Brasil; RS= Rio Grande do Sul.

and character states were defined by reviewing an extensive list of bibliographic references of Bromeliaceae (Eames & Macdaniels 1947; Uphof & Hummel 1962; Tomlinson 1969; Smith & Downs 1974; Reitz 1983; Strehl 1983; Benzing 2000; Arruda & Costa 2003; Forzza 2005; Scatena & Segecin 2005; Souza *et al.* 2005; Strehl & Beheregaray 2006; Horres *et al.* 2007; Santos-Silva *et al.* 2013 and Krahl *et al.* 2013), which resulted in a matrix with 35 characters, of which 14 were quantitative and 21 qualitative (Table 2).

The resulting matrices were submitted to statistical tests using Pasw Statistics 18, SPSS (SPSS Inc., Chicago IL, USA). Simple descriptive statistics (mean, standard deviation, median, coefficient of variation and standard error) were calculated for each quantitative character. Quantitative character states, as well as mean and standard deviation values, are shown in Table 2. Characters were also evaluated for normality and homoscedasticity, while the significance of interspecific variability was estimated for each character using ANOVA (normalized morphometric characters) and the Kruskal-Wallis's test for the non-normalized morphological characteristics (Table S2). Characters were removed from the matrix when significant variability was not detected ($p>0.05$).

Two multivariate approaches were employed to identify morphological discontinuities among taxa and to detect characters that could contribute to the delimitation of the *Dyckia selloa* complex.

The first approach consisted of a Principal Coordinate Analysis (PCoA) of all analyzed characters, both quantitative and qualitative. In this analysis, the characters were discretized into categories and standardized by the Normatization Method - Object Principal. On a second approach, the quantitative characters were subsequently submitted to Discriminant Analysis (DA) to discriminate groups/species of *Dyckia*. For this analysis, species were defined by probabilities, estimated from the size of the established groups (case-wise test), through the covariance matrix and Fisher's linear predictive model. Thus, the functions that best discriminate the sampled groups were obtained (Table S3).

Results

Morphological and anatomical characters

The analyzed species possess rosette phyllotaxis with succulent, rigid, lanceolate leaf blades ranging from erect to revolute. Aspects of leaf morphology can be seen Figures 1, 2 and Fig. 3, including details of external morphology, morphology in transverse section, and details of spines and epidermis trichomes (Table 2).

Leaf length exhibited great variation among the analyzed species, with *D. rigida* having the longest at 1002 mm ± 184.9 (mean ± SD) (Fig. 2M) and *D. choristaminea* the shortest at 99 mm ± 23.55 (Fig. 2Y). Analysis of the leaf in transversal section allowed the identification of different shapes, these shapes could be elliptical (Figs. 1F, 2Z*)*, narrow elliptical (Figs. 1N, 1V, 1Z, 2B, 2F, 2J, 2R, 2V), short obovate (1J, 1R) or linear (2N, 2D'). Leaf blade color was found to vary from green to grayish-green and whitish and the blades were covered with peltate scales on both sides (Fig. 1K). The leaf margins possess grayish-green (Fig. 1K, 2G, 2S, 2W), brown (Fig. 1G, 1S, 2K, 2O, 2A') or black spines (Fig. 1C, 1O, 1W, 1A', 2C, 2E', 3A), that are often rigid, with the exception of *D. delicata*, which develops flexible spines (Fig. 1K). Undulations are present on the abaxial leaf surface in transverse section, forming a costal zone and, consequently, establishing intercostal zones (Fig. 3B-F). Costal zone possesses only ordinary epidermal cells whereas the intercostal zone has also specialized cells: trichomes and stomata located together and aligned (Figs. 3D-F and 3E). The leaves are hypostomatic with tetracytic stomatal complexes situated above the level of other epidermal cells and guard cells with equivalent periclinal thickening (Figs. 3D-F). The adaxial leaf surface is generally flat or slightly undulating, causing the formation of depressions in which there is a reduced number of layers of mechanical hypodermis and the insertion of peltate trichomes (Fig. 3G). All analyzed species have unistratified epidermis on both surfaces, with thickening of the anticlinal and inner periclinal walls of the cells, reduced lumen and the presence of silica bodies (Fig. 3D, Table S1).

A mechanical hypodermis is evident in the mesophyll of both leaf sides (Fig. 1B,1I and 3K and 3J), which is where the greatest mean thickness occurs in *D. agudensis* (10.23 µm ± 2. and the least in *B. antiacantha* (3.4 µm ± 0.98). This hypodermis presents pits and differentiates strata in all species analyzed, and these strata were firstly observed with the phloroglucinol test (Fig. 3B). Specific histochemical tests permitted the identification of the presence of different cell wall thickening (Table S1). The outermost cells of the hypodermis possess cell walls with coniferilic acid-type (guaiacil) lignin compounds (Fig. 3I). The innermost cells of this hypodermis have cell walls with pectic thickening (Fig. 3J). In transverse section, water-storage parenchyma is present below the mechanical hypodermis of the adaxial side where it occupies on average two thirds of the leaf mesophyll (see histochemical test in Table S1). The thickness of this parenchyma varies among species, with it being the greatest in *D. alba* (34 µm ± 6.9) (Fig. 1F) and the thinnest in the outgroup taxon *B. antiacantha* (4.2 µm ± 9.0) (Fig. 2D'). Differences were observed in the shape of the cells of the water-storage parenchyma, which resulted in its classification into two strata. The 1st stratum appears from two to three layers of cells closest to the mechanical hypodermis of the adaxial side and is formed by isodiametric cells. This stratum was thickest in *D. alba* (4.3 µm ± 0.43) (Fig. 1F) and thinnest in *D. delicata* (1.05 µm ± 0.42) (Fig. 1J). The cells that make up the 2nd stratum of this tissue extend

Table 2. Mean ± standard deviation values for each morphological and anatomical character used in *Dyckia* species analyses and states for each categorical character.

