Stamen morphoanatomy of *Dyckia* Schult.f. (Bromeliaceae, Pitcairnioideae) species: new data for taxonomic use

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ABSTRACT

This study presents a morphoanatomical analysis of *Dyckia ibicuiensis*, *D. polyclada* and *D. racinae* stamens. Flowers at anthesis were desiccated and their stamens were processed using common microtechniques for light microscopic analysis. Morphologically, the androecium in these species is differentiated by the disposition of the stamens around the gynoecium and by general anther characteristics. Included stamens, with antesepalous filaments different from those of the antepetalous, anthers in a radial disposition, always curved, and sporangia facing the gynoecium characterize *D. ibicuiensis* and *D. racinae*. This configuration results from the filament connation, as well as special anatomical characteristics of the anthers, such as the connective with thickenings. In addition, these species are differentiated by the U-shaped thickening in the endothecium. *D. polyclada* is characterized by its small, free and exserted stamens, with a very short common tube, its anthers, that are not curved or organized around the gynoecium, and its divergent sporangia. The anatomical and morphological characteristics identified here are important for characterization of these species. Considering that the androecium is important in the delimitation of *Dyckia*, the increased use of these data should be equally important for other species and infrageneric groupings.

Keywords. anatomy, androecium, anther, common tube, connective, *Dyckia*, endothecial thickening, stamen vasculature

Introduction

Dyckia is a very diverse genus of Pitcairnioideae (Bromeliaceae), with over 160 terricolous or saxicolous species, with adaptations to xerophytism, distributed across South America, especially in Brazil (Smith & Downs 1974; Leme *et al.* 2012; Forzza *et al.* 2015; Gouda *et al.* 2015). Phylogenetic hypotheses based on morphological (Forzza 2001) and molecular (Krapp *et al.* 2014) traits have confirmed the monophyly of the genus. However, there are still difficulties regarding both the circumscription of some species and the proposition of infrageneric groupings

(Krapp *et al.* 2014). This genus has a large number of endemic or rare species (Martinelli *et al.* 2008), and is considered problematic from a taxonomic point of view, either because of insufficient records of specimens, or due to overlaps in key analytical characteristics (Smith 1967; Versieux & Wendt 2007). For this reason, *Dyckia* has been of interest in morphoanatomical studies of reproductive organs in order to define the species more consistently (Fagundes & Mariath 2010; Dorneles *et al.* 2014; Martins *et al.* 2015; Oliveira *et al.* 2015; Santos-Silva *et al.* 2015) and to serve as a basis for understanding infrageneric relations and making phylogenetic inferences, which are rare and hampered by

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the homogeneity of traits in this group, due to its recent radiation (Krapp *et al.* 2014).

Characteristics of floral morphology, such as the presence of extranuptial nectaries and, mainly, fusion of the stamens, are important in the definition of the genus, which is phylogenetically and morphologically quite similar to *Encholirium*. However, the androecium is usually little pronounced in the original descriptions of *Dyckia* species, with emphasis in some species on the connation and relative size. Nevertheless, the importance of the stamens is not restricted to the delimitation of the genus, having been referred to in epithets, such as in *Dyckia choristaminea* ("dyckia of separated stamens"), *D. tubifilamentosa* ("dyckia of filaments in tube"), *D. exserta* ("dyckia of exserted stamens"), demonstrating the plasticity of this organ in the group, whose floral characteristics are considered quite homogeneous.

Anatomical analysis of reproductive organs has been used for taxonomic and phylogenetic purposes in Bromeliaceae and *Dyckia* (Sajo *et al.* 2005; Fagundes & Mariath 2010; Nogueira *et al.* 2015; Santos-Silva *et al.* 2015), due to not only the descriptive and diagnostic potential but also to the value of this kind of analysis in evolutionary considerations or the relative homogeneity of some often used morphological traits. In *Dyckia*, in addition to general anatomical traits of the flower (Dorneles *et al.* 2014; Martins *et al.* 2015), stamen anatomy, especially of the anther, has been investigated in some works from ontogenetic (Sajo *et al.* 2005; Mendes *et al.* 2012), taxonomic (Dorneles *et al.* 2014; Martins *et al.* 2015) and histochemical (Oliveira *et al.* 2015) points of view, especially in endemic or rare species. This type of analysis has contributed to a more precise characterization and delimitation of the respective species of *Dyckia*. The original descriptions of the species analyzed in the present study, *Dyckia racinae*, *D. ibicuiensis* and *D. polyclada*, presented only general aspects of flowers (Smith 1988; 1989; Strehl 1997) and few specific elements. The difficulties in identification not only exacerbate the condition of 'critically endangered' of *D. ibicuiensis*, but also hamper the classification of *D. racinae* and *D. polyclada* into the accurate category of endangerment, due to insufficient data and a lack of knowledge about the distribution and/or their respective population states (DOE RS 2014).

