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Flower and floral trichome morphology of species of *Dyckia* Schult. f. (Bromeliaceae, Pitcairnioideae), and their importance to species characterization and genus taxonomy

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ABSTRACT

This paper presents a morphological analysis of the flower and floral trichomes of three rare species of *Dyckia*: *Dyckia ibicuiensis*, *D. polyclada* and *D. racinae*. Flowers at anthesis were collected from natural populations and subjected to morphometric and microscopic analysis. Among the most representative features for *Dyckia* are: morphometrics of individual floral parts; the general configuration of the androecium and gynoecium; the degree of fusion of the stigmatic lobes; the morphology of the ovules, especially in relation to the chalazal appendix; and the presence and constitution of peltate trichomes in the perianth, which exhibited a polymorphism not previously reported for *Dyckia*. The characters were effective at describing each species, proposing phylogenetic inferences and recognizing infrageneric groupings. We propose two species groups, which are consistent with previous hypotheses about the relationships among the species of the genus. The objective of this study was to provide floral morphological data useful for characterizing these three rare species, delimiting the genus and forming phylogenetic hypotheses.

Keywords: androecium, *Dyckia*, *Encholirium*, floral morphology, gynoecium, peltate trichome, perianth, Pitcairnioideae

Introduction

Morphological and anatomical studies using different approaches have provided effective instruments for characterization of species and elucidation of taxonomic and phylogenetic problems in Bromeliaceae (Varadarajan & Gilmartin 1987; Benzing 2000; Sajo *et al.* 2004; Proença & Sajo 2007; Santos-Silva *et al.* 2015; Nogueira *et al.* 2015), a predominantly neotropical diverse family. Given the structural complexity of reproductive organs in this taxon, morphological studies of different flower organs have allowed for inferences of evolutionary, systematic, ecological and physiological orders in diverse genera of the family (Varadarajan & Brown 1988; Brown & Gilmartin 1989; Brown & Terry 1992; Sajo *et al.* 2004; Fagundes & Mariath 2010).

One of the most diverse genera of Bromeliaceae, *Dyckia* currently encompasses more than 160 species distributed in South America, mainly in Brazil, many of which are rare or endemic, under threat of extinction or often with insufficient populational data (Smith & Downs 1974; Leme

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et al. 2012; Dorneles *et al*. 2014; Gouda *et al*. 2016). This genus is included in Pitcairnioideae, and presents unique morphological features within the subfamily, which allow for its circumscription as a monophyletic genus (Forzza 2001). This hypothesis was later confirmed by molecular studies, which have not yet solved the complex infrageneric relations between the species of this diverse genus (Krapp *et al*. 2014), but have advanced in the proposition of hypotheses regarding the evolution of morphological characters and confirmed probable geographical groupings (Pinangé *et al*. 2016).

While closely related to *Encholirium* ecologically and morphologically, *Dyckia* differs mainly because it encompasses plants that produce a lateral peduncle and present few floral diagnostic features, such as fusion of the stamens (Forzza 2005). If, on the one hand, *Dyckia* differs from its sister group in details of the floral architecture, the delimitation of its species can be even more subtle, not only due to the homogeneity of morphological characters in various species of the taxon (Krapp *et al*. 2014), but also due to historical problems related to the lack of characters in the original descriptions and analytical keys. This results from incomplete *typus* or even the lack of analysis of specimens in their natural environment (Smith 1967; Versieux & Wendt 2007). Since the correct delimitation of the species, as well as possible infrageneric groupings in *Dyckia*, is hampered from insufficient morphological data, comparative morphological studies, especially in natural populations, are essential for future delimitations of taxa and propositions of phylogenetic hypotheses. The efforts made toward floral anatomical and morphological analysis in *Dyckia* have provided important data for species characterization and inferences of infrageneric groupings (Dorneles *et al*. 2014; Oliveira *et al*. 2015; Carvalho *et al*. 2016).

The species analyzed in the present study, *Dyckia polyclada*, *D. racinae* and *D. ibicuiensis* are endemic to the southern region of Brazil. Besides their sparse floral features, few details are known about the morphology of their flowers and floral trichomes in natural populations, mainly for the two first species. In addition, *D. ibicuiensis* presents an official conservation status of critically endangered (DOE RS 2014), while there are insufficient population data for the other two species, a common condition for several species of the genus, whose sparse distribution and misidentification of specimens limit taxonomic investigations, as well as possible conservation strategies. Therefore, this study aimed to morphologically characterize the flower and floral trichomes of these *Dyckia* species, and to provide characters with potential taxonomic and phylogenetic value for this genus, using each floral whorls in the analysis.

Materials and methods

Botanical material

Inflorescences of ten specimens of *Dyckia ibicuiensis* Strehl., nine specimens of *D. racinae* L.B. Sm. and ten specimens of *D. polyclada* L.B. Sm. were collected in natural populations, respectively, in: Morro Itaquatiá, São Pedro do Sul, RS, Brazil (29°42.941’S/ 53°43.087’W); São Pedro do Sul, RS, Brazil (29°35’22.2’’S/ 54°49’49.4’’W); Distrito de Santo Antão, Santa Maria, RS, Brazil (29°42.911’S/ 53°43.093’W). The exsiccatae were deposited in the Herbarium of the Botanical Garden of the Universidade Federal de Santa Maria (SMDB), under registration numbers SMDB 13840, SMDB 15923 and SMDB 15924.

Morphometric analysis and terminology

Morphometric data were obtained from random individuals, using an average of 30 flowers at anthesis for each species, obtained from median portion of the inflorescences. The lengths of the pedicel, flower and petals were measured. In the androecium, the total length of the stamen, anther and filament were measured, in addition to the ratio of filament length and total stamen length, the height of connation including the common tube with petals, the ratio between the height of connation and total stamen length, the height of the common tube, and the widths of the antepetalous and antesepalous filaments. In the gynoecium, the length from the base of the ovary to the apex of the stigma was measured. In addition, some different lengths of floral parts were compared in proportion: relative size of floral bract, relative size of the sepals and relative size of the style. The terminology employed for the flower follows Smith & Downs (1974) with corrections proposed by Scharf & Gouda (2008). For the peltate trichomes, the terminology follows Benzing (1976).

Preparation for light microscopy

For the analysis in light microscopy, the plant material was fixed in a solution containing 1% glutaraldehyde and 4 % formaldehyde (McDowell & Trump 1976) in 0.1 M sodium phosphate buffer, pH 7.2 (Gabriel 1982), followed by rinsing in the same buffer, rinsing in distilled water and, finally, dehydration in an ethanolic series (O’Brien & McCully 1981), up to 70 % ethanol.

Mature flowers were analyzed and dissected under a stereomicroscope. The stigmas were isolated from dissected flowers and photographed in liquid medium. To improve the contrast, they were previously stained with 1 % safranin. For analysis using Differential Interferential Contrast Microscopy (DIC), fixed sepal portions and peltate trichomes were stained with safranin. Peltate trichomes were manually isolated and analyzed in temporary preparations in a solution ethanol/glycerin 1:1 (Johansen 1940). In addition, ovules were isolated manually and clarified using 3 % sodium hypochlorite (Johansen 1940), and also prepared in ethanol/glycerin.

