Dyckia racinae L. B. Sm. (*Bromeliaceae*): morphological description emphasizing the reproductive structures[1]

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ABSTRACT – This study presents an analysis of the external morphology and anatomy, especially of the micromorphology of reproductive organs that are important for characterizing *Dyckia racinae* L.B.Sm. The presence of a parietal U-shaped thickening in the endothecium and in the connective differ from other *Dyckia* species. Characteristics of pollen grains and ovules, indicated by micromorphology of the sporoderm and structure of the chalazal appendix, respectively, are similar to other species, and useful for characterizing the genus. Preferences for rocky soils, besides leaf characteristics and inflorescence structure, approximate *D. racinae* to *D. cabrerae* Smith & Reitz in the main dichotomous keys for the genus. Considering that *Dyckia racinae* is endemic in Rio Grande do Sul, and that the original description of the species was proposed based on a single cultivated individual, it is clear that the characteristics described in the present study, based on individual species analyzed in their natural environment, are important botanical contributions.

Keywords: anther, endemic species, ovule, taxonomy

RESUMO – ***Dyckia racinae*** **L. B. Sm. (*Bromeliaceae*): descrição morfológica enfatizando as estruturas reprodutivas.** Este trabalho apresenta uma análise da morfologia externa, interna e, principalmente, da micromorfologia de órgãos reprodutivos, importantes para a caracterização de *Dyckia racinae* L.B.Sm. A presença de espessamentos parietais em U no endotécio e no conectivo difere de outras espécies de *Dyckia Schult.* Características dos grãos de pólen e dos óvulos, como micromorfologia da esporoderme e estrutura do apêndice calazal, respectivamente, são similares a outras espécies, sendo úteis para a caracterização do gênero. Preferências por solos pedregosos, além de características das folhas e estrutura da inflorescência, permitem aproximar *D. racinae* a *D. cabrerae* Smith & Reitz, nas principais chaves analíticas para o gênero. Considerando-se que *Dyckia racinae* é endêmica no Rio Grande do Sul, e que a descrição original da espécie foi proposta com base em um único indivíduo em cultivo, entende-se que as características descritas no presente estudo, com base em indivíduos analisados em seu ambiente natural, figuram como importantes contribuições botânicas.

Palavras-chave: antera, espécie endêmica, óvulo, taxonomia

INTRODUCTION

Consisting of approximately 58 genera (Givnish *et al.* 2011), *Bromeliaceae* is considered one of the most important neotropical families (Benzing 2000). However, there are still taxa with a significant number of indeterminate species (Benzing 2000), among which we can cite genus *Dyckia* Schult. f. (Martinelli *et al.* 2008), that belongs to the subfamily *Pitcairnioideae* (Smith & Downs 1974, Givnish *et al*. 2011). The lack of taxonomically useful traits (Leme *et al*. 2012), determined by natural causes, as recent radiation of *Dyckia* (Krapp & Weising 2011, Krapp *et al*. 2014), or by methodological problems, such as incomplete botanical material in herbaria (Smith & Downs 1974), imprecise field information, few studies based on live material (Krapp *et al*. 2014) or studies based on material that is being cultivated (Smith 1988), explain the high rate of inaccurate identifications found for this genus.

The morphology of the reproductive organs can be a major source of data for classification at several infrafamily levels. Studies on the morphological characterization of the stigma (Brown & Gilmartin 1984, 1989), septal nectaries (Varadarajan & Brown 1988), and petal appendages (Brown & Terry 1992) in *Bromeliaceae,* show that these traits have great taxonomic potential.

The description of the structure and processes that involve the formation of the sporangia and the gametophyte can be applied in phylogenetic and taxonomic analyses (Endress 2005, 2011), where the characterization of the microsporangium and pollen grain (Furness & Rudall 1999, 2001) and the carpel structure and the megasporangium, when used jointly, may become potentially informative (Fagundes & Mariath 2014). However, in a great number of genera of *Bromeliaceae*, information about the anatomy of reproductive organs and embryology is scarce and incomplete, and *Dyckia* may be included in this context (Furness & Rudall 1999, 2001, Fagundes & Mariath 2010, 2014).