Considering the lack of information about the stamen in *Dyckia*, more detailed analyses of this organ are important in research on traits with potential phylogenetic and taxonomic use. Thus, the present study aims to characterize morphoanatomically the androecium of *D. racinae*, *D. ibicuiensis* and *D. polyclada*, highlight relevant characteristics of the stamens and anthers for each species and compare them using these characteristics.

Materials and methods

The processing and analysis of floral samples was carried out at the Laboratório de Botânica Estrutural (LABOTE) of the Departamento de Biologia at the Universidade Federal de Santa Maria (UFSM). Inflorescences of six specimens of *D. ibicuiensis* Strehl, ten specimens of *D. racinae* L.B. Sm. and ten specimens of *D. polyclada* L.B. Sm. were collected from natural populations 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) and Distrito de Santo Antão, Santa Maria, RS, Brazil (29°42.911'S/ 53°43.093'W), respectively. The plant material was deposited at the herbarium of UFSM, under the registration numbers SMDB 13840, SMDB 15923 and SMDB 15924.

Morphometric data were obtained from random individuals, using stamens of 10 to 20 flowers at anthesis for each species, at different heights of inflorescence. The total size of the stamen, the length of the anther and the filament, the ratio between filament length and total length of the stamen, the height of connation, the height of connation relative to the total size of the stamen and the width of the filament were measured. The terminology used is in accordance with Smith & Downs (1974).

For the light microscopy, the botanical 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 washing in the same buffer, washing in distilled water and finally dehydration in an ethyl alcohol series (O'Brien & McCully 1981). The material was pre-infiltrated in a 2-hydroxyethyl methacrylate (HEMA) and pure ethanol solution (1:1) during 12h, followed by infiltration in HEMA for approximately 4h and embedding in a Teflon holder until polymerization was completed (Gerrits & Smid 1983). The anthers and filaments were sectioned to 3μm in thickness using a Leica RM2245 rotary microtome. Toluidine blue O in 0.05% sodium benzoate buffer, pH 4.4 was used to stain the histological slides (Sidman *et al.* 1961).

For analysis of fibrous thickening, portions of anthers were macerated using a 3:1 solution of hydrogen peroxide and glacial acetic acid, with subsequent temporary preparation in alcoholic glycerine (Johansen 1940) and analyzed using Differential Interference Contrast (DIC).

For analysis of vascularization, fixed anthers and filaments were clarified using 3% sodium hypochlorite (Johansen 1940).