The photographic documentation and analyses were carried out using a stereomicroscope Leica M80 with DFC295 image capture system and microscope Zeiss AxioImager A2 with digital photo system Zeiss Axiocam RMC. Photoshop ™ was used to process photos and figures.

Taxonomic sampling and phylogenetic analysis

For the phylogenetic inference and analysis of the evolutionary characters, seven species of Bromeliaceae were included, six of which were species of the genus *Dyckia* Schult. f.: *Dyckia choristaminea* Mez, *D. ibicuiensis* Strehl, *D. racinae* L.B. Sm., *D. maritima* Baker, *D. polyclada* L.B. Sm. and *D. selloa* (K. Koch) Baker; and one which was of an external group: *Encholirium horridum* L.B. Sm. Of these, *D. choristaminea* is morphologically similar to *D. ibicuiensis* and *D. racinae*; *D. maritima* and *D. selloa* are morphologically similar to *D. polyclada*. For species that were part of the comparative analysis, data from their original descriptions were used, as well as data from taxonomic revisions listed in Tab. 1.

For the phylogenetic analysis, a matrix of 18 morphological characters was created, with characters encoded as discrete, unordered, and without weight, with the Mesquite software (Maddison & Maddison 2016). Only variable characters were included, 15 of which were potentially parsimony-informative and three non-informative. The characters used for the analysis are shown in Tab. 1. The matrix was exported for analysis in the software PAUP 4.0 Beta version for Windows™ (Swofford 2002), for inference with Maximum Parsimony method. An exhaustive search of trees was conducted, with generation of initial trees by random stepwise addition and searching trees algorithm TBR. The missing data were treated by the program as uncertainties. Rooting of the trees was carried out through an external group. Also in the PAUP software, a Consistency Index (IC), Retention Index (IR) and majority consensus were calculated. A bootstrapping test with 1000 replicates was also conducted. Generated trees were saved and used later in the Mesquite software to map the characters. Final pictures were produced with Photoshop™.

Results

Flower morphology

The three species analyzed in this study presented complete trimerous flowers with a short pedicel and a bract. The sepals are imbricate and smaller than the petals, which are also imbricate (Fig. 1A-D). The androecium is composed of six stamens, three antesepalous, adnate to the sepals and petals, and three antepetalous, adnate only to the petals. Thus, there are two whorls of stamens, which form, in conjunction with the base of the petals, a typical common tube (Fig. 1A-H). The gynoecium (Figs. 1F-H, 2A-F) presents a superior ovary (Fig. 1F-H) and a conduplicate-spiral stigma (Fig. 2A, C, E). The ovules are anatropous and bitegmic, with a chalazal appendix and are inserted axially in the ovary (Fig. 2B, D, F). In addition, the flowers have a nectary that differentiates mainly in the floral receptacle, although it may extend above the base of the ovary (Fig. 1F-H).

The flowers of *D. polyclada* present a mean length of 1.0 cm, with an almost imperceptible pedicel and a bract that is much smaller than the sepals (Fig. 1A) (Tab. 1). The sepals are green, ovate to suborbicular and slightly connate at the base, not exceeding half the length of the petals (Fig. 1A, F). The corolla has a narrow opening, and is almost completely obstructed by the androecium and gynoecium (Fig. 1A, F). The petals are 0.7 to 0.8 cm long, spatulate, yellow, with obtuse apices near to each other during anthesis (Fig. 1A, F). The stamens present a mean length of 1.0 cm; are exserted and free above the common tube with petals, which is the shortest in comparison with the other two species (Tab. 1) (Fig. 1F). The stamens bear dorso-basifixed anthers that are 0.3 cm long on average, rectilinear, slightly articulated with divergent sporangia and arranged freely above the apex of the corolla (Fig. 1A, B, F). The antesepalous and antepetalous filaments are thin and constitute approximately 65 % of the total height of the stamen and are similar (Tab. 1). The gynoecium is exserted, 1.0 cm in length, with a style longer than the ovary and a stigma positioned at the same level with the anthers (Fig. 1F) (Tab. 1). The stigma presents lobes that are generally free among each other, with the edges of each lobe slightly fringed (Fig. 2A). The ovules are ovate, with short chalazal region, due to the presence of a small appendix (Fig. 2B). At pre-anthesis, there is a progressive elongation of both androecium and gynoecium, which are included before this period (Fig. 1A).

Dyckia racinae presents flowers that are 1.4 to 1.6 cm long with a short pedicel during anthesis (Tab. 1) (Fig. 1C, G). The floral bract is approximately the same size as the sepals (Tab. 1). The calyx exceeds half the length of the corolla and presents ovate sepals that are slightly connate at the base and greenish in this region, with yellowed apices (Fig. 1C, G) (Tab. 1). The corolla has a wide opening (Fig. 1C), with petals that are 1.0 to 1.2 cm long, obtrulate, yellow and have attenuated and curved apices (Fig. 1C) (Tab. 1). The stamens are included, 0.9 to 1.0 cm long and connate up to 1.0 mm above the common tube with petals, which is just over 1.0 mm (Tab. 1). The connation tube extends to the median-superior height of the ovary (Fig. 1G). Each stamen bear a dorsifixed anther, 0.4 cm long on average, curved dorsally at maturity, with sporangia facing the gynoecium.

Table 1. Morphology and morphometry of floral parts, pedicel and bract of *Dyckia* species. SL: stamen length. AL: anther length. FL: filament length (including common tube with petals). FL/SL: mean percentage ratio between filament length and total stamen length. CON: connation height (including common tube with petals). CON/SL: mean percentage ratio between connation height and total stamen length. CT: common tube height. FW: filament width immediately above connation region. FW(AP): antepetalous filament width. FW (AS): antesepalous filament width. GL: gynoecium length. Codes and respective characters used in the phylogenetic analysis: **Bract** - Relative length: smaller than the sepals (0); same length as the sepals (1); larger than the flower (2); smaller than the pedicel (3). **Sepal** - Color: green (0); green at the base and yellow at the apex (1); yellow (2). Shape: carinate (0); ovate (1). Apex: pungent (0); obtuse (1). Relative size: smaller than half the corolla (0); larger than half the corolla (1). Indument: present only at the margin (0); present in the entire dorsal region (1); absent (2). **Petal** - Color: yellow (0). Shape: obtrulate (0); spatulate (1); ovate (2). Indument: absent (0); present at the margin (1). **Androecium** - Position relative to the corolla: exserted (0); included (1). Connation: free stamens above the common tube (0); connate stamens above the common tube (1); free stamens not adnate to the petals (2). **Anther** - curved dorsally (0); rectilinear (1). **Ovule** - Wing: reduced (0); visible (1); long caudate (2). **Style** - Size relative to the ovary: smaller (0); larger (1). **Stigma** - Lobes: 3-erect (0); joined in a spiral (1); laminate and joined in a spiral (2). Position relative to the corolla: included (0); exserted (1). Measured in centimeters, except for percentages. *For *D. selloa* only data from complete flowers were used.