Dyckia racinae L.B.Sm. is an endemic Brazilian species, whose distribution is limited to the Pampa biome in Rio Grande do Sul, state of Southern Brazil (Forzza *et al*. 2014), and twenty-five years after it was collected for the first time, in 1988, it was recorded again in the same state. The fact that no other characterization was found for *D. racinae*, besides the description by Smith (1988), based on cultivated material from Rio Grande do Sul state, make the results described here an important contribution.

The purpose of this study was to characterize *Dyckia racinae*, emphasizing the reproductive structures, contributing to extending its morphological description and adding information to the genus taxonomy. Besides, these data are supplied on the basis of the location of the species in Rio Grande do Sul, and also on general aspects about the natural environment in which it occurs.

MATERIAL AND METHODS

The specimens of *Dyckia racinae* were collected in the municipality of São Pedro do Sul, RS, Brazil, at the following geographical coordinates: 29° 35' 22,2'' S and 54° 49' 49,4'' W (Figs. 1, 2). The collection location was open, with no shading, with shallow and stony soil. The voucher is deposited in the SMDB Herbarium under accession number 13840.

According to Moreno (1961), the climate of this region, using the Köppen classification, is the subtropical type Cfa with rainfall well distributed throughout the year, mean temperatures above 22°C in the warmest month and less than 18°C in the coldest month, when frost may frequently occur, especially at higher altitudes. Relative humidity of the air varies from 75-85% during the year. The works by Reitz (1983) and Smith & Downs (1974) provided a basis to select the morphological traits evaluated in this study.

Dyckia racinae flowers, at different stages of development, were analyzed under light microscopy and fixed in 1% glutaraldehyde and 4% formaldehyde in sodium phosphate 0.1M pH 7.2 buffer (Gabriel 1982, McDowell & Trump 1976), to which Tween20 2ml/L was added (adapted from Freudenstein *et al*. 2002). After fixing, the material was submitted to a vacuum, followed by washing in the same fixative buffer, washing in distilled water, washing in an aqueous solution with 2ml/L of Tween20, followed by dehydration in an ethylic series and later pre-infiltrated into 2-hydroxyethyl methacrylate (HEMA) and absolute ethanol followed by infiltration in HEMA. Thereafter, samples were placed in embedding moulds containing HEMA until polymerization occurs (Gerrits & Smid 1983).

Sections 3µm-thick were made on a Leica RM2245 rotary microtome, stained with Toluidine Blue O (0.05% pH 4.4) in sodium benzoate buffer (Feder & O'Brien 1968). In order to detect insoluble polysaccharides, a histochemical test was performed using the Periodic Acid-Schiff's reaction (PAS)

(O'Brien & McCully 1981). The analyses and observations of photomicrographs of the slides were performed under a brightfield Leica DM2000 light microscope. For analysis and the photographic record of the complete flower and dissected floral organs, already fixed flowers were used (as previously described) and dehydrated with ethanol 70ºG, and then stored in a liquid medium. The analysis, dissection and photographic record were performed under a Leica M80 stereoscopic microscope. For both microscopes mentioned, the photographic records were performed through the Leica DFC 295 digital capture system.

For analysis by scanning electron microscopy (SEM), the material was processed according to Gersterberger & Leins (1978) and submitted to critical point drying using BAL-TEC, CPD 030 equipment. Next, the samples were mounted on aluminum stubs and covered with gold in a BAL-TEC, SCD 050 sputter coater. Analysis and creation of electron micrographs were performed using a JEOL 6060 microscope at 20 kV. The procedures for processing and analysis of the botanical material under electron microscopy were performed at the Laboratory of Plant Anatomy and Electron Microscopy Center of the Federal University of Rio Grande do Sul.