Photographic documentation and analysis were performed using a Leica DM2000 microscope® and Stereomicroscope Leica M80 with DFC295 image capture system and Zeiss AxioImager A2 with a Zeiss Axiocam RMC digital photographic system.
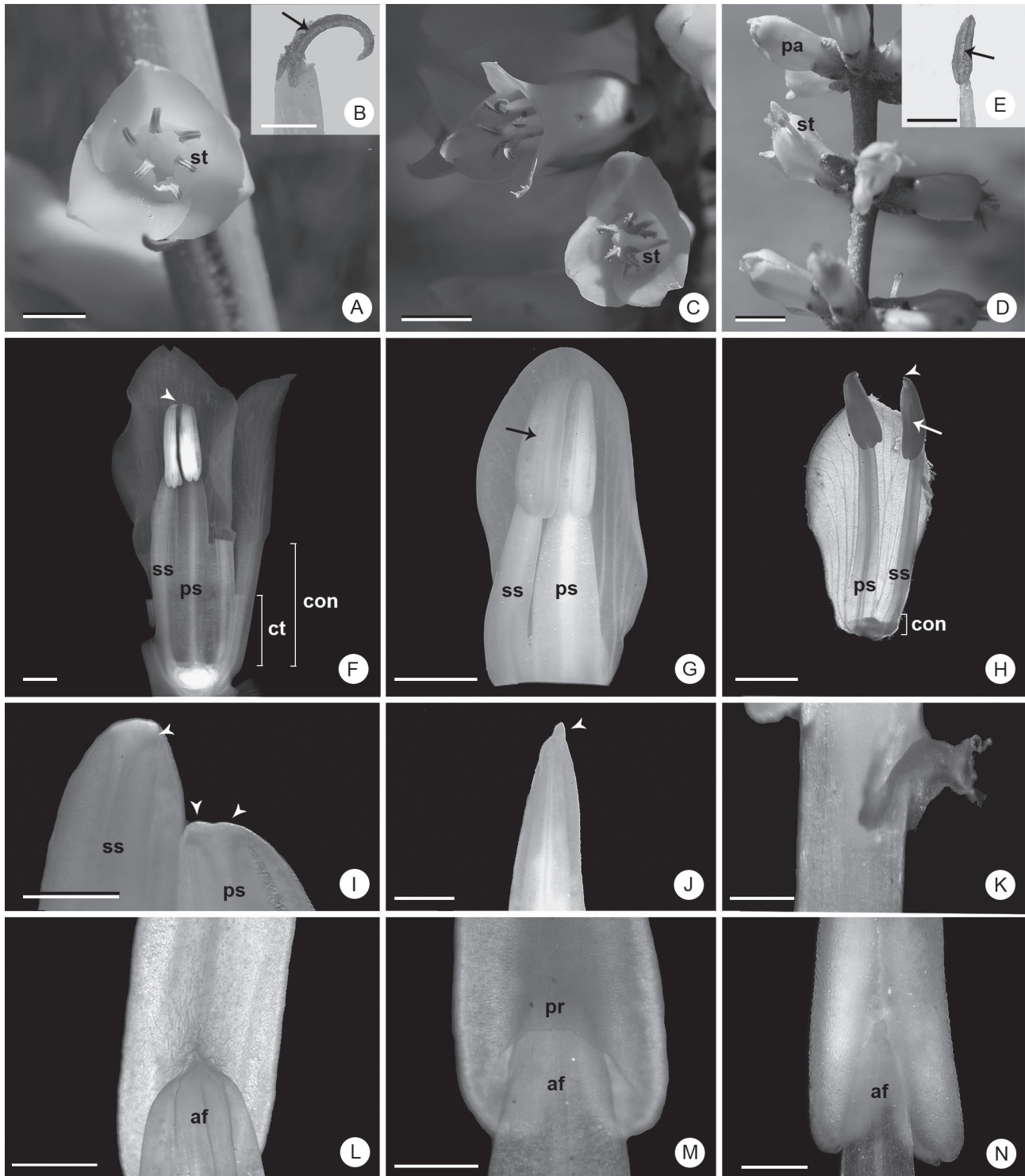
Photoshop™ was used to process the images.

Results and discussion

The species analyzed present an androecium with six stamens, three antesepalous and three antepetalous (Fig. 1A-E), which are adnate to the corolla forming a typical

Figure 1. General aspects of the stamens of *Dyckia* species. A, B, F, I, L: *D. ibicuiensis*. C, G, J, M: *D. racinae*. D, E, H, K, N: *D. polyclada*. A, C, D: General appearance of the flower *in vivo*. B, E: detail of dehiscent anther. F, G, H: general appearance of antepetalous and antesepalous stamens after chemical fixation. G: cross section of filaments occurred near the distal region of the common tube. I, J: rostra. K: projection of tissue at the base of the anther of *D. polyclada*. L, M, N: detail of the region of filament insertion. Arrows: region of dehiscence; arrow heads: rostra; **af**: apex of the filament; **con**: connation; **ct**: common tube with the petals; **pa**: flower at pre-anthesis; **pr**: projection around the apex of the filament; **ps**: antepetalous stamen; **ss**: antesepalous stamen; **st**: stamen. Bars: A, C, D: 5 mm; B, E, F-H: 2 mm; I-N: 500 μm.