Characters/Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
	D. ag	*D. al*	*D. de*	*D. do*	*D. he*	*D. ma*	*D. my*	*D. ni*	*D. po*	*D. re*	*D. ri*	*D. se*	*D. to*	*D. ch*	*B.an*
Leaf: L (mm)	361 ± 148	206 ± 43.8	182 ± 44.17	235 ± 46.2	113 ± 15.7	47 ± 7.26	478 ± 232.7	656 ± 148.1	318 ± 111	267.1 ± 88.4	1002 ± 184.9	266 ± 68.2	282 ± 107.3	99 ± 23.55	1246 ± 209.4
Leaf: W (mm)	14.45 ± 2.8	17.7 ± 3.4	8.71 ± 2.4	19.7 ± 3.79	8.75 ± 3.56	1.92 ± 0.29	19.2 ± 3.68	23.2 ± 7.96	17.4 ± 3.17	15.1 ± 3.1	24.2 ± 5.1	16.7 ± 3.4	12.6 ± 2.93	4.6 ± 1.07	23.1 ± 6.5
TS - Leaf: H (mm)	4.66 ± 0.94	5.5 ± 1.8	3.9 ± 0.57	4 ± 0.66	3.6 ± 0.97	0.35 ± 0.11	4.7 ± 1.16	5.8 ± 4.44	8.9 ± 11.38	3.7 ± 0.49	0.33 ± 0.07	3.8 ± 0.63	4 ± 1.76	2.9 ± 0.738	2 ± 29.2
TS - Leaf width *x* height ratio (mm)	0.34 ± 0.11	0.31 ± 0.06	0.47 ± 0.12	0.21 ± 0.05	0.44 ± 0.09	0.19 ± 0.08	0.25 ± 0.06	0.22 ± 0.11	0.30 ± 0.11	0.26 ± 0.06	0.15 ± 0.06	0.24 ± 0.08	0.32 ± 0.11	0.74 ± 0.20	0.09 ± 0.02
TS - Mechanical hypodermis AD: H (µm)	10.23 ± 2.1	7.1 ± 3.0	4.6 ± 2.3	5.6 ± 1.9	4.0 ± 1.5	0.69 ± 0.19	4.9 ± 2.0	6.0 ± 98000	4.0 ± 2.0	3.1 ± 0	8.0 ± 2.0	5.0 ± 2.0	4.0 ± 2.0	5.0 ± 2.0	3.4 ± 98
TS - Water-storage parenchyma (total) AD: H (µm)	22.63 ± 4.01	34.0 ± 6.9	20.61 ± 5.01	23 ± 6.4	20.46 ± 3.05	19.9 ± 6.0	24.3 ± 4.0	23.4 ± 8.8	27.3 ± 9.9	19.9 ± 2.9	11.4 ± 4.6	15.8 ± 5.6	20.8 ± 9.3	18 ± 3.8	4.2 ± 9.0
TS - Water-storage parenchyma AD 1st stratum: H (µm)	1.33 ± 0.46	4.3 ± 043	1.05 ± 0.42	1.4 ± 0.44	1.3 ± 0.29	2.3 ± 0.98	1.6 ± 7.0	3.2 ± 1.3	1.4 ± 7.0	1.1 ± 5.0	3.6 ± 4.2	1.3 ± 5.0	1.5 ± 0.8	1.7 ± 1.0	3.7 ± 1.2
TS - Water-storage parenchyma AD 2nd stratum: H (µm)	21.3 ± 3.89	32 ± 7.2	19.95 ± 5.1	22 ± 6.48	19.2 ± 3.1	15.63 ± 7.5	22.7 ± 3.5	20.3 ± 9.1	25.8 ± 10	18.9 ± 2.4	8.4 ± 4.7	14.3 ± 5.6	19.3 ± 8.9	16.7 ± 3.0	6.0 ± 9.0
TS - Chlorenchyma total: H (µm)	12.86 ± 2.25	13 ± 1.5	12.78 ± 1.23	10.35 ± 0.80	8.1 ± 2.84	10.4 ± 3.2	15.8 ± 4.5	14.5 ± 3.7	17.8 ± 4.1	11.2 ± 1.4	9.9 ± 1.9	11.7 ± 1.0	10.6 ± 3.7	6.9 ± 2.0	6.7 ± 1.5
TS - Vascular bundle: H (µm)	1.38 ± 0.31	1.9 ± 0.24	1.43 ± 0.26	2.6 ± 0.62	1.5 ± 0.39	2.2 ± 0.6	1.7 ± 0.2	2.3 ± 0.5	1.7 ± 0.3	1.4 ± 0.4	2.1 ± 0.5	1.8 ± 0.5	1.6 ± 0.4	1.1 ± 0.4	1.8 ± 0.4
TS - Armed parenchyma cells: H (µm)	9.45 ± 1.78	9.2 ± 3.2	8.52 ± 1.63	8.8 ± 0.67	5.3 ± 0.81	8.9 ± 1.6	11.9 ± 2.4	10.6 ± 1.5	12.3 ± 2.3	8.8 ± 1.5	6.8 ± 1.5	9.3 ± 1.6	7.9 ± 2.6	5.0 ±1.2	2.2 ± 1.0
TS - Water storage parenchyma AB: H (µm)	8.87 ± 1.59	9.2 ± 1.5	7.5 ± 1.75	7.2 ± 0.72	4.5 ± 0.94	7.2 ± 1.1	10.63 ± 3.0	9.2 ± 1.2	11.2 ± 2.4	7.0 ± 1.0	5.5 ± 1.23	8.0 ± 0.8	7.0 ± 2.4	4.3 ± 1.0	3.6 ± 0.9
TS - Mechanical hypodermis AB: H (µm)	1.08 ± 0.22	2.0 ± 3.6	1.5 ± 2.83	0.68 ± 0.19	0.49 ± 0.22	9.0 ± 3.0	7.0 ± 2.0	1.0 ± 3.0	9.0 ± 2.0	4.0 ± 2.0	9.0 ± 1.0	7.0 ± 2.0	7.0 ± 3.0	5.0 ± 2.0	6.0 ± 2.0
External morphology of the leaf blade: (0) lanceolate (1) revolute (2) intermediate	0	0	1	0	2	0	0	0	0	0	0	0	0	2	0
Leaf shape in TS: (0) Elliptical (1) Narrow elliptical (2) Short obovate (3) Linear	1	0	2	1	2	1	1	1	1	1	3	1	1	0	3
Color of the spine: (0) black (1) brown (2) grayish-green	0	1	2	1	1	0	0	0	2	1	0	1	2	2	0
Trichomes in the epidermis Ad face: (0) Absent (1) Present in the sulcus (2) Present, not restricted to the sulcus	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Trichomes in the epidermis Ab face: (0) Absent (1) Present in the sulcus (2) Present, not restricted to the sulcus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AD Surface: (0) Smooth (1) with sulcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AB Surface: (0) Smooth (1) with sulcus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 2. Cont.