		D. polyclada	*D. racinae*	*D. ibicuiensis*	*D. maritima* (Smith & Downs 1974)	*D. selloa** (Smith & Downs 1974)	*D. choristaminea* (Smith & Downs 1974)	*Encholirium horridum* L.B. Sm. (Forzza 2005)
Pedicel	Lenght	<0.1	0.1	0.5-0.7	-	-	-	1.0-1.5
Bract	Relative size	0	0	2	0	0	1	3
	Indument	1	0	1	1	0	1	0
Flower	Lenght	1.0	1.4-1.6	2.0-2.4	1.2	1.0-1.2	1.8-2.4	1.9
Sepal	Color	0	1	2	0	0	1	2
	Shape	1	1	0	1	1	1	1
	Apex	1	0	0	1	1/0	0	1/0
	Relative size	0	1	1	0	0	1	0
	Indument	1	0	1	1	-	-	2
Petal	Lenght	0.7-0.8	1.0-1.2	2.0	0.7	1.2	1.8	1.5-2.2
	Color	0	0	0	0	0	0	0
	Shape	1	0	0	1	1	0	2
	Indument	1	0	0	-	-	-	0
Androecium	Relative position of the stamen	0	1	1	0	1	1	0
	Connation	0	1	1	0	0	0	2
Anther	Curvature	1	0	0	1	1	0	1
	SL	0.8-1.0	0.9-1.1	1.4-1.8	1.2	-	-	1.5-2.2
	AL	0.25-0.3	0.4-0.45	0.4-0.5	0.3	0.25	-	0.7
	FL	0.5-0.7	0.6-0.7	1.0-1.5	0.9	-	-	-
	FL/SL(%)	65.0	55.0	74.0	75.0	-	-	-
	CON	0.03-0.05	0.21-0.3	0.7-1.0	-	-	-	-
	CON/SL (%)	5.0	25.5	56.5	-	-	-	-
	CT	0.05	0.15	0.35	-	-	-	-
	SW (AP)	0.04-0.05	0.17	0.25	-	-	-	-
	SW (AS)	0.04	0.09	0.1	-	-	-	-
Gynoecium	GL	1.0	0.8-1.1	1.3-1.4	1.2	-	1.6	1.5
Ovule	Wing	0	1	1	0	0	1	2
Style	Size relative to the ovary	1	0	0	1	1	0	1
Stigma	Lobes	0	1	1	0	0	1	2
	Relative position	1	0	0	1	1	0	1

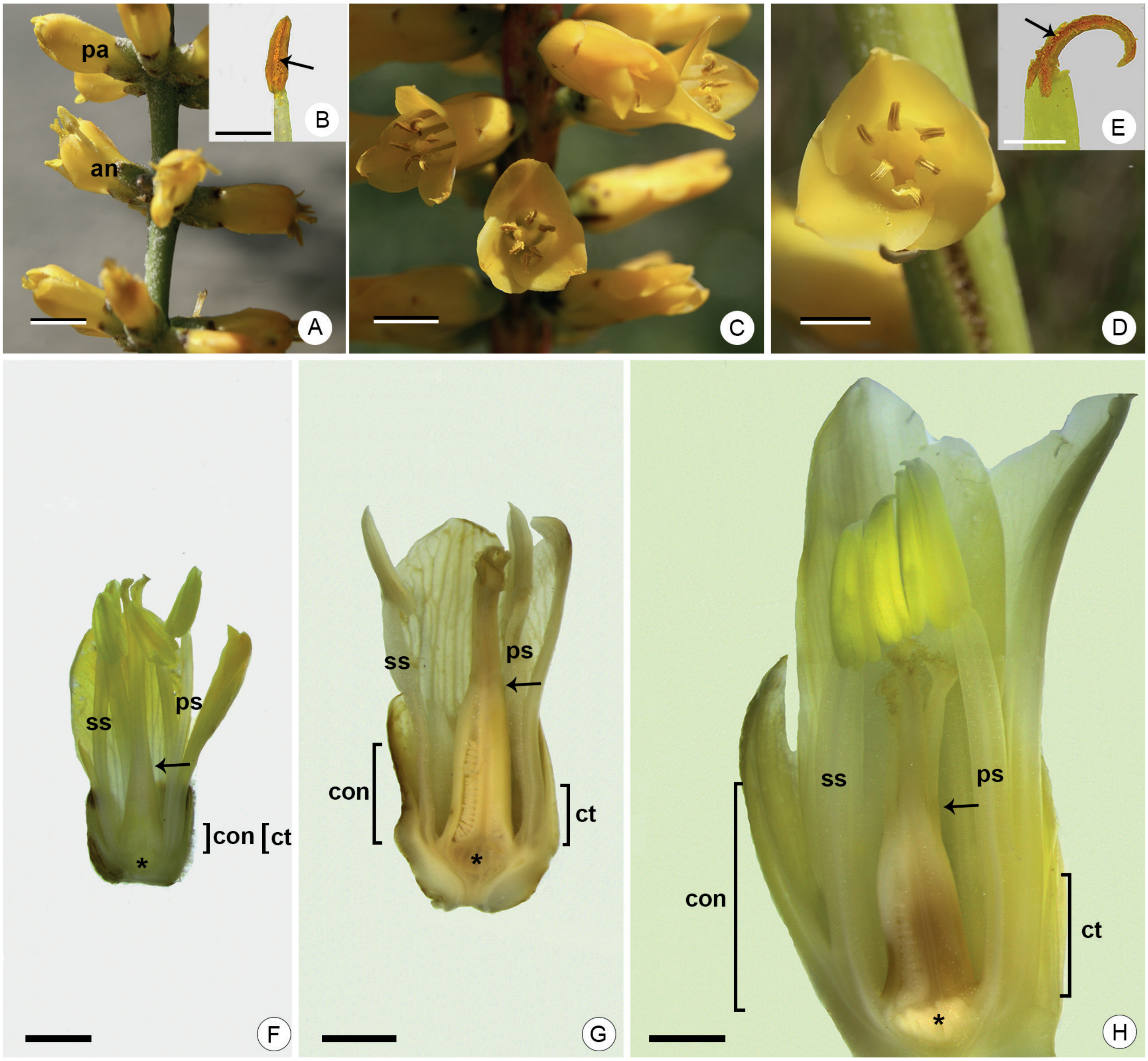

Figure 1. *Dyckia* flowers in natural environment and longitudinal section of fixed flowers. A, B, F: *D. polyclada*. C, G: *D. racinae*. D, E, H: *D. ibicuiensis*. A, C, D: flowers in natural environment. B, E: fresh anthers in dehiscence. Arrows indicate stomium rupture. F-H: fixed material, longitudinally sectioned flowers. *: septal nectary; arrows: edge of the ovarian region; **an**: flower in anthesis; **con**: stamen connation; **ct**: common tube with petals; **pa**: flower in pre-anthesis; **ps**: antepetalous stamen; **ss**: antesepalous stamen. Bars: A, C, D: 5 mm; B, E, F-H: 2 mm.