RESULTS

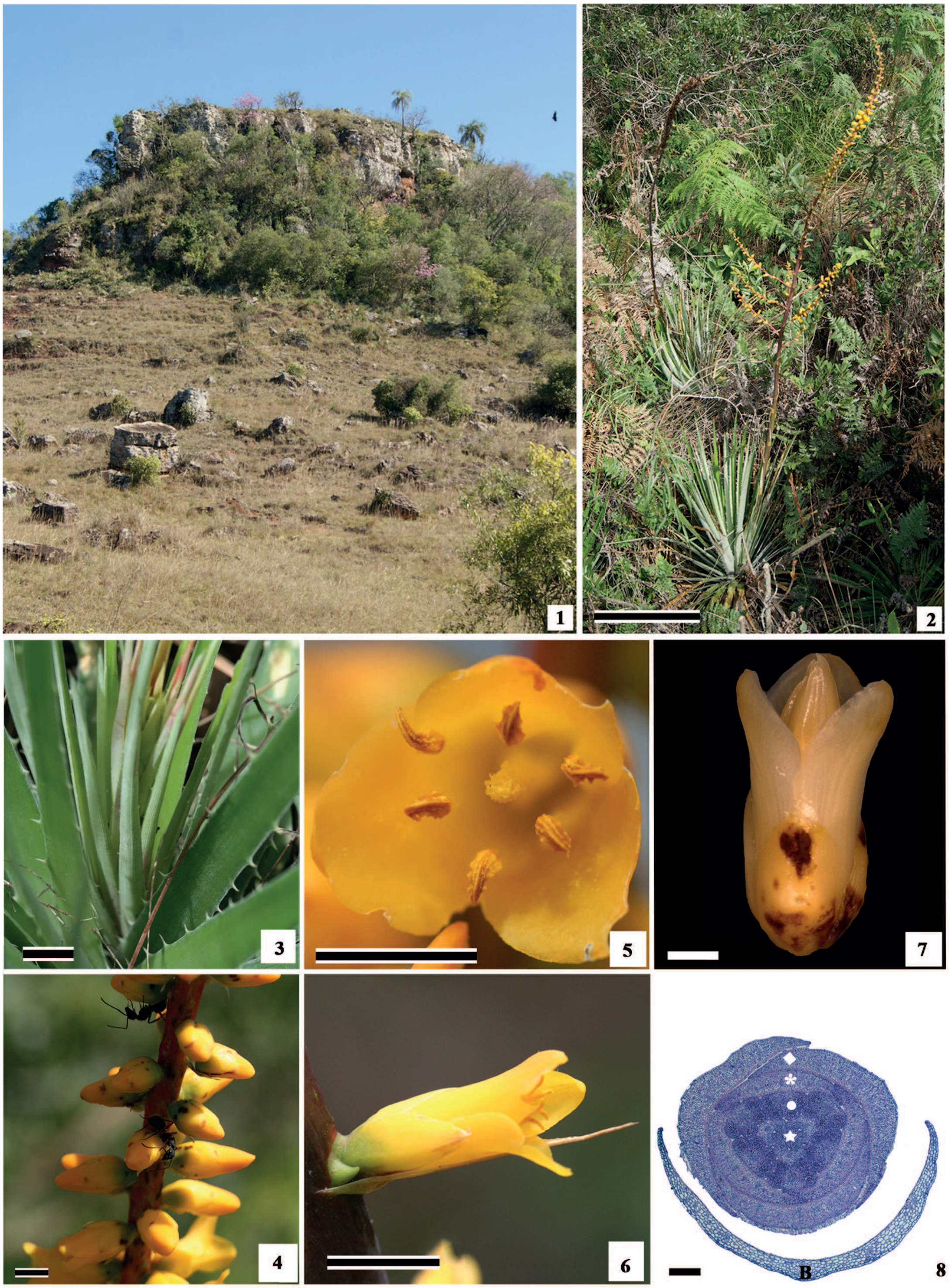
Morphological description of *D. racinae* emphasizing the reproductive structures

The analyzed plant presented at about 1.40 m tall, with leaves rosulated (Figs. 2, 3), basal 37.8-40.5 cm long x 1.3-1.6 cm wide x 0.18-0.27 cm thick. The median sized leaves were 34.3-35.8 cm x 1.3-1.4 cm x 0.16-0.21 cm, and the apical leaves were 29.2-33.3 cm x 1.2-1.3 cm x 0.12-0.14 cm, erect, borders with straight to antrorse thorns, abaxial surface densely lepidote, and adaxial surface glabrous to sparsely lepidote. The scape was slightly arched (Fig. 2), cylindrical, reddish, glabrous (Fig. 4); scape bracts were 3.8 x 0.5 cm long smaller than the glabrous, acuminate internodes.