Characters/Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
	D. ag	*D. al*	*D. de*	*D. do*	*D. he*	*D. ma*	*D. my*	*D. ni*	*D. po*	*D. re*	*D. ri*	*D. se*	*D. to*	*D. ch*	*B.an*
Classification of the leaf according to the position of the stomata: (0) Hypostomatic (1) Amphistomatic (2) Epistomatic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Type of Stomata: (0) Paracytic (1)Tetracytic	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Crystals/silica bodies in epidermis: (0) Absent (1) AD (2) AB (3) Both sides	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Proportion of water-storage parenchyma adaxial side in relation to the abaxial side: (0) 2:1 (1) 3:1 (2) 1:2	1	2	1	1	1	0	0	1	0	0	0	0	0	1	2
Hypodermis in AD: (0) Absent (1) 1 layer (2) 2 layers (3) 3 or more layers	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3
Chlorenchyma projections: (0) Absent (1) Present	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
Number of vascular bundles in TS of the leaf	61	74	30	74	41	81	63	83	51	63	87	62	45	23	78
Arrangement of vascular bundles in relation to the presence of fiber cap: (0) Ph:X/Ph/Ph/Ph:X; (1) Ph:X/Ph/Ph/Ph/ Ph:X; (2) Ph:X/Ph/Ph/Ph:X and Ph:X/Ph/Ph/Ph/ Ph:X	0	1	1	1	1	0	1	0	1	0	1	2	1	1	0
Phloem shape: (0) Triangular (1) Semiorbicular	1	0	1	0	1	1	1	1	0	1	1	0	0	1	1
Non-vascular fibers: (0) Absent (1) Present	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fibers surrounding the vascular bundle: (0) 1 - 2 caps (2) 2 caps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Raphides: (0) Absent (1) Between the vascular bundles (2) Above the vascular bundle (3) Below the vascular bundle	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2
Type of parenchyma in AB: (0) water-storage absent (1) water-storage interleaved with aerenchyma channels (2) water-storage continuous from bundle to abaxial side	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1
Shape of armed parenchyma cells AB: (0) Lobe-shaped (1) Star-shaped (2) Intermediate	0	0	0	0	0	2	0	2	0	0	2	0	0	0	1
Sclerenchyma fibers: (0) Absernt (1) Present in the bundle (2) Present in the epidermis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2

Notes: TS = Transversal Section; L = length; W= width; H = height; AB = Abaxial surface; AD = Adaxial surface; Ph = phloem; X = xylem; Proportion of water-storage parenchyma on the adaxial side in relation to the abaxial side: 2:1 = two parts: one part; 3:1 = three parts: one part; 1:2 = one part: two parts. Arrangement of vascular bundles in relation to the presence of fiber cap: Ph:X/Ph/Ph/Ph:X = Phloem:Xylem/Phloem/Phloem/Phloem:Xylem; Ph:X/Ph/Ph/Ph/Ph:X = Phloem:Xylem/Phloem/Phloem/Phloem/ Phloem:Xylem.1 = *Dyckia agudensis*; 2 = *D. alba*; 3= *D. delicata*; 4= *D. domfelicianensis*; 5= *D. hebdingii*; 6 = *D. maritima*; 7= *D. myriostachya*; 8= *D. nigrospinulata*; 9 = *D.* aff *polycladus*; 10= *D. retroflexa*; 11 = *D. rígida*;12= *D. selloa*; 13= *D. tomentosa*; 14= *D. choristaminea*; 15= *Bromelia antiacantha*.

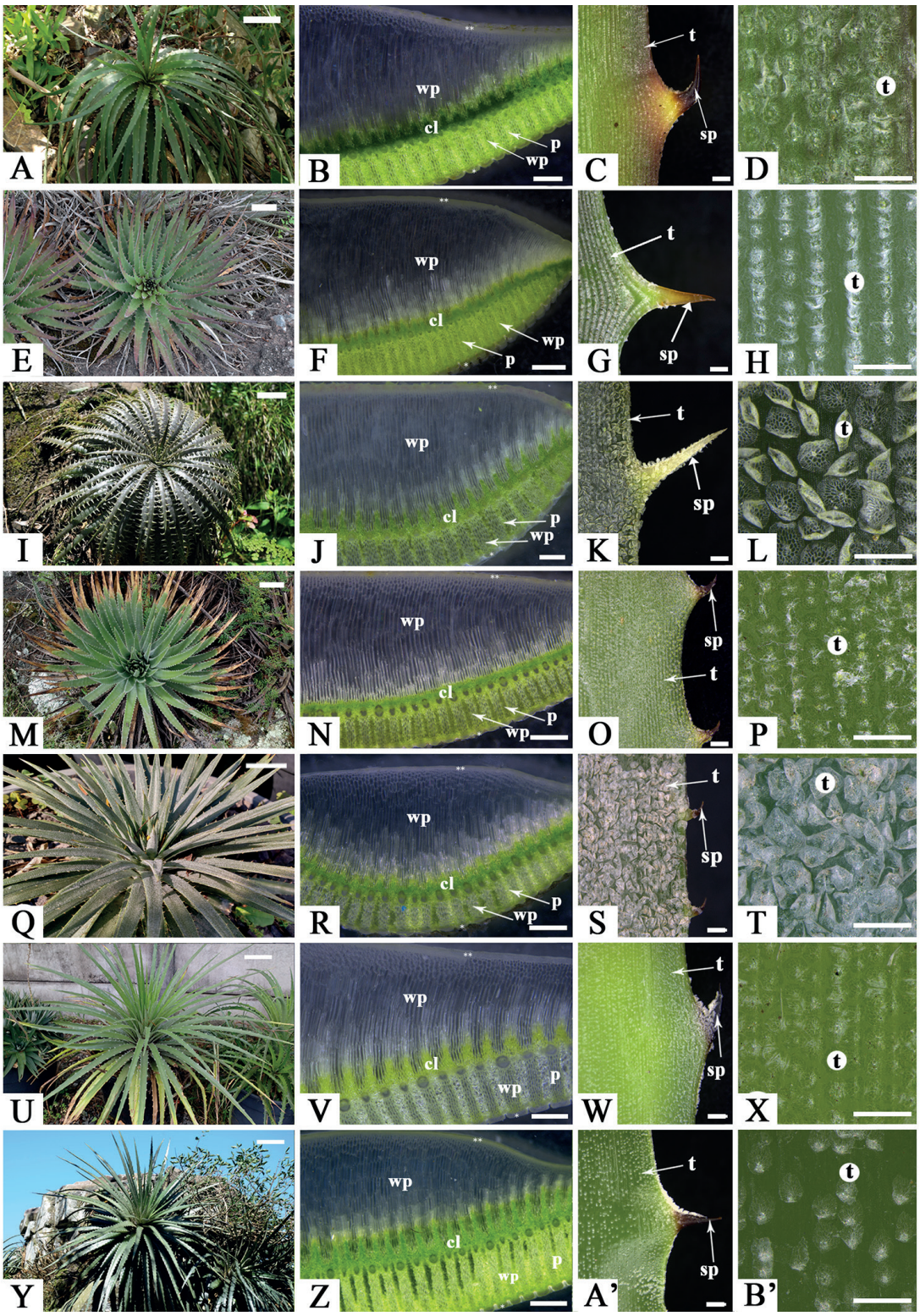

Figure 1. External and internal morphology of species of *Dyckia*: **A-D** - *D. agudensis*; **E-H** - *D. alba*; **I-L** - *D. delicata*; **M-P** - *D. domfelicianensis*; **Q-T** - *D. hebdingii*; **U-X** - *D.* aff. *maritima*; **Y-B'** - *D. myriostachya*. Evidencing in **A, E, I, M, Q, U** and **Y** - the general appearance of the plants; in **B, F, J, N, R, V** and **Z** - leaf shape in transverse section under stereomicroscopy without staining; in **C, K, O, S, W** and **A´** - detail of adaxial leaf side showing spines and trichomes; in **G** - detail of abaxial leaf side showing spines and trichomes; and in **D, H, L, P, T, X** and **B´** - detail of the adaxial leaf side and trichome arrangement. (**cl** = chlorenchyma; **sp** = spine; **wp** = water-storage parenchyma; **ap** = armed parenchyma; **t** = trichome), ** mechanical hypodermis in adaxial side, * mechanical hypodermis in abaxial side. Scale bar: C, F, G, J, K, N, O, P, S, V, W, Z and A' = 1 mm; B, D, H, L, T, R, X and B' = 0.5 mm; E, I = 2 cm; M, Q, U = 4 cm; and Y= 1 cm.