Due to the tubular structure of the base of the stamens, the anthers are positioned radially (Fig. 1C, G). The filaments are flattened dorsoventrally, with wider antepetalous filaments than antesepalous ones, and correspond to about 60 % of the total length of the stamen, including in this percentage the height of the common tube (Tab. 1). The gynoecium is included, with a style smaller than the ovary and stigma at about the same level as the anthers (Fig. 1G). The stigmatic lobes are united in a tight spiral, which is delimited by the fringed edges of each of the lobes (Fig. 2C). The ovules are discoid, with a thick outer integument and a prominent chalazal appendix, which forms a wing that narrows to the base of the funiculus (Fig. 2D).

Dyckia ibicuiensis presents flowers 2.0 to 2.4 cm long, with pedicels 0.5 to 0.7 cm in anthesis (Tab. 1). The bracts vary in size in different flowers and individuals, generally reaching or exceeding the length of the flower (Fig. 1D). The calyx exceeds half the length of the flower and is composed of yellowish sepals, slightly carinate, with a pungent apex and free above the base (Fig. 1H). The petals are 2.0 cm on average, are yellow, carinate or obtrulate and have divergent apices, forming angles of approximate 120° in relation to the gynoecium (Fig. 1D). The stamens are included, 1.4 to 1.8 cm long and connate 0.3 to 0.4 cm above the common

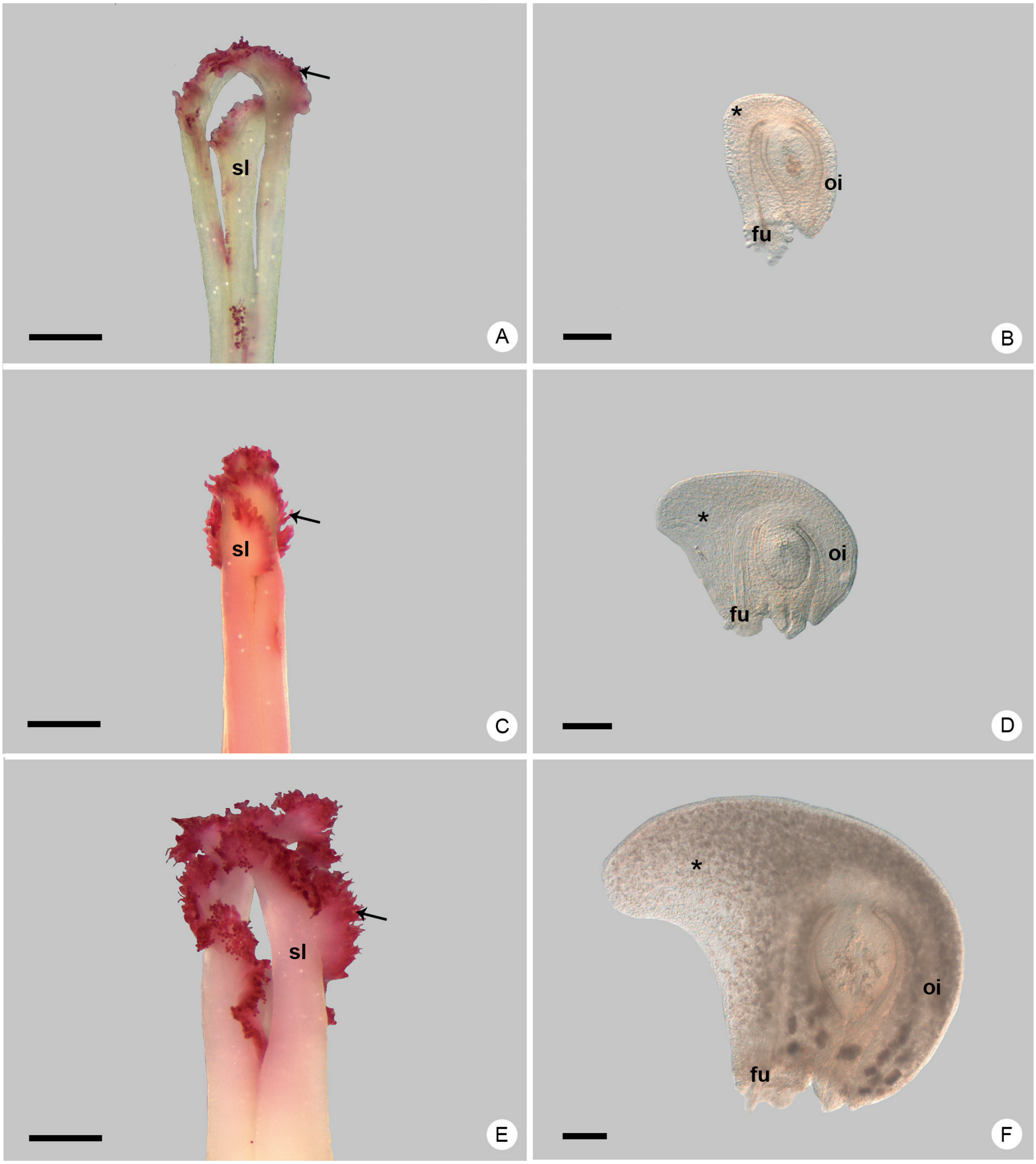

Figure 2. Stigmas and ovules of *Dyckia* species. A, B: *D. polyclada*. C, D: *D. racinae*. E, F: *D. ibicuiensis*. A, C, E: stigmas under stereomicroscopy. B, D, F: ovules in Differential Interference Contrast (DIC). *: chalazal appendix; **fu**: funiculus; **oi**: outer integument; **sl**: stigmatic lobe; arrows: edge of the stigmatic lobe. The stigmas are stained with Safranin. Bars: A, C, E: 500 μm; B, D, F: 100 μm.

tube with petals, which is long, measuring 0.3 cm. The conation tube extends beyond the height of the ovary (Fig. 1H). Each stamen has a dorsifixed anther, 0.4 cm long on average, curved dorsally at maturity, with sporangia facing the gynoecium (Fig. 1D, E). At anthesis, the stamens are positioned radially (Fig. 1D, H). The filaments are wide, flattened dorsoventrally and correspond to approximately 70 % of the total length of the stamen, including the common tube. The antepetalous filaments are wider than the antesepalous (Tab. 1). The gynoecium is included, with a stigma below the anthers (Fig. 1H). The stigmatic lobes are united in a spiral at maturity, which tends to remain