common tube with the petals (Smith & Downs 1974). Thus, there are two stamen whorls, which can be connate

only at the base or above the common tube (Fig. 1F-H). The stamens are included in *D. racinae* and *D. ibicuiensis*

and exserted in *D. polyclada* (Fig. 1A-H). *D. racinae* and *D. ibicuiensis* present an evident common tube with the petals (Fig. 1F); in *D. polyclada* this tube is short or imperceptible (Fig. 1H). The anthers are triangular, dorsifixed, introrse and bithecous, with longitudinal dehiscence (Fig. 1F-H), and present a pointed rostrum (Fig. 1 H-J), which is bilobed in the antepetalous stamens of *D. ibicuiensis* (Fig. 1I). The general characteristics of the androecium, in the three species analyzed, corroborate previous data for *Dyckia* (Smith & Downs 1974; Reitz 1983) in large taxonomic studies of the genus. However, the original descriptions of the species analyzed here (Smith 1988; 1989; Strehl 1997), merely reported the height relative to the corolla and the occurrence of connation.

Morphometrically, the stamens differ by total size, size relative to the corolla, extent of connation and length and width of the filaments (Tab. 1). *D. ibicuiensis* and *D. racinae* present the largest stamens, with antepetalous filaments wider than the antesepalous filaments and with a greater extent of connation (Tab. 1). The filament in these two species represent 74% and 55%, respectively, of the total size of the stamen (Tab. 1). *D. polyclada* presents the smallest stamens and smallest extent of connation. In addition, the filaments are thin and the antepetalous and antesepalous filaments are similar (Tab. 1). The filament comprise more than 60% of the total size of the stamen (Tab. 1). Based on interpretation of the data, it is possible to infer that despite these ratios being unique for each species, there is no direct relationship between relative size of the filament and length relative to the corolla, which may be related to the length and curvature of the petals. Nevertheless, it is observed that the filaments continue to lengthen after anthesis in *D. polyclada*, while the petals cease to grow in this period (Fig. 1D), a common phenomenon in several angiosperms according to Greyson (1994). This characteristic could be investigated in future studies, associated to temporal development patterns of flower organs, during anthesis. Similarly, it is not possible to evaluate the relationship between the longer common tube and stamens, with greater extent of connation, and the relative sizes observed which differentiate *D. racinae* and *D. ibicuiensis* from *D. polyclada*, since the common tube with petals may also lengthen late, without leading to exposition of the stamens. It is important to note that relative measurements are rarely used in descriptions of species, despite generally being

robust, as they do not present overlaps and they provide good comparisons.

At anthesis, the stamens of *D. ibicuiensis* and *D. racinae* are regularly positioned around the carpels, giving the anthers a radial organization, with zones of dehiscence facing toward the gynoecium (Fig. 1A-C). This radial disposition is made possible by the greater extension of connation of the stamens, and by the greater opening of the corolla in these species, due to the dorsal curvature of the petals in *D. racinae* and *D. ibicuiensis* (Fig. 1A, C). In contrast, *D. polyclada* presents an irregular stamen distribution (Fig. 1D), mainly due to the smaller opening of the corolla and by the short connation. In addition, *D. ibicuiensis* and *D. racinae*, *in vivo*, show a marked dorsal curvature of the anthers (Fig. 1A-C), while in *D. polyclada*, there is only a slight curvature (Fig. 1D-E). After fixation, or when the anthers are rehydrated, there is a return to the rectilinear shape observed prior to dehiscence (Fig. 1F-G). This curvature does not appear to be a result of differential growth processes, but rather a result of typical dehydration of the anther during dehiscence, in association with its mature tissues. This consideration is supported by the fact that after rehydration, the anthers resume the typical pre-anthesis form. Changes in morphology, as well as position of the anther during anthesis have been observed in other plant groups (Dettke & Santos 2011), including *Dyckia* (Rogalski *et al.* 2009) and are related mainly to strategies of pollen grain dispersal and reproductive systems adopted by the species. As indicated by Rogalski *et al.* (2009), in addition to the radial arrangement of the anthers, which turn the sporangia toward the gynoecium, this curvature also characterizes a self-pollination system commonly observed in other Bromeliaceae genera, including *Dyckia* (Smith & Downs 1974; Benzing 2000).