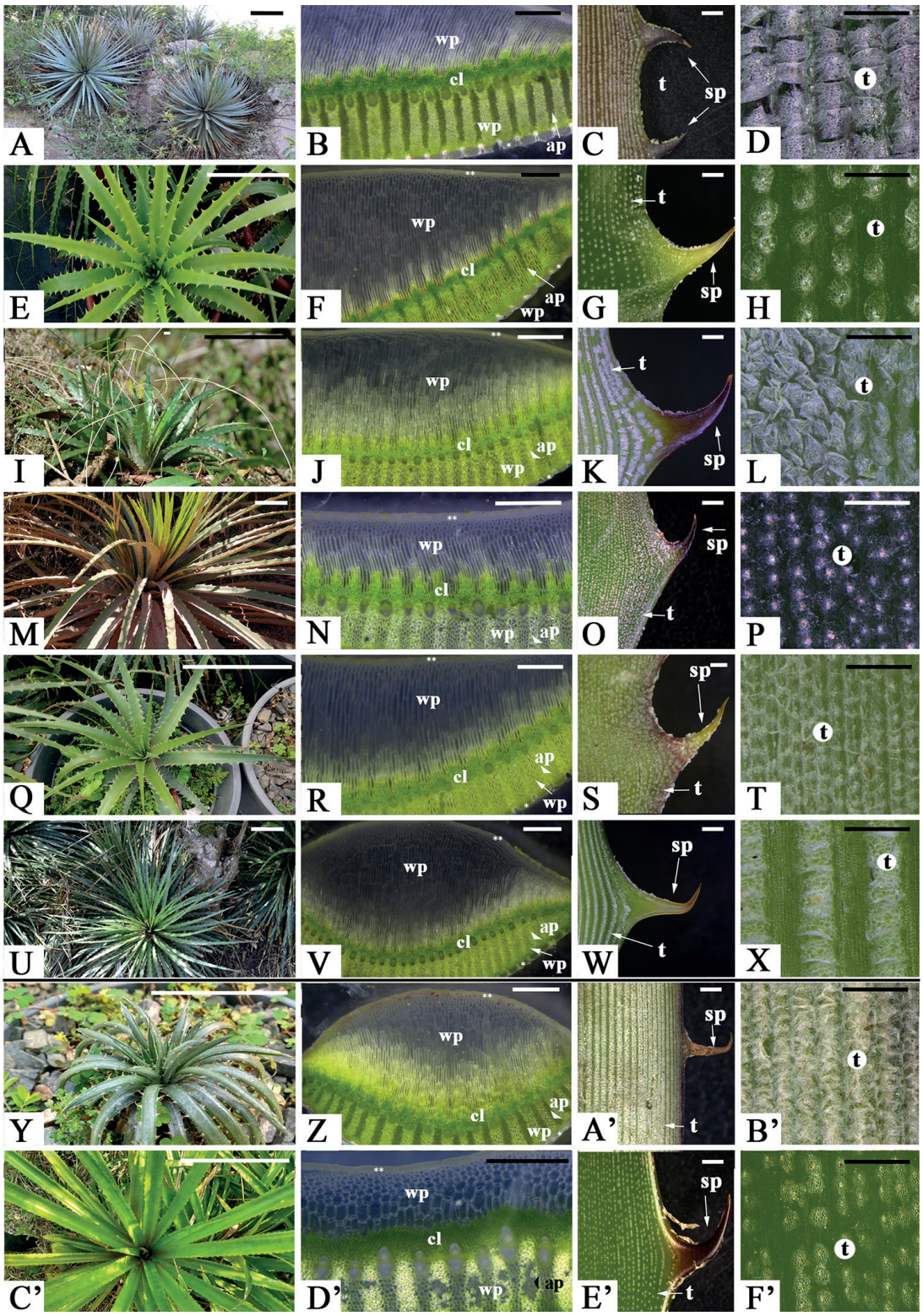

Figure 2. External and internal morphology of species of *Dyckia* and *Bromelia* **A-D** - *D. nigrospinulata*; **E-H** - *D. polyclada*; **I-L** - *D. retroflexa*; **M-P** - *D. rigida*; **Q-T** - *D. selloa*; **U-X** - *D. tomentosa*; **Y-B´** - *D. choristaminea*; **C´-F´** - *Bromelia antiacantha*. Evidencing in **A, E, I, M, Q, U, Y** and **C´** - the general appearance of plants; in **B, F, J, N, R, V, Z** and **D´** - a leaf shape in transverse section under stereomicroscopy without staining; in **G, O, S,** and **E´** - detail of adaxial leaf side showing spines and trichomes; in **C**, **K. W** and **A´** - detail of abaxial leaf side showing spines and trichomes; in **H, P** and **F´**- detail of the adaxial leaf side and trichome arrangement; and in **D, L, T, X** and **B´** - detail of the abaxial leaf side and trichome arrangement. (**cl** = chlorenchyma; **sp** = spine; **wp** = water-storage parenchyma; **ap** = armed parenchyma; **t** = trichomes. Scale bar: A, E, I, M, Q, U, Y and C´ = 2 cm; B, C, D, F, G, J, K, N, O, P, S, V, W, A´, D´, E´ and F´ = 1 mm; H, L, R, T, Z, X and B´= 0.5 mm.