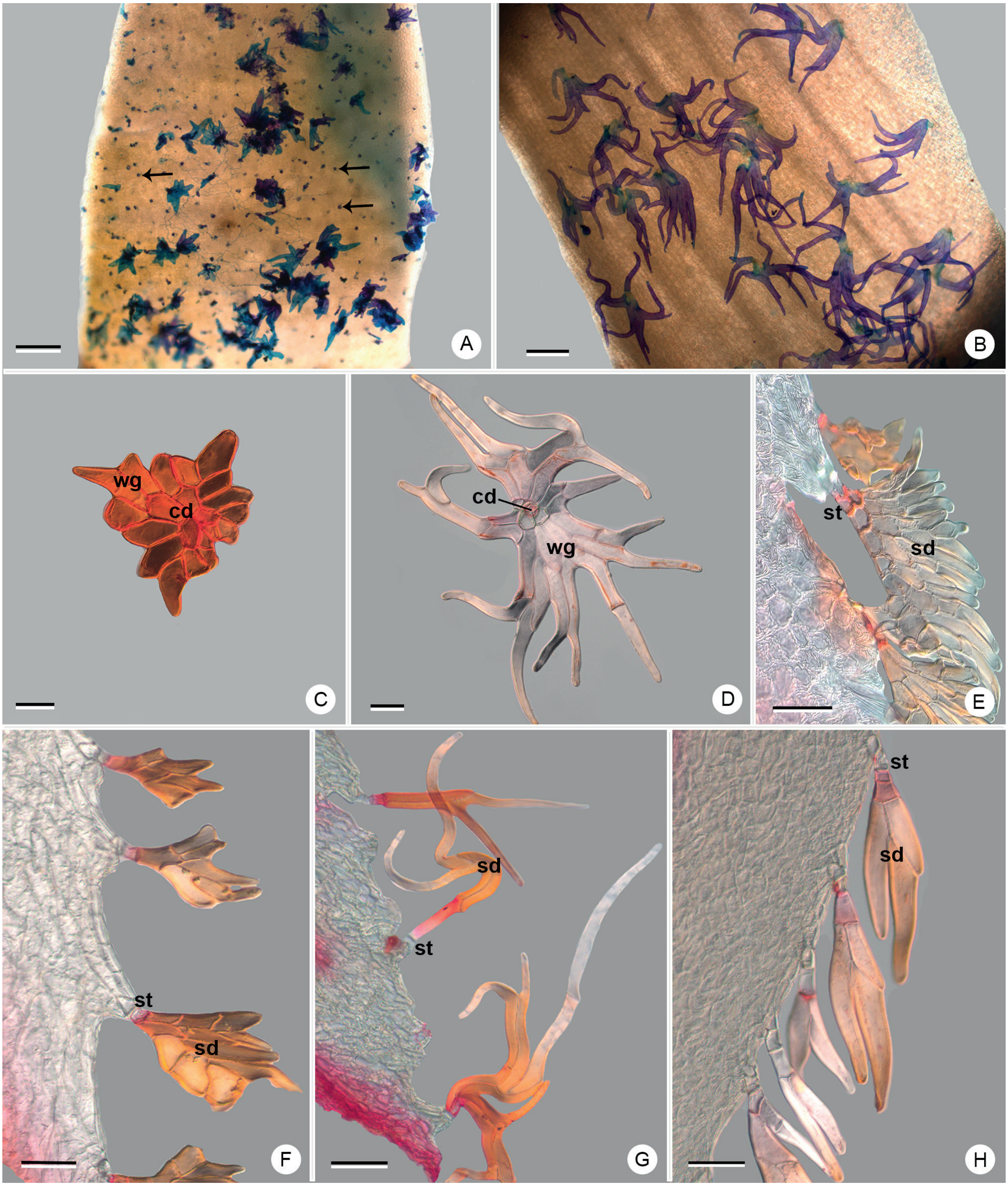

Figure 3. Peltate trichomes of the perianth in *Dyckia* species under Differential Interferential Contrast (DIC). A, C, E, F: *D. polyclada*. B, D, G: *D. ibicuiensis*. H: *D. racinae*. A, B: general aspect of the abaxial face of the sepal. Arrows indicate trichome insertion point. C, D: peltate trichomes from the sepal lamina. E: trichomes from the margins of the petal. F-H: trichomes from the margins of sepals. cd: central disc cells; st: stalk; sd: shield; wg: wing. The trichomes are stained with Toluidine Blue (A, B) and with Safranin (C-H). Bars: A, B: 200 µm; C, D: 20 µm; E-H: 50 µm.

loose at the basal region (Fig. 2E). In addition, the margins of the lobes are serrate, which constitutes a membranous fringe (Fig. 2E). The ovules are asymmetrical from the lateral view, with a voluminous thick outer integument and a wide appendix in the chalazal region, which forms a wing that corresponds to about half the width of the ovule (Fig. 2F).

Perianth trichomes

Peltate trichomes may occur on the peduncle, bracts, sepals and infrequently on the petals, in the species analyzed in this study. The gynoecium and androecium are typically glabrous (Fig. 3A-H). The peltate trichomes, when present on the flowers, may present dimorphism when considering the abaxial face of the sepal lamina relative to the margin of sepals and petals. On the sepal lamina, the trichomes are made up of a stalk perpendicular to the shield, which is formed by the central disc and the wing. In the margin trichomes, the stalk is inserted laterally to the shield, without an apparent central disc (Fig. 3C-H).

Dyckia polyclada presents indument on the peduncle and the floral bracts, as well as on the sepals and petals (Tab. 1). The peltate trichomes are densely distributed on the abaxial face of the sepal and are present on both the lamina and the margin of this organ (Fig. 3A, C, F). The trichomes of the lamina have a shield with an irregular contour, where the cells of the wing are always short and there is not a clear distinction between the edges of the central disc cells and the cells of the wing (Fig. 3C). The stalk is slightly eccentric and is inserted perpendicularly to the shield (Fig. 3C). The trichomes of the margin have a palmate shield with short cells, and the stalk is oriented parallel to the shield, which provides a flat organization, parallel to the abaxial face (Fig. 3F). The petals are glabrous on the lamina, but possess trichomes on the margin, similar to those of the sepals, however with a shield two to three times wider (Fig. 3E).

The peduncle and floral bracts of *D. ibicuiensis* are lepidote, as are the sepals. Their trichomes are sparsely distributed and are located both on the lamina (Fig. 3B, D) and margin (Fig. 3G). The trichomes of the lamina have an irregular contour and are two to three times larger compared to those of *D. polyclada* (Fig. 3B), with a distinction between cells of the central disc, which usually number four and are isodiametric, and the wing cells, which vary in number and are always elongated (Fig. 3D). The stalk is inserted eccentrically and is perpendicular to the cells of the shield. The trichomes of the margin are quite elongated, some with several millimeters (Fig. 3G). The shield typically presents elongated cells, oriented in dichotomous poles, infrequently with a single cell forming the shield. In these trichomes, the stalk is oriented on the same plane of the shield cells, parallel to the abaxial face (Fig. 3G).

Dyckia racinae presents glabrous peduncle and floral bracts (Tab. 1) as does the lamina of the sepals. However, trichomes can be observed on the margin of this organ (Fig. 3H). These possess a shield with elongated cells that are short compared to *D. ibicuiensis*, and generally not dichotomous, despite the shields often being formed by a single elongated cell, or even by two divergent cells (Fig. 3H). The stalk is oriented on the same plane of the shield, parallel to the abaxial face (Fig. 3H).