Irregular projections of tissue were observed from the base of the theca of some stamens in *D. polyclada* (Fig. 1 K). This structure possesses variable and asymmetric forms. Since the presence of these projections is not constant, its taxonomic utilization is not viable, but nevertheless is a new record for the species and genus.

The insertion point of the filament is different in each species (Fig. 1L-N). In *D. ibicuiensis* and *D. polyclada* the apical region of the filament is exposed (Fig. 1L, N), unlike *D. racinae*, where this region is covered by the projection of the connective and sporangia around the apex of the

Table 1. Morphometry of the stamens of *Dyckia ibicuiensis*, *D. racinae* and *D. polyclada*. SL: stamen length. AL: anther length. FL: filament length (including the common tube with the petals). FL/SL: approximate mean ratio of filament length relative to total stamen length CON: connation height (including the common tube with petals). CON/SL: mean ratio between connation height and total stamen length. FW: filament width immediately above the connation point. FW (AS): filament width of antesepalous stamens. FW (AP): filament width of antepetalous stamens. SL, AL, FL, CON and FW measurements in cm; CON/SL and FL/SL: percentage values.

	SL	AL	FL	FL/SL	CON	CON/SL	FW (AS)	FW (AP)
D. ibicuiensis	1.4-1.8	0.4-0.5	1.0-1.5	74.0	0.7-1.0	56.5	0.1	0.25
D. racinae	0.9-1.1	0.4-0.45	0.6-0.7	55.0	0.21-0.3	25.5	0.09	0.17
D. polyclada	0.8-1.0	0.25-0.3	0.5-0.7	65.0	0.03-0.06	5.0	0.05	0.05-0.06

filament, similar to a groove (Fig. 1M). These species also differ by the shape of the filament apex, both antesepalous and antepetalous, which are obtuse in *D. ibicuiensis* and *D. racinae* (Fig. 1L-M) and acute in *D. polyclada* (Fig. 1N). In all the species, there is a gradual narrowing of the filament towards the apex (Fig. 1L-N). The insertion point of the filament is often associated with the movement of the anther in the reproductive period (D'Arcy 1996; Dettke & Santos 2011). The morphology of this region suggests constraint of lateral and dorso-ventral movements of the anther in *D. ibicuiensis* and *D. racinae*, regardless of its curvature. In *D. polyclada*, on the other hand, there seems to be articulation of the anthers, which, combined with the free exserted stamens, the rectilinearity of the anthers and the divergent sporangia, may be evidence of variations in the pollination process.

Another important feature is the differential morphology between the antepetalous and the antesepalous stamens of *D. ibicuiensis* and *D. racinae* (Fig. 1F-G) (Tab. 1). Differences between stamen whorls, as indicated by Greyson (1994), are related to initiation time and differential spacing between the antepetalous and antesepalous stamens during the early stages of floral development. These different widths of filaments in each whorl may demonstrate different patterns of floral development between these species and *D. polyclada*, whose filaments presented only a slight dimorphism, making their use in future taxonomic analyses relevant for both revisions of species of genus and for new descriptions. In the anthers, it is noteworthy that there is a forked rostrum only in the antepetalous stamens in *D. ibicuiensis*, which may be used as a diagnostic trait.

The anatomy and histology of the mature anthers in a cross-section of the species analyzed here was in line with typical expectations for *Dyckia* and other Bromeliaceae (Johri *et al*. 1992; Sajo *et al*. 2005; Mendes *et al*. 2012), with regard to the presence of a connective with central vascular bundle and four sporangia with parietal layers consisting of epidermis, endothecium and middle layer (Fig. 2A-F). Details of the distribution and constitution of these tissues, however, showed important anatomical and histological differences among the species.

In the cross section, the middle portion of the anthers presents typical histological sites, the connective and the sporangial site (Fig. 2A-F). The connective in *D. ibicuiensis* and *D. racinae* (Fig. 2A-D) presents a relatively more developed mid-dorsal region of the anther, unlike that in *D. polyclada* (Fig. 2E-F). The sporangial site comprises four sporangia facing the gynoecium in *D. ibicuiensis* and *D. racinae* (Fig. 2A, C), where in *D. polyclada* the sporangia assume a divergent position (Fig. 2E).