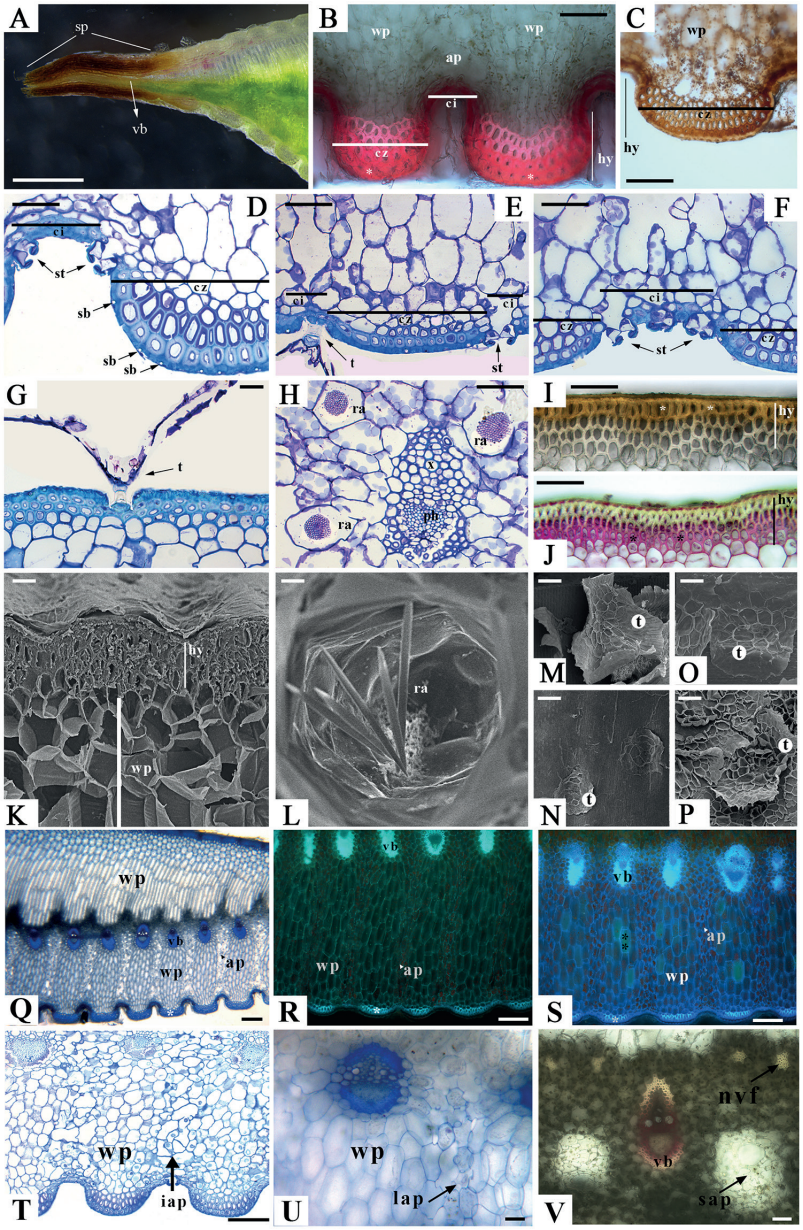

Figure 3. Leaf anatomy of studied species in transverse section. **A -** Stereomicroscopy transverse section of vascularized spine in *D. agudensis*. **B**-**C** - Detail of abaxial side: showing positive reaction of lignin detection in the hypodermis (acidified phloroglucinol test) in *D. nigrospinulata,* with indications of intercostal zone (iz) and costal zone (cz) (**B**); and negative reaction (Maüle's test) for syringyl lignin in *D. alba* (**C**). **D-H** - Transverse section stained with Toluidine Blue O: detail of the stomata and trichomes and silica bodies (**D-F**) on abaxial side; detail of trichomes and hypodermis on adaxial side (**G**); and detail of the vascular bundle and raphides (**H**); **D** - *D. alba*; **E** - *D. hebdingii*; **F-H** - *D. delicata*. **I-J** - details of the hypodermis in the adaxial side with chlorine-sulfite test in *D. rigida* (**I**) and Ruthenium Red in *D. domfelicianensis* (**J**). **K-P** - SEM images: **K** - details of hypodermis of adaxial side and water-storage parenchyma in *D. agudensis*; **L** - idioblast and raphide in *D. agudensis*; **M** - trichomes and stomata in intercostal area of abaxial side in *D. nigrospinulata*; **O** - trichomes in intercostal area of adaxial side in *D. agudensis*; **N** - trichomes in intercostal area of adaxial side in *D. selloa*; **P** - trichomes in intercostal area of abaxial side in *B. antiacantha;* **Q** - Toluidine Blue staining in transverse section of *D.* aff. *maritima*, highlighting the mechanical hypodermis of the abaxial side with asterisc*;* **R** - Water-storage and armed parenchyma cells autoflorescence in *D. hebdingii;* **S** – Test with LYCH apoplastic tracer florescence showing positive results for water-storage parenchyma in *D. hebdingii*, with black asterisk for water identification; **T** – Intermediated armed parenchyma cells staining with toluidine blue in *D.* aff. *maritima;* **U** – Lobed-shaped parenchyma cells with toluidine blue in *D. selloa;* **V** - Star-shaped parenchyma with phloroglucinol test in *B. antiacantha* (* white = indicates thickening of mechanical hypodermis; **black= apoplastic tracer; **nvf** = non-vascular fibers; **hy** = hypodermis; **ap =** armed parenchyma; **ph =** phloem; **id =** idioblast; **ra =** raphides; **sb** = silica bodies; **sp =** spine; **st** = stomata; **t =** trichomes; **vb =** vascular bundle; **wp =** water-storage parenchyma; **x =** xylem; **iz** intercostal zone; **cz =** costal zone; **lap**= lobe-shaped armed parenchyma; **sap**= star-shaped armed parenchyma; **iap**= intermediate armed parenchyma). Scale bar: A = 1 mm; B, C, I, J, M, O and U = 50 µm; D, E, F, G, H and L = 20 µm; K, N, P, R, S and T = 100 µm; Q = 200 µm.

to the vascular bundles and are anticlinally-elongated, with the greatest thickness in *D. alba* (32 µm ± 7.2) (Fig. 1F) and the least in *B. antiacantha* (6.0 µm ± 9.0) (Fig. 2D´).

Water-storage, proved with apoplastic tracer (Fig. 3R-S), and armed parenchyma cells are present in the region of the mesophyll between the vascular bundles up to the abaxial side (e.g. Fig. 3Q). The greatest and least thickness of both tissues in this region are for *D. polyclada* (11.2 µm ± 2.4) (Fig. 2R) and *B. antiacantha* (3.6 µm ± 0.9) (Fig. 2D'), respectively. In these species, the cells of the water-storage parenchyma are polygonal, which may or may not be anticlinally-elongatedand extending to the costal region of the abaxial side (Fig. 3Q). The armed cells of the armed parenchyma may have short or long cells extending to the intercostal area of the abaxial face of the epidermis. The types of the armed parenchyma cells are star-shaped armed parenchyma (Fig. 3V), lobe-shaped armed parenchyma (Fig. 3U), and intermediate armed parenchyma (Fig. 3T).

In transverse section, the chlorenchyma cells possess an isodiametric shape, with the greatest mean thickness of this tissue being for *D. polyclada* (17.8 µm ± 4.1) (Fig. 2R) and least for *B. antiacantha* (6.7 µm ± 1.5) (Fig. 2D´) and are distributed among the vascular bundles in the center of the leaf blade, yet still containing idioblasts with raphides (Figs. 3H, 3L). Chlorenchyma reaches part of the water-storage parenchyma in the species *D. agudensis, D. choristaminea, D. delicata, D. hebdingii; D.* aff. *maritima, D. myriostachya, D. polyclada, D. retroflexa, D. rigida, D. selloa* (e.g. Fig. 2R), and accompanies the bundles in *D. alba, D. domfelicianensis, D. nigrospinulata and D. tomentosa,* and *B. antiacantha* (e.g. Fig. 2D´). *Bromelia antiacantha* was the only species to present non-vascular fibers (Fig. 3V) located at the chlorenchyma tissue.