Phylogenetic analysis

Three equally parsimonious trees were produced with 32 steps, one of which was chosen for this paper. The consistency index was 0.813 and the retention index was 0.769. Most of the clades presented bootstrap support, although only a few presented good support (*D. maritma* + *D. polyclada* = 80; *D. choristaminea* + *D. racinae* + *D. ibicuiensis* = 95) (Fig. 4). The position of *D. selloa* cannot be considered well resolved. Its cluster with the clade [*D. choristaminea* + *D. racinae* + *D. ibicuiensis*] presented low support (54), as well as low frequency among the most parsimonious trees (Fig. 4). The wings of ovules, the morphology of stigmatic lobes, stamen connation and shape of the petals are among the most phylogenetically informative characters, being mapped using one of the parsimonious trees (Fig. 5).

Discussion

The flowers in *Dyckia* are trimerous and heterochlamydeous, with imbricate sepals free above the base and smaller than the petals, which are also free and imbricate. The androecium is composed of two whorls of stamens, three antesepalous and three antepetalous, which form a typical common tube with the corolla, generally connate at the base, and either free or connate above the common tube with petals (Smith & Downs 1974). In older reviews and descriptions (Smith & Downs 1974; Smith 1988; 1989) there are frequent contradictions related to the two whorls of stamens in flowers of *Dyckia*, especially regarding adnation. Therefore, we emphasize that in the species analyzed here both whorls are adnate to the petals, at least at the base, but the antesepalous stamens are also adnate to the sepals, an important detail of the floral architecture, used in more recent descriptions (Büneker *et al*. 2015; 2016). The gynoecium has a conduplicate-spiral stigma (Brown & Gilmartin 1989) and normally a superior ovary or, in some species, semi-inferior (Bernardello *et al*. 1991; Forzza 2005). In addition, there is an infralocular nectary in the region of the receptacle, common to the genus (Bernardello *et al*. 1991). In relation to these general floral features, the species of this study confirm what is typically expected for *Dyckia*, however they present morphological details that allow them to be differentiated and relate to other species of the genus.

The most evident and least variable morphological and morphometric differences in the flower among the three

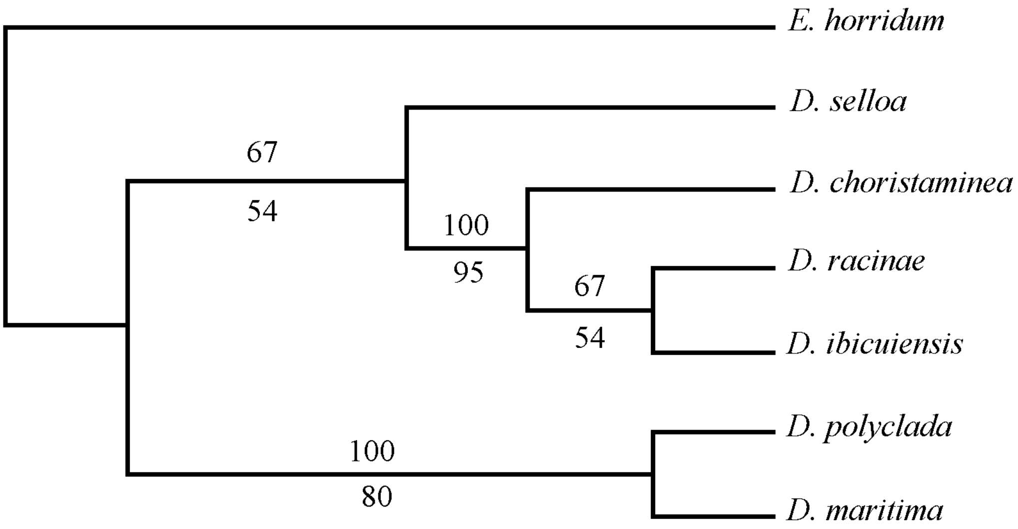

Figure 4. One of the three most parsimonious trees inferred for morphological data. Majority rule consensus is presented above the branches, bootstrap values are presented below the branches. Values lower than 50 are not presented.

species analyzed are related to the size of the flower and the conformations of the corolla, androecium and gynoecium. *Dyckia polyclada* has small flowers (1.0 cm), with a narrow opening of the corolla. This structure is possible not only due to the absence of dorsal curvature in the petals, but also due to the short common tube with petals, the thin filaments, and the absence of connation above this tube. On the other hand, *D. ibicuiensis* presents large flowers (2.0 – 2.4 cm), with a wide opening of the corolla due to the curvature of the petals and the stamens that are positioned radially, which is possible due to the tubular conformation of the filaments, in turn due to the elevated common tube with petals, and to the great extension of connation above the tube, where the filaments are wider and more dimorphic in each stamen whorl, unlike *D. polyclada*. *D. racinae* presents intermediate measurements relative to the other two species, despite the general structure of the flower, small compared to other dyckias, resembling that of *D. ibicuiensis*. The radial arrangement of the stamens reported for these species, as well as characters of the anthers, such as curvature and position of the sporangia, has been dealt with in other studies involving *Dyckia* species (Rogalski *et al*. 2009), and are related to a system of self-pollination. Thus, while there are no similar studies in species related to *D. polyclada*, there may be peculiarities in the events of pollination in this species, due to occlusion of the corolla, as well as the distinct configuration of the androecium, features that may allow more inferences, not only of a reproductive nature, but also taxonomic, if assessed in other species. As for the relative position of the stamens and stigma in relation to the corolla, the morphometric data of the present study suggest that the exserted stigma in *D. polyclada* is due to the long style, however the exsertion of the stamens is not directly related to the relative size of the filament, since *D. ibicuiensis* presented the greatest values for this measure, but has included stamens, as was also observed by Carvalho *et al*. (2016). We underline, however, that the exsertion in *D. polyclada* occurs after anthesis, due to the apparent increase in the lengths of the androecium and gynoecium, a common phenomenon in angiosperms, which can occur by cellular elongation, or even by the presence of intercalary meristems at the base of these organs (Greyson 1994). In the other two species, this late elongation may not occur, or may be synchronized with the growth of the corolla, which demonstrates the need for studies on the development of floral parts in the post-anthesis period in these species, as well as in *D. polyclada*, in order to infer relationships between differential growth of such parts and the final structure of the flower.

Phylogenetic analyses, both with morphological (Forzza 2001) and molecular data (Krapp *et al*. 2014), have confirmed the monophyly of *Dyckia*, while also reinforcing the paraphyly of *Encholirium*, its sister group. Krapp *et al*. (2014) discuss possible geographical groupings, among other hypotheses, for the infrageneric relations between species of *Dyckia*. However, according to the same author, unresolved clades and polytomies make it more difficult to establish reliable groupings and the cause of this difficulty has been attributed to misidentification of specimens sampled, as well as problems with the specificity of molecular markers used, as well as by the probable recent radiation of the group. Pinangé *et al*. (2016), in a recent paper, discussed probable infrageneric groupings in *Dyckia*, and mapped some important morphological characters using a polymorphic molecular marker. The authors corroborate the hypothesis of geographical clades, but emphasize the importance of additional morphological analyzes for poorly resolved subclades, especially of species from southern