The connective, in a broad sense, is a tissue that comprises the entire area inside the anther, except for the sporangial tissue (D'Arcy 1996). In addition, it is the site of vascular bundle differentiation and, thus, sporangial nutrition. It also provides support for the anther and assists

in its dehiscence, in addition to being a site for storage and mobilization of metabolites (Clément *et al*. 1994; Oliveira *et al*. 2015).

In the present study, the presence of fibrous thickenings allowed for differentiation between two regions in the connective (Fig. 2A-F). The region without fibrous thickenings is central and made up of a collateral vascular bundle, with two parallel xylem poles, surrounded by parenchymatous tissue, similar to a sheath, with four to six cell layers extending to the septal region (Fig. 2A-F). The sheath, and its extensions to the septal sporangia are probably associated with the transfer of nutrients to the sporangia. In addition, they possibly aid in anther support, especially in *D. polyclada*, where this tissue forms almost the entirety of the connective (Fig. 2E-F). The connective dorsal and ventral regions in *D. ibicuiensis* and *D. racinae* are bulky and have cells with fibrous thickenings (Fig. 2B, D), unlike in *D. polyclada* (Fig. 2E), where this region is limited to a few thick cell layers (Fig. 2E-F). Considering the anthers in cross-section, the dorsal region in *D. ibicuiensis* and *D. racinae* is protruding (Fig. 2A, D), and in *D. polyclada* it is always concave (Fig. 2E), due to the connective bulk. Apparently, the greater accumulation of mechanical tissue in the dorsal and ventral region of the connective is important for the conformation of the anther in the anthesis period. Hufford & Endress (1989) and Dettke & Santos (2011) described the dehiscence of the anthers in different species as a result of the association between mechanical tissues of the connective and the endothecium, which likely occurs in the present study. The mechanical function is associated with the forces generated by differential dehydration of cell walls of mechanical tissues, as described by D'Arcy (1996). Thus, the dorsal curvature observed in the anthers after dehydration was interpreted as a result of deformation of the connective in *D. racinae* and *D. ibicuiensis*. In *D. polyclada*, the presence of few cells in the connective region and a more limited distribution of thickenings allow for this interpretation. Another result of the volume of the connective in the species analyzed in this work is related to the convergent position of the sporangia, facing the ventral region, especially in *D. ibicuiensis* and *D. racinae*, where this tissue orientates the growth and direction of the sporangia. In *D. polyclada*, whose connective is basically limited to the parenchymal region surrounding the vascular bundle, there is no mechanical restriction on sporangial development, which takes a lateralized position. From a taxonomic point of view, it is important to check this anatomical trait in future descriptions or infrageneric considerations, given its importance in the final structuring of anthers and its constancy in different flowers and different individuals.

As for fibrous thickenings, although Manning (1996) suggested that only those originating in the endothecium are taxonomically useful, in this study, thickenings found in the connective are similar to those of the endothecium. Some authors such as Hufford & Endress (1989) and D'Arcy

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(1996) have interpreted these portions of the connective as an endothecial-type of hypodermis or as a tabular configuration of the endothecium, due to the fact that the presence of fibrous thickenings is also associated with the opening of the anthers.

As for the sporangial region (Fig. 3), in the mature anther, there only remains cells from the epidermis, fibrous endothecium and some cells from the middle layer, despite there being an increase of cell layers in regions of transition from the sporangia to the connective (Fig. 3A, C, D).