Among the analyzed species, *D. rigida* (Fig. 2N) was found to have the greatest number of vascular bundles in transverse section with 83 units while *D. choristaminea* (Fig. 2Z) was found to have the least with 23 units. These vascular bundles, inserted within the chlorenchyma of the mesophyll, are distributed in a linear plane through the mesophyll and are formed by collateral bundles (Fig. 3H) with one or two caps of fibers, depending on the caliber.

Multivariate analyses

Principal Coordinates Analysis (PCoA)

The results of the PCoA demonstrated that the morphological variation observed for the analyzed species could be adequately summarized using the first two dimensions (Fig. 4). The species formed a large grouping, except for the outgroup taxa *D. choristaminea* and *B. antiacantha*, which were discriminated from the species of the *D. selloa* complex. The first dimension (D1) represented 34.5% of the total variance (Cronbach's alpha=0.925) and the second dimension (D2) 20.1% (Cronbach's alpha=0.835), for a total of 54.6% of the total observed variation.

The values obtained for D1 and D2 showed that 21 of the 35 characters used in the analysis have high discrimination values (discrimination measures: DM > 0.7) in at least one of the first two dimensions, with the DM of seven characters being greater than 0.7 in both dimensions (Table

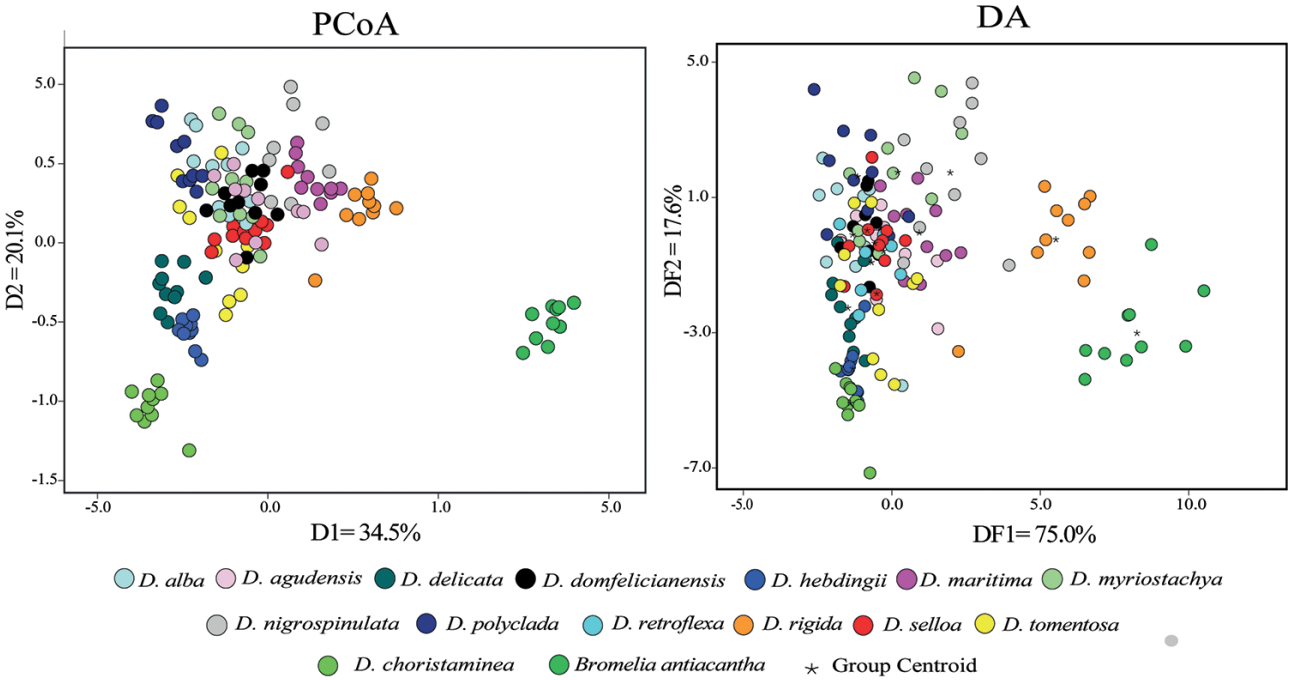

Figure 4. Two-dimensional scatter plots obtained from morphological character analyses: on the left, graph of principal coordinates analysis (PCoA); on the right, discriminant analyses (DAs). The proportions of morphological variation captured by each of the two main dimensions of PCoA (D1 and D2) and the two discriminant functions (DF1 and DF2) are shown in the two scatter plots s, left and right, respectively. Colored circles indicate the analyzed species described in Table 1.

S3). The qualitative characters that contributed the most to both D1 and D2 were spine color, non-vascular fibers, leaf shape in transverse section, chlorenchyma projections, shape of armed parenchyma cells in abaxial side and external leaf blade morphology. The analysis revealed ten morphometric characters with high discrimination values including leaf length and width, leaf height in transversal section, proportion of total chlorenchyma length x width, total length of water-storage parenchyma, length of 2nd stratum of water-storage parenchyma, total height of chlorenchyma, length of armed parenchyma cells, height of vascular bundle, and abaxial water-storage parenchyma and number of bundles in transverse section. The set of analyzed characters had high discrimination values capable of delimiting species from the outgroup taxa *D. choristaminea* and *B. antiacantha*.

Discriminant Analysis (DA)

Discriminant analysis performed with the set of 14 quantitative characters showed that 92.6% of the total variation can be explained by the first two discriminant functions, Discriminant Function 1 (DF1=75.0%) and Discriminant Function 2 (DF2=17.6%), as shown in the ordering Table S3. The DA scatter plot revealed the separation of the outgroup taxon *B. antiacantha* accompanied by *D. rigida* of the *D. selloa* complex (Fig. 4). Despite this subtle separation, the other analyzed species were grouped in the *D. selloa* complex, including the outgroup taxon *D. choristaminea*.

The two discriminant functions were strongly correlated (canonical correlation: DF1 = 0.951; DF2 = 0.889) and highly significant (DF1 to DF2: Wilks' Lambda = 4.55×10^{-54}; DF2 to DF1: Wilks' Lambda = 6.87×10^{-79}). Based on the correlation coefficients obtained between each character and the discriminant functions, the highest absolute correlations were detected for four variables: DF1, leaf length and for DF2, leaf width, proportion of total chlorenchyma parenchyma length x width and armed parenchyma cells (Table S3). Although the set of characters has a high discriminant value, which enabled the separation of *B. antiacantha* and *D. rigida* from the other species of *Dyckia* sampled, the species from the *D. selloa* complex were grouped with the other outgroup taxon *D. choristaminea*.