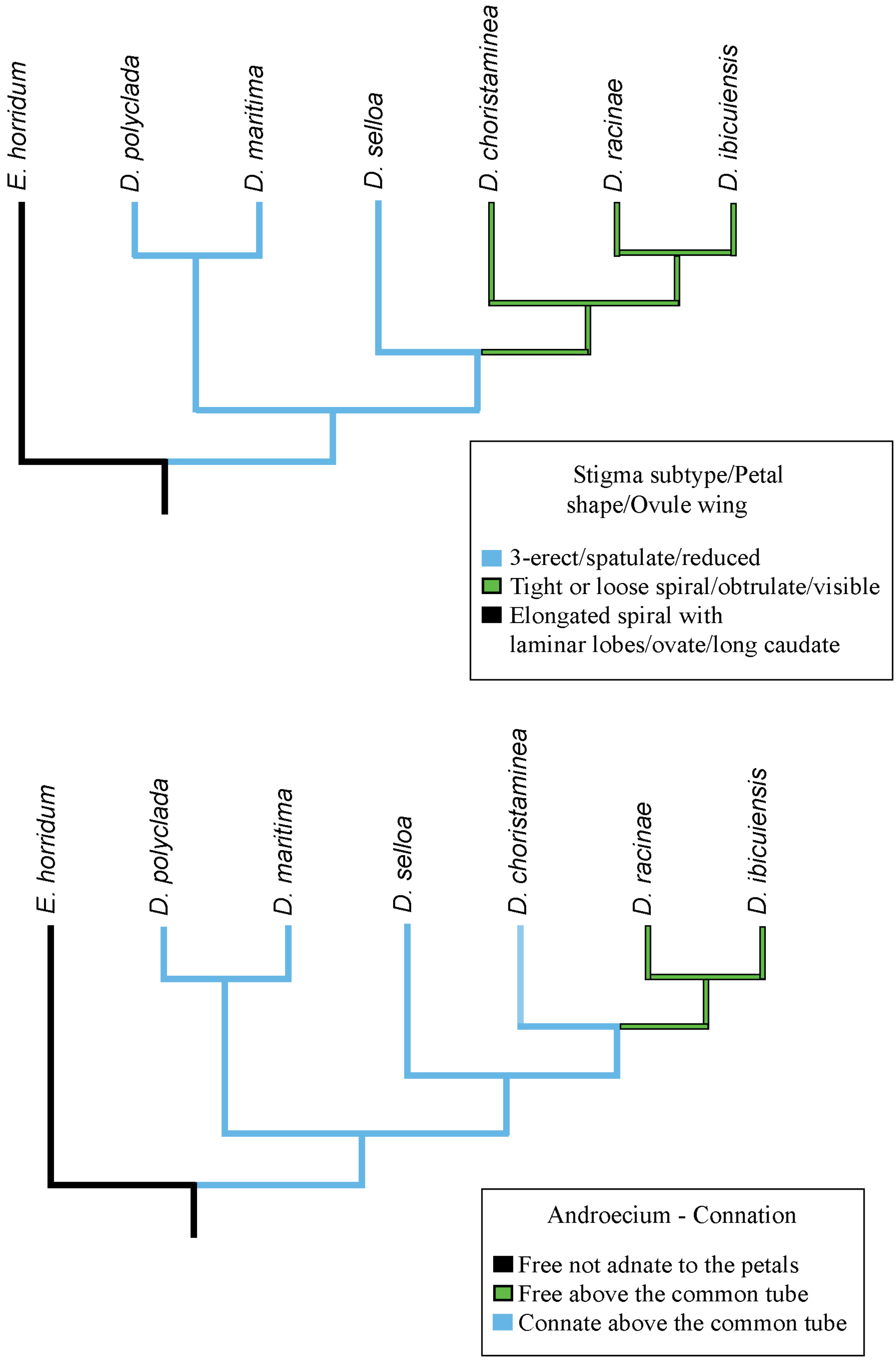

Figure 5. Some of the main phylogenetically informative morphological characters mapped in one of the three most parsimonious trees inferred.

Brazil. The parsimonious tree proposed in the present study, although encompassing a small number of species, allows the circumscription of clades with a certain degree of consistency, relatively well supported by most morphological characters used in our analysis. Among these characters, some can be highlighted because they are in agreement with some still informal hypotheses of groupings in *Dyckia*, besides being an alternative to complement molecular analyses in the future, if used with a larger number of species.

In the original description of *D. polyclada* (Smith 1989), in which there are few diagnostic data about the floral components, this species is compared to *D. selloa*, whose floral and inflorescence features, quite distinct from other dyckias, initially led to the proposal of a separate genus, *Prionophyllum*, which would also include *D. maritima*. Indeed, there are great similarities between individuals of *D. polyclada* and *D. selloa*, especially regarding the individual size, the small size of the flower, and the androecium with free stamens and gynoecium with exserted stigma. However, it is to *D. maritima* that this species possesses greater affinity, especially in floral features, which feature multiple overlays that led to their grouping in this study. *Dyckia racinae*, which was also compared to *D. selloa* in its diagnosis (Smith 1988), resembles it only macromorphologically and floral characters are clearly distinct, especially those related to the corolla, the androecium and gynoecium. Apparently, this species, as well as *D. ibicuiensis*, represents a morphologically distinct group, in conjunction with *D. choristaminea* and other *Dyckia* species not analyzed in this study, while *D. selloa*, despite being morphologically similar to *D. maritima* and *D. polyclada*, presented an uncertain position, perhaps due to the absence of several morphological data, a similar condition pointed out by Pinangé *et al*. (2016) as for *Dyckia hebdingii*, a species morphologically related to *D. selloa* and *D. maritima* with an uncertain position due to low statistical support. Although not originally included in *Pryonophyllum*, this species, like *D. polyclada*, can be grouped with *D. maritima* in the future, with more investigations of morphological characters and evaluation of its taxonomic utility and correlation with molecular data.

The androecium, which in this study was found to be one of the most important whorls for comparative purposes, was treated in detail by Carvalho *et al*. (2016), in an anatomical and morphological analysis. Among the most important features described by the authors, the different types of fibrous thickenings found in endothecium are important as they allowed the establishment of a relationship between *D. racinae* and *D. ibicuiensis*. Other important features are the arrangement of the anthers in relation to the gynoecium and their curvature, provided by the connation of the filaments, and anatomical features of the connective. In our analysis, the connation of the stamens was found to be of comparative value, since free stamens are observed in species that are very distinct morphologically, which indicates a possible independent evolution of this character in the group. Similarly, the character state "stamens connate above the common tube", according to Pinangé *et al*. (2016), would have evolved several times in the genus. In fact, the connation of filaments above the common tube, present in *D. racinae* and *D. ibicuiensis* reach the extreme in *D. tubifilamentosa*, a species from Northeastern Brazil, probably unrelated to such species, or any other dyckias (Wanderley *et al*. 2014). Since the connate stamens is a feature that distinguish *Dyckia* from *Encholirium* (a closely related genus, which usually has free stamens) (Forzza 2005; Krapp *et al*. 2014), an analysis of this whorl in a larger number of species may contribute to both the understanding of the relationships between the two genera, as well as to inferences about the evolution of this whorl in *Dyckia*.