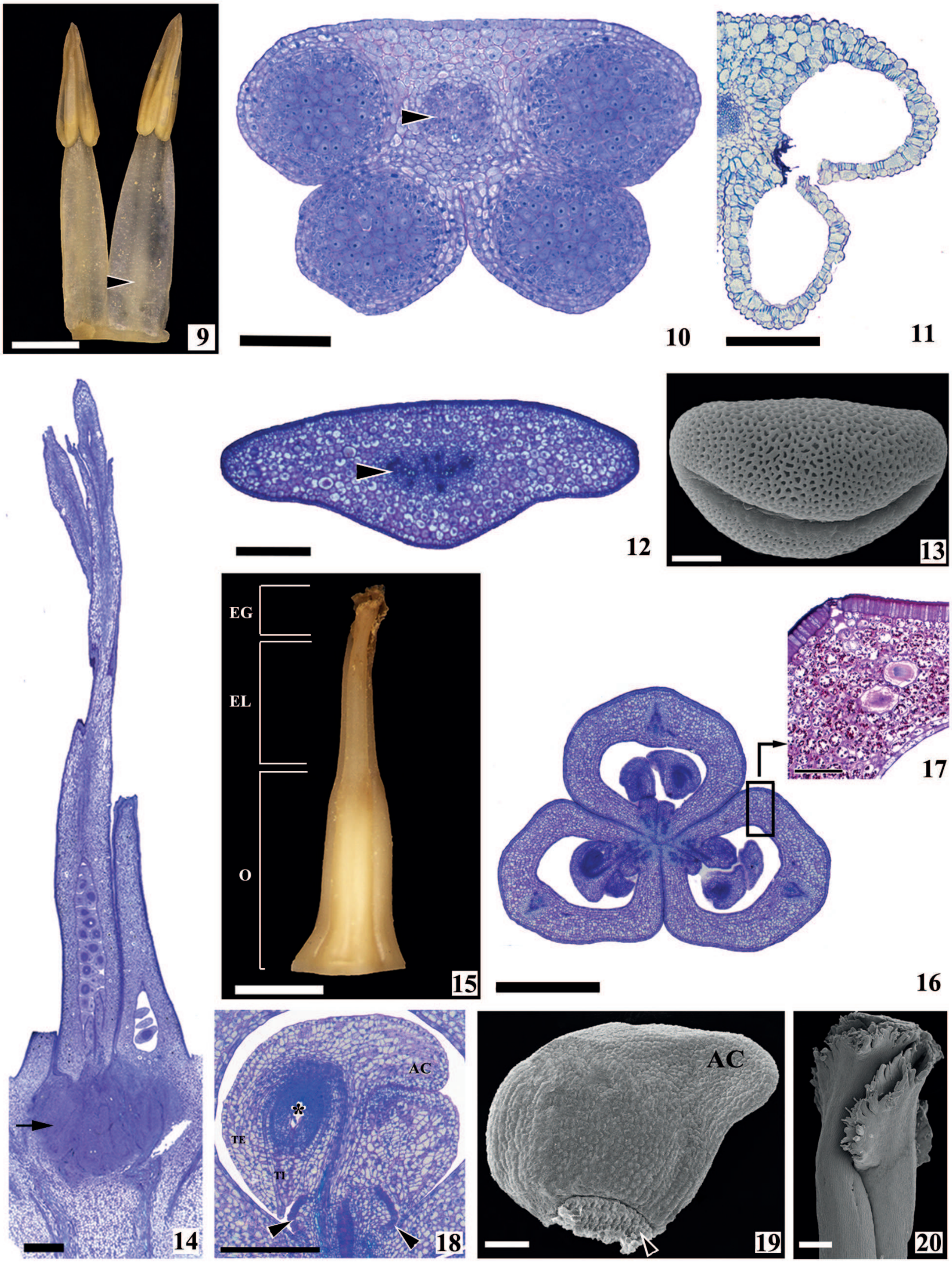
The inflorescences are compounds and lax, with few basal secondary branches (Fig. 2). Uniform flowers, perfect, trimerous, heterochlamydous (Figs. 5-8), patent, pedicels 0.1-0.2 x 0.15-0.25cm, persistent floral bracts, 0.6 x 0.2 cm long, smaller than the flower, reaching until mid-calyx, glabrous, acute. Free sepals, 6-7 x 3-4 mm, oval-lanceolate, obtuse, glabrous to sparsely pilous at the apex, slightly asymmetrical because of the lateral displacement of the apex, at most, until it is half the size of the petals (Figs. 6, 7), greenish when fresh (Fig. 4) and yellowish-brown when dry, with brown irregular-sized and shaped spots at the base (Fig. 7). Free petals, 12-13 x 5-6mm, obovate to spatulate, obtuse, glabrous, whitish margins and yellow-gold surface, fused at the base with the stamens forming the filament-tube to reach 0.05 cm high.