Discussion

Morphological and anatomical characters

The analyses carried out here found morphological characteristics that are shared between species of the *D. selloa* complex and other species of Bromeliaceae, such as: spine color, presence of trichomes on both sides of the leaf, lignified mechanical hypodermis, layers of water storage parenchyma, cells morphology in armed parenchyma cells, and chlorenchyma disposition in the mesophyll (Tomlinson 1969; Smith & Downs 1974; Reitz 1983; Benzing 2000; Proença & Sajo 2007; Voltolini *et al.* 2009; Santos-Silva *et al.* 2013; Krapp *et al.* 2014; Pinangé *et al.* 2016; Schütz *et al.* 2016). *Dyckia* possesses leaf anatomical characteristics common to other genera of the xeric clade of Pitcairnioideae, such as the presence of mechanical hypodermis and water-storage parenchyma on both sides (Givnish *et al.* 2007; 2011; Gomes-da-Silva *et al.* 2017).

Species of the *D. selloa* complex exhibit great plasticity in external leaf morphology, ranging from lanceolate to revolute with intermediate morphologies. In *Dyckia*, rosettes do not form cisterns, which makes for a larger contact surface to acquire water and nutrients from the xeric environment of these species (Smith & Downs 1974; Reitz 1983; Benzing 2000; Proença & Sajo 2007; Dettke & Milanez-Gutierre 2008; Voltolini *et al.* 2009; Aoyama *et al.* 2012). The absence of cistern in *Dyckia* indicates a variation related to environmental conditions compared to other xeric members of Bromeliaceae.

Analysis of leaf morphology and anatomy showed the occurrence of spines in *Dyckia* through visualization of the continuous vascular system (Tomlinson 1969; Reitz 1983; Benzing 2000). Aculeae and spines are similar, pointed elements on the surface of plant organs. However, an aculea is an exclusively epidermal structure, while a spine can result from the modification of a branch, leaf, stipule, or root. Such structures are vascularized and firmly attached to the plant body (Ferri *et al.* 1978). Spines are normally found on leaf margins within Bromelioideae and Pitcairnioideae and are rigid and organized along the entire leaf margin (Tomlinson 1969). Although rare, aculeus have been recorded in Bromeliaceae, on the leaves of *Aechmea calyculata* Baker (Favretto & Geuster 2017). Species of the *D. selloa* complex have extraordinarily strong spines with a horny, black-brown constitution and the apex of the leaf blade can end in a spine, in agreement with Reitz (1983). As for their color, spines in *Dyckia* are usually white or the same color as the leaf (Smith & Downs 1974; Reitz 1983). The extensive sampling in this study showed variation in the color of these structures, enabling the separation of the *D. selloa* complex into two groups: one containing *D. agudensis*, *D.* aff. *maritima*, *D. myriostachya*, *D. nigrospinulata*, and *D. rigida*, which have black spines; and one containing *D. alba*, *D. delicata*, *D. domfelicianensis*, *D. hebdingii*, *D. polyclada*, *D. retroflexa*, *D. selloa* and *D. tomentosa*, which have brown spines. Many terrestrial bromeliad species invest in spines as a mechanical defense against herbivores (Benzing 2000). This is an important feature to be included not only in analyses for the circumscription of the *D. selloa* complex itself, but in analyses of other taxonomic studies as well.

Another interesting character is the position of stomata. The present results show that stomata in *Dyckia* are located above the level of the epidermis. This character has already

been used to separate the genera of Pitcairnioideae into two clades, a xeric clade and a mesic clade (Santos-Silva *et al.* 2013). The present results indicate that the *D. selloa* complex should be positioned within the xeric clade of Pitcairnioideae. In addition, the epidermis of the analyzed species of the *D. selloa* complex has numerous trichomes, aligned on both sides and sharing intercostal space with stomata on the abaxial side, a result already presented for other species in the family (Tomlinson 1969; Krauss 1948; Smith & Downs 1974; Benzing 2000). Some authors claim that the main function of trichomes in *Dyckia* would not be absorption, as they are present in terrestrial species, in addition to not having a water storage tank in the center of the rosettes and raise the hypothesis that the trichomes are used as an efficient strategy for light refraction and water economy and reserve (Smith & Downs 1974; Aoyama & Sajo 2003; Scatena & Segecin 2005; Proença & Sajo 2007). Some authors consider trichomes to act in the retention of absorbed water and its distribution to other cells of the epidermis, thereby improving absorption, reducing transpiration, and providing mechanical protection (Tomlinson 1969; Aoyama & Sajo 2003; Scatena & Segecin 2005; Proença & Sajo 2007).

Species of the *D. selloa* complex possess, in the mesophyll, a mechanical hypodermis with differentiated strata and with variation in the deposition of pectins and lignins in the thickening of the wall of these cells. Pectin permeates the entire mechanical hypodermis on both sides of the epidermis while the two layers just below the epidermis possess greater lignin deposition. The pectic composition of the walls of the hypodermis can highlight a hydrophilic behavior for water retention and the presence of communications in the anticlinal walls (micropores), as reported for *D. brevifolia* Baker by Lobo (2007), indicating a probable water transport function. Thus, the presence of pectic layers in the inner part of the mechanical hypodermis of the studied species suggests that they function in the aid of water absorption and in leaf turgor maintenance, in addition to preventing leaf blade dehydration, as these plants survive in a xeric environment (Lyshede 1978; Lobo 2007). On the other hand, the identification of a secondary thickening rich in coniferyl lignin characterizes this hypodermis as a sclerenchymatous tissue present on both sides of the leaves of the analyzed *Dyckia* species. This lignification is associated with the rigidity of the walls close to the epidermis and can provide protection and maintenance of leaf structure to prevent dehydration of individuals exposed to intense solar radiation and protection against herbivory (Krauss 1948; Lobo 2007). In addition, mechanical hypodermis can serve as a taxonomic character since this character was previously identified in *Encholirium* and *Dyckia*, distinguishing these from the remaining genera of the subfamily (Santos-Silva *et al.* 2013; Guarçoni 2015). More comprehensive analyzes of this character, in this complex of the genus *Dyckia* could be used as discriminators for groups of species.

The occurrence of succulent leaves by species from xeric environments is one of the adaptations to resist water stress due to lack of water and is related to the presence of water storage tissue (Tomlinson 1969). Within Bromeliaceae, this tissue is distributed in the form of a water-storage parenchyma, with water reserve cells that may or may not contain chloroplasts, mucilage, and large vacuoles (Fahn & Cutler 1992). In *Dyckia*, this water-storage parenchyma may be present facing either the adaxial side or the abaxial leaf side, between the set of cells of the armed parenchyma cells, as also observed for leaves of species of the genus *Encholirium* (Santos-Silva *et al.* 2013). In other genera of the subfamily, this tissue is only present near the adaxial side (Pita 1997; Vailati 2009; Voltolini *et al.* 2009; Santos-Silva *et al.* 2013). The species of *Dyckia* analyzed here have a well-defined proportion of water-storage parenchyma to armed parenchyma cells, while chlorenchyma differs in its disposition, being able to follow bundles or reach part of the water-storage parenchyma of the adaxial side. The presence of armed parenchyma cells has already been reported in other species of Bromeliaceae, along with differences in the sizes of intercellular spaces (e.g., Santos-Silva *et al.* 2013). The observation of this characteristic also in *D. brevifolia* and *D. distachya* (Lobo 2007; Voltolini *et al.* 2009), indicates that armed parenchyma cells could enlarge intercellular spaces, favoring the accumulation of CO_2. This tissue disposition is strategic in the species of the present study, as the diffusion of CO_2 into intercellular spaces contributes to the absorption of photosynthetic carbon in thick and hypostomatic leaves (Parkhurst 1994).