One of the first propositions of infrageneric groupings in *Dyckia*, albeit artificial, when considering the small number of features evaluated, was the result of a morphological analysis of seeds (Strehl & Beheregaray 2006), which allowed the proposition of two groups: the first, with species of narrow-ovate seeds and little developed chalazal appendix was called "maritima complex", which would include *D. maritima, D. selloa* and *D. retardata*, and a second group that would be subdivided, leading to two other complexes: the "leptostachya complex", with wide-ovate seeds and a large chalazal appendix, including *D. ibicuiensis*, *D. choristaminea*, *D. distachya*, *D. leptostachya* and *D. reitzii*, and the "remotiflora complex", with subcircular seeds, including *D. elisabethae* and *D. remotiflora*. *Dyckia ibicuiensis*, which has already been related to *D. choristaminea* by Strehl & Beheregaray (2006) in the "leptostachya complex", helps to corroborate the possibility of taxonomic use of ovules in *Dyckia*, since they maintain the most important morphological features for comparative purposes after maturity, as the relative size of the chalazal appendix. Since the ovule maintains its basic morphology upon maturation, with the exception of the progressive increase in volume, it is possible to include *D. polyclada* in the "maritima complex", not only based on this feature, but also, as previously discussed, due to a series of floral similarities to species of the old genus *Prionophyllum*, also part of this complex. As for *D. racinae*, the subcircular shape of the ovule allows it to be related to the "remotiflora complex", however, we opt to consider in our comparisons only the presence of a developed wing in the ovule as the main feature.

As for the stigma, Brown & Gilmartin (1989) classified it as a conduplicate-spiral type in *Dyckia*, but they emphasize the possibility of subclassifications, especially at the species level. In the present study, it is important to note the stigma with free lobes in anthesis observed in *D. polyclada*. Brown & Gilmartin (1984) observed similar subtypes in *D. maritima* and *D. selloa* (treated as *Prionophyllum*), called 3-erect. *D. ibicuiensis* and *D. racinae* also presented singularities in their stigma morphology. Among the most evident morphological characters, we can highlight the size of the lobes and the degree of torsion between them, as well as the extension and ornamentation of the fringe on the margin of each lobe, features that have already been found to be taxonomically useful, especially at the species level (Brown & Gilmartin 1989). Although it is not usually used for phylogenetic hypotheses, the stigma is useful for comparative purposes and was phylogenetically representative in the present analysis. In addition, as an example of the plasticity of this organ in the genus, we can cite *D. tubifilamentosa*, a recently described species, whose elongated stigma region with juxtaposed lobes, among other floral features, initially led to its classification as a genus distinct from *Dyckia*, possibly related to *Encholirium* (Wanderley *et al.* 2014), which underlines the possibility of taxonomic use of this organ, not only at the species level, but also in taxonomic analyses at the genus level.

The perianth, mainly in terms of morphometry and petal and sepal trichome morphology, was found to be important in the species comparison. The petal morphology was one of the main comparative characters, not only phylogenetically, but also because of the importance of this organ in the final organization of the stamens. In addition, the lengths and relative sizes of perianth parts were noteworthy. Although the peltate trichomes of the perianth were not phylogenetically informative, probably due to a lack of data, they were effective in the characterization of the species analyzed, given the complexity and variability of their constituents. The peltate trichomes are of great ecological and physiological importance in Bromeliaceae, besides being considered synapomorphic in the family (Benzing 1976; Benzing 2000) and have been analyzed especially in nomophyles, where they may take on the function of absorbing water and nutrients, aiding in the dissipation of solar radiation (Benzing *et al.* 1985), or even acting to protect against pathogens (Pierce *et al.* 2001). These trichomes, sometimes treated as foliar scales (Varadarajan & Gilmartin 1987), are made up of a stalk that supports the shield, which is considered more important from a comparative point of view (Benzing 2000). In addition, the distribution and density of trichomes on the foliar surface, in addition to their structure, are important characters in the delimitation of taxa (Proença & Sajo 2007). Pitcairnioideae is characterized by trichomes whose shield may be radially symmetric or asymmetric, formed by one to four cells in the central disc, and either with or without an evident delimitation between these and the wing cells (Varadarajan & Gilmartin 1987). Despite being well documented in the literature, especially in taxonomic studies, the peltate trichomes of nomophyles have been the main focus of morphological comparative analyses and, to our knowledge, there are no studies specifically focusing on the indument of reproductive organs in Bromeliaceae. In *Dyckia*, the presence of indument in the peduncle, bracts and sepals is important in the characterization of some species (Smith & Downs 1974), and even for the proposition of infrageneric groupings (Büneker *et al.* 2016), although details of the constitution of this indument are generally neglected. In the species analyzed here, in addition to the presence of indument in the bracts and different floral parts, there are details of the structure of the peltate trichomes, mainly in the cells of the shield that are useful in the characterization of species, of note, the presence of polymorphic trichomes in the perianth, which were previously unknown in *Dyckia*. It can thus be seen that, besides evaluating the presence of induments in different floral and extrafloral parts, the independent structural evaluation of different regions of these organs may provide important data for species characterization. In addition, we can emphasize that the peltate trichomes of the perianth found on the margins of sepals and petals are structurally different from those commonly found in nomophyles of *Dyckia* and their production is unrelated to the presence of indument in the lamina, which makes them useful for comparative purposes when they are present, even in species with apparently glabrous flowers, such as *D. racinae*. Moreover, the perianth trichomes, common to several *Dyckia* species, are rare in *Encholirium*, with the exception of two species (Forzza 2005), which demonstrates its usefulness not only in species characterization but also in the expansion of characters that delimit the genus.

The present study provides useful morphological information for the characterization of rare *Dyckia* species, whose brief descriptions with few details have caused difficulties in the interpretation and survey of potential characters for taxonomic use. Among the most important characters, we can highlight those related to the morphometry of individual floral parts, especially related to the corolla and androecium, the relative size of these parts, the differential morphology of stigmas and ovules. In addition, we underline the importance of the indument and structure of peltate trichomes of the perianth, a whorl which has been widely neglected in structural studies on the genus. With regard to the phylogenetic analysis, given the reduced number of species analyzed, greater inferences about infrageneric groupings require a broader analysis. Nevertheless, the two most parsimonious groups proposed here are in agreement with previous hypotheses of relationship, demonstrating the usefulness of the characters found here in future studies. Comparatively, we consider *D. polyclada* similar to the species treated by Strehl & Behregaray (2006) as the "maritima complex", especially *D. maritima*, with which it shares a series of features discussed in the present study. The general floral morphology of *D. ibicuiensis* appears to be similar to several species of *Dyckia* included in the other two complexes proposed by Strehl & Beheregaray (2006), however, it is necessary to evaluate a larger number of species to make more robust inferences of relations, as well as to understand the apparently intermediate characters of *D. racinae*. Finally, we propose that the morphological data dealt with here be used widely in *Dyckia*, both in the description of new species and in the expansion of distinctive features of the genus or in the proposition of infrageneric groupings, as they deal with the whole flower and are viable for use in phylogenetic analyses.

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