The androecium is slightly heterodynamous, with six stamens, free from each other above the common tube with the petals, included, laterally visible, with a maximum height equivalent to that of the corolla (Figs. 5-7); basifixed anthers, introrse, glabrous, opening by longitudinal slits, triangular, acute and curved at the apex, cordate at the base (Figs. 7, 9), tetrasporangiate (Fig. 10); filaments of the opposite petal stamens broader and slightly longer compared to the opposite sepals, a condition which confers a small difference in height between the stamens (Figs. 7, 9). The anatomy of the stamens is identical; the parenchymatous filament, with raphids throughout its length and a simple epidermis. A collateral vascular bundle occurs in the filament and in the anther (Figs. 9, 10, 12). Basic type sporangium formation, that gives rise to an epidermis, endothecium, two middle layers and tapetum (Fig. 10). In the mature anther, only the epidermis and endothecium with U-shaped thickening are present (Fig. 11). The U-shaped thickening develops in the connective cells (Fig. 11). The pollen grains are released in monads and with a single aperture (Fig. 13); in the sporoderm, the sexine is reticulated; the sexine pattern reduces abruptly closed to the ectoaperture, where it is not seen (Fig. 13).

The gynoecium is syncarpous and trimerous. The carpels are connate only in the ventral regions. Ovary is superior with three loculus, approximately 20 ovules per locule, and axillary placentation (Figs. 15, 17); style of different lengths in relation to the ovary (Fig. 14); triple stigma, with free margins and an conduplicate-spiral pattern (Fig. 20), with the presence of pluricellular and uniseriated glandular trichomes. Histologically, carpels are present in the ovarian region, simple epidermis on both faces, the mesophyll is parenchymatous and cells accumulate starch (Fig. 17). Each carpel has a dorsal vascular bundle and two ventral vascular bundles (Fig. 16). In the abaxial epidermis, the cells are radially elongated, with a thick external periclinal wall and dense cytoplasm (Fig.16). The abaxial epidermis of the ovary and style presents stomata (Fig. 17). The ventral epidermis cells are tangentially elongated, with thin walls (Fig. 17). The ovules are anatropous (Fig. 18), bitegmic, and crassinucellate (Fig.18), with a chalazal appendage (Figs. 18, 19); the micropyle is formed by the inner integument (Fig. 18); the obturator epidermis is composed by papillate cells, with a structure similar to a glandular epidermis (Fig. 18); the ovules occur in two series from the base to the apex of the locule (Fig. 14), which emerge alternately from the margins of each carpel and are fitted in by the chalazal appendage. Raphids occur in the ovary mesophyll (Fig. 16), in the style and in the stigma. A predominantly infralocular nectariferous tissue is observed (Fig. 14). These nectariferous tissues develop as a labyrinth cavity that reaches the septal region, with a multiple and glandular epidermis, with a well-developed vasculature.