Some authors have identified water-storage parenchyma facing the abaxial leaf side in *D. dystachya* and *D. encholirioides*, located between the layers of armed parenchyma cells (Pita 1997; Voltolini *et al.* 2009; Vailati 2009). Although Lobo (2007) reported that for *D. brevifolia*, water-storage parenchyma is present only near the adaxial side, other studies have shown that species of *Dyckia* and *Encholirium* have this parenchyma on both leaf sides (Santos-Silva *et al.* 2013), which was confirmed here for the species of the *D. selloa* complex. Water-storage parenchyma is present in some species of the genera *Pitcairnia*, *Fosterella* and *Deuterocohnia*, and in most species, this parenchyma only occurs on the adaxial side (Santos-Silva *et al.* 2013). Allied to this, the literature reports that species of *Dyckia* possess only armed parenchyma cells with cellular projections with short arms (Santos-Silva *et al.* 2013), while the present study was able to identify the occurrence of both short and long arms.

The presence of raphides in the chlorenchyma of species of the *D. selloa* complex is a common feature to both vegetative and reprodutive organs of the family (Tomlinson 1969; Aoyama & Sajo 2003; Scatena & Segecin 2005; Souza *et al.* 2005; Lobo 2007; Proença & Sajo 2007; Dettke & Milanez-Gutierre 2008; Vailati 2009; Voltolini *et al.* 2009; Silva & Scatena 2011; Krahl *et al.* 2013; Guarçoni 2015). The presence of these calcium oxalate structures can make

plants less palatable to predators and thus prevent herbivory (Mauseth 1988). Despite the presence of raphids being used in these taxonomic studies, our analysis did not show that it is an important character to discriminate species.

The vascular bundles of the *D. selloa* complex species are collateral and with one or two caps of fibers, as reported for other *Dyckia* species (Santos-Silva *et al.* 2013). This character may also be useful in characterizing groups of species, especially considering the number of layers of these caps. All *Dyckia* species do not present non-vascular fiber bundles when compared to *B. antiacantha* which has non-vascular fiber bundles, constituting a possible character for future taxonomic analysis.

Multivariate analysis and delimitation of the **Dyckia selloa** *complex*

The scatter plot obtained from the first two PCoA axes, incorporating all analyzed species, clearly shows that the *D. selloa* complex is morphologically distinct from the other taxa. The selected outgroup taxa, *B. antiacantha* and *D. choristaminea*, were discriminated from the other species by the first two axes of PCoA, with the characters most contributing to this delimitation being leaf length and spine color. The scatter plot also shows the proximity of *D. choristaminea* to the sampled species of the *D. selloa* complex, especially *D. hebdingi* and *D. delicata*, resulting from shared characteristics related to spine color (such as brown spines), upturned leaves and trichomes on both leaf sides. *Dyckia* aff. *maritima*, *D. nigrospinulata, D. myriostachya* and *D. rigida* group closely on the scatter plot, possibly because they share characters related to black spine color and leaf length.

The scatter plot of the first two discriminant functions clearly shows that the *D. selloa* complex groups with the outgroup taxon *D. choristaminea*. On the other hand, *B. antiacantha* and *D. rigida* appear discriminated from the other species in the right portion of the scatter plot, mainly by DF1, which is based mainly on characters such as leaf length and proportion of water-storage parenchyma. Despite the high values of discrimination and correlations obtained, the discriminant function analysis was not able to delimit the *D. selloa* complex using morphometric data.

From data observed in this work, combined with the incongruence between the multivariate analyses in the delimitation of species belonging to the *D. selloa* complex, in addition to the distinct seminal rudiment data obtained in Breitsameter (2017), we suggest maintaining the *D. selloa* complex based on morphological qualitative data, with the possibility of dividing the species into two groups. The first group would consist basically of the same species group treated like *D. maritima* complex *sensu stricto* (except for *D. agudensis* and *D. retroflexa*) of Büneker *et al.* (2015), consisting of species morphologically similar to *D. maritima* that have as main feature the black leaf spines. This first group we observe is formed by the species *D. agudensis, D.* aff. *maritima, D. myriostachya, D. nigrospinulata* and *D. rigida*. The second group would be composed of the species *D. alba, D. domfelicianensis, D. selloa, D. retroflexa, D. polyclada* and *D. tomentosa*, for which a new name could be created to contrast to with *D. maritima* complex *sensu stricto,* as established before, showing that there are at least two species groups within the *D. selloa* complex. The fact that the species *D. delicata* and *D. hebdingii* share very similar leaf morphoanatomical characteristics with the outgroup taxon *D. choristaminea*, show their distinctions between the other species of the complex.

Choosing the optimal methodological approach is essential for identifying the boundaries between sets of species and inferring the number of species in a complex (Rieseberg & Burke 2001; Sites & Marshall 2003; De Queiroz 2007). Morphological characters are the original tools used by taxonomists to identify and discriminate species (Cronquist 1981; Dahlgren *et al.* 1985). However, many studies focus on few characteristics, with no attention to morphological variation in a morphometric and multivariate context. The past few decades have seen researchers develop several methods for recognizing new species or testing species hypotheses (Wiens 2007; Naciri & Linder 2015). However, these tools for species delimitation often involve expensive molecular and computational methods that are demanding of specialized work. In this sense, the morphological and anatomical approaches applied in the present study, mainly based on qualitative characters, proved to be robust for delimiting the *D. selloa* complex.

The analyses carried out here indicate that characters related to leaf morphology and anatomy can be useful for delimiting species or groups of species, but they should be applied with caution in taxonomic decisions involving the genus *Dyckia*. Characters related to the armed parenchyma cells shape and spine color proved to be important for the *D. selloa* complex, and so we indicate their importance for future studies. There also remains a need to include different technical approaches – including reproductive structures and palynology – that make analyses more sensitive and enable the delimitation of species complexes in *Dyckia*.

Supplementary material

The following online material is available for this article:
Table S1. Histochemical tests used for the identification of cellular compounds in leaf anatomy of *Dyckia* species.
Table S2. Analysis of variance in morphological characters among *Dyckia* species using one-way ANOVA (1) and Kruskal-Wallis test (2).
Table S3. Discrimination measures for the first and second dimension (D1 and D2) of Principal Coordinates Analysis (PCoA) and structure matrix with correlation coefficients for the first two discriminant functions (DF1 and DF2) of discriminant analyses (DA) performed on two taxonomic subsets of morphological characters.

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