Figs. 1-8. *Dyckia racinae* (*Bromeliaceae*). **1.** Hill in São Pedro do Sul, RS: overview of the collection site; **2.** General aspect of the plant with inflorescence; **3.** Detail of the basal leaves; **4.** Detail of the inflorescence: flowers in pre-anthesis; **5.** Flower in anthesis: view of stamens and stigma; **6.** Flower in anthesis: side view; **7.** Flower in anthesis: view of the spots on the sepals; **8.** Median cross section of the flower bud in pre-anthesis. Losangle (sepal), asterisk (petal), circle (stamen) and star (ovary). Bars = **Fig. 2** = 40 cm; **Figs. 3-6** = 9 mm; **Fig. 7** = 2 mm; **Fig. 8** = 200µm.



Figs. 9-20. *Dyckia racinae* (*Bromeliaceae*). **9.** Stamens, morphology and detail of the union between the filaments. Arrow head (vascular bundle); **10.** Cross section of anther, showing the four sporangia. Arrow head (vascular bundle); **11.** Cross section of the mature anther; **12.** Cross section of filament. Arrow head (vascular bundle); **13.** Mature pollen grain with a single aperture (SEM); **14**. Longitudinal section of entire gynoecium, arrow indicating nectary. **15**. Gynoecium: EG (stigma), EL (style) and O (ovary). **16**. Cross section of gynoecium. Frame delimiting the carpel leaf area. **17**. Detail of carpellar leaf, showing stomata in the epidermis; PAS positive starch and presence of raphids in the mesophyll;**18.** Longitudinal section of ovule. Arrow head (obturator), asterisk (gynophyte), AC (chalazal appendage), TE outer integument and TI (inner integument); **19.** Ovule (SEM). Arrow head (obturator), AC.; **20.** Stigma (SEM). Bars: **Figs. 9, 14** = 2 mm; **Fig. 17** = 500µm; **Figs. 11, 15, 20** = 200µm; **Figs. 10, 12, 16** = 100µm; **Fig. 19** = 50µm; **Fig. 18** = 20µm; **Fig. 13** = 5µm.

DISCUSSION

This study presents a contribution to the morphological characterization of *D. racinae*, with data related to the external morphology of the flower, anatomy, and embryology, including the micromorphology of the pollen grains and ovules, broadening the original description of the species supplied by Smith (1988). In this description, based on cultivated material, the author does not go into details on anatomy and embryology and only presents general vegetative and reproductive aspects of the species.

There is little information about floral and embryological anatomy in *Dyckia*, but works on *D. pseudococcinea* L.B.Sm. (Conceição *et al*. 2007, Mendes *et al*. 2012) and *D. hebdingii* L.B.Sm. (Sajo *et al*. 2005) about anthers and pollen grains can be cited for *D. maritima* Baker (Fagundes & Mariath 2010), emphasizing the fruits but also presenting information about the ovules. Thus, considering the present study, currently there is anatomical information regarding the sporangia and embryology only for four species in one genus with 159 species (Krapp *et al*. 2014, Leme *et al*. 2012).

In *Dyckia pseudococcinea* (Mendes *et al*. 2012), the anthers have characteristics similar to those presented in *D. racinae*. The exception, however, is the type of endothecium thickening, that is, U-shaped in *D. racinae*, and helical in *D. pseudococcinea* (Mendes *et al*. 2012) and in *Dyckia hebdingii* (Sajo *et al*. 2005). However, regarding the presence of U-shaped thickenings in the connective cells can only be seen in *D. racinae*. Considering that anthers are organs exposed to the environment, and that the species occur in different environments, *D. pseudococcinea* being typical of the Atlantic Coast restingas (Martinelli *et al*. 2008, Mendes *et al*. 2012), and *D. racinae* found in the stony soil in the Pampa biome (Forzza *et al*. 2014), it is suggested that the increase of mechanical tissues in the anthers in *D. racinae* is an adaptation to the environment. The structure of the pollen grains, including the micromorphology of the sporoderm, is very similar among *Dyckia pseudococcinea* (Mendes *et al*. 2012), *Dyckia hebdingii* (Sajo *et al*. 2005), and *D. racinae* - a fact that apparently would not be useful for the diagnostic characterization of the species.

The embryological characteristics in *Dyckia pseudococcinea* (Mendes *et al*. 2012), *Dyckia hebdingii* (Sajo *et al*. 2005), and in *D. racinae* are very similar to those described for Bromeliaceae (Johri *et al*. 1992, Lakshmanan 1967, Furness & Rudall 1999, 2001, Fagundes & Mariath 2014). Embryological characteristics are considered those connected to the transformations that occurred in the reproductive cells by sporogenesis and gametogenesis. Embryological studies conducted by Furness & Rudall (1999) demonstrate that the embryological characteristics are homogeneous in the families of monocotyledons and may characterize them. Among the *Dyckia*, as previously described, differences are seen in the sporangia, such as the types of parietal thickenings in the endothecium and the occurrence of cells with parietal thickenings in the connective, and this may be used in the taxonomic classification of the species. Thus, embryological characteristics can be used to characterize family or genus, while the anatomy of the sporangia can be used to identify species.

The ovule is anatropous, bitegmic and crassinucellate in *D. racinae*, confirming what has already been described for *Bromeliaceae* by Johri *et al.* (1992) and Fagundes & Mariath (2014). In *Bromeliaceae*, many species have a chalazal appendage, which varies in shape and size (Sajo *et al*. 2004a, Fagundes & Mariath 2014). In *D. racinae* the shape and size of the chalazal appendix is very similar among ovules of a same flower and among different flowers. The chalazal appendix originates the asymmetrical lateral wing described for the seeds in *D. racinae* (Smith 1988). A chalazal appendix, very similar to that observed in *D. racinae*, occurs in *D. pseudococcinea* (Conceição *et al*. 2007). The papillate epidermis of the obturator in *D. racinae* was also observed in *D. maritima* and *Pitcairnia flammea* Lindl. (Fagundes & Mariath 2010), where the authors indicated that this characteristic was typical in *Pitcairnioideae,* although similar in *Bromelioideae* and different in *Tillandsioideae.* When one checks the phylogeny of *Bromeliaceae*, it is seen that the *Pitcairnioideae* and *Bromelioideae* are closer from the point of view of evolution when compared to *Tillandsioideae* (Givnish *et al*. 2011), which might justify the structural similarities.

The conduplicate-spiral stigma described for *Dyckia* (Brow & Gilmartin 1989), as well as the septal nectaries (Sajo *et al*. 2004b), shows a similarity to data collected on the anatomy of sporangia and embryological data, and requires additional studies to increase the number of data points for presenting broad comparisons with emphasis on the taxonomy. So far, characteristics of stigmata and septal nectaries would not be useful in helping to determine various species of *Dyckia*.

As already mentioned, there is little data available in the literature on *D. racinae*, and nothing is found in the analytic keys cited in Reitz (1983) and in Smith & Downs (1974), traditionally used to identify species of *Dyckia.* However, based in part on the results obtained here, *D. racinae* can be positioned in analytical keys, close to *Dyckia cabrerae* L.B.Sm. & Reitz*,* considering that both present a preference for stony environments and both have erect leaves, densely lepidote on the abaxial surface; pauciracemose and lax inflorescences; and glabrous scape with bracts smaller than their internode. *Dyckia cabrerae* is distinguished from *D. racinae* by presenting larger flowers, 5 cm long pedicels; acute apex sepals, 8-13mm long.; rhomboid, clearly unguiculate petals (Reitz 1983, Smith & Downs 1974). Among the floral anatomical traits described here, little can be explored taxonomically, due to lack of information in the literature. However, the analysis of the anther anatomy, indicating the presence and absence of parietal thickenings in the connective cells, would be sufficient, for instance, in distinguishing between *D. racinae* and *D. pseudococcinea*.

This new information helps identify *D. racinae* more precisely, and it is useful in taxonomy, taking into account the difficulties encountered in delimiting the species of this genus. Considering that *Dyckia* is a genus with several taxonomic problems, one can see such complications as insoluble if a larger number of species is not studied, emphasizing the anatomy of the sporangia or in embryological development. Recent molecular information has become essential to understanding the phylogenetic relationships at different taxonomic and taxon levels, such as *Dyckia*, but the morphological characteristics, being more easily obtained and compared, ensure additional information for the efforts to determine the species.

Additionally, we understand that the information presented also helps in the understanding of the status of the conservation of *D. racinae*, whose general distribution is currently unknown. This is essential for more elaborate and effective conservation strategies.

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