When comparing the three species, the epidermis is striated, with thickened and cutinized external periclinal
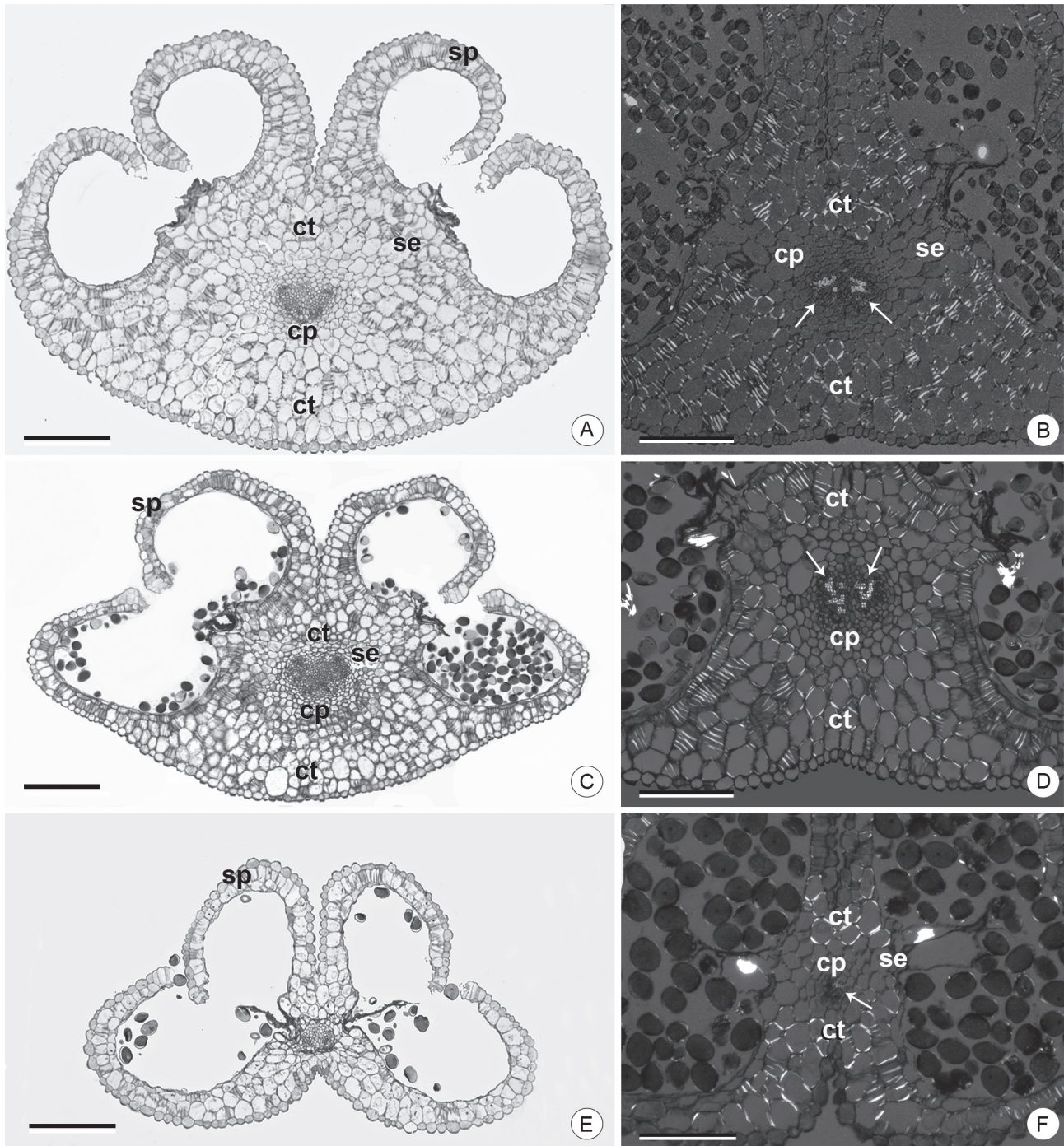

Figure 2. Anthers of *Dyckia* species in median cross section. A, B: *D. ibicuiensis*. C, D: *D. racinae*. E, F: *D. polyclada*. A, C, E: bright field microscopy (images on the same scale). B, D, F: polarized light microscopy. Arrows: vascular bundle xylem poles; **cp**: connective region with parenchymatous cells; **ct**: connective region with thickened cells; **se**: bundle sheath extension; **sp**: sporangium. Bars: A, C, E: 200 μm; B, D, F: 100 μm.

walls, making the organ surface irregular (Fig. 3B). In addition, it presents cells with anticlinal elongation in the region adjacent to the stomium (Fig. 3A, C, D). Lobo *et al.* (2013) consider these characteristics of *Dyckia* epidermis to reflect a xeromorphic condition common to the genus,

being that the taxonomic use of this layer is limited due to its structural simplification in the stamens.

The endothecium is the thickened subepidermal layer of the anthers, responsible, mainly, for aiding in its dehiscence (D'Arcy 1996). This function is associated directly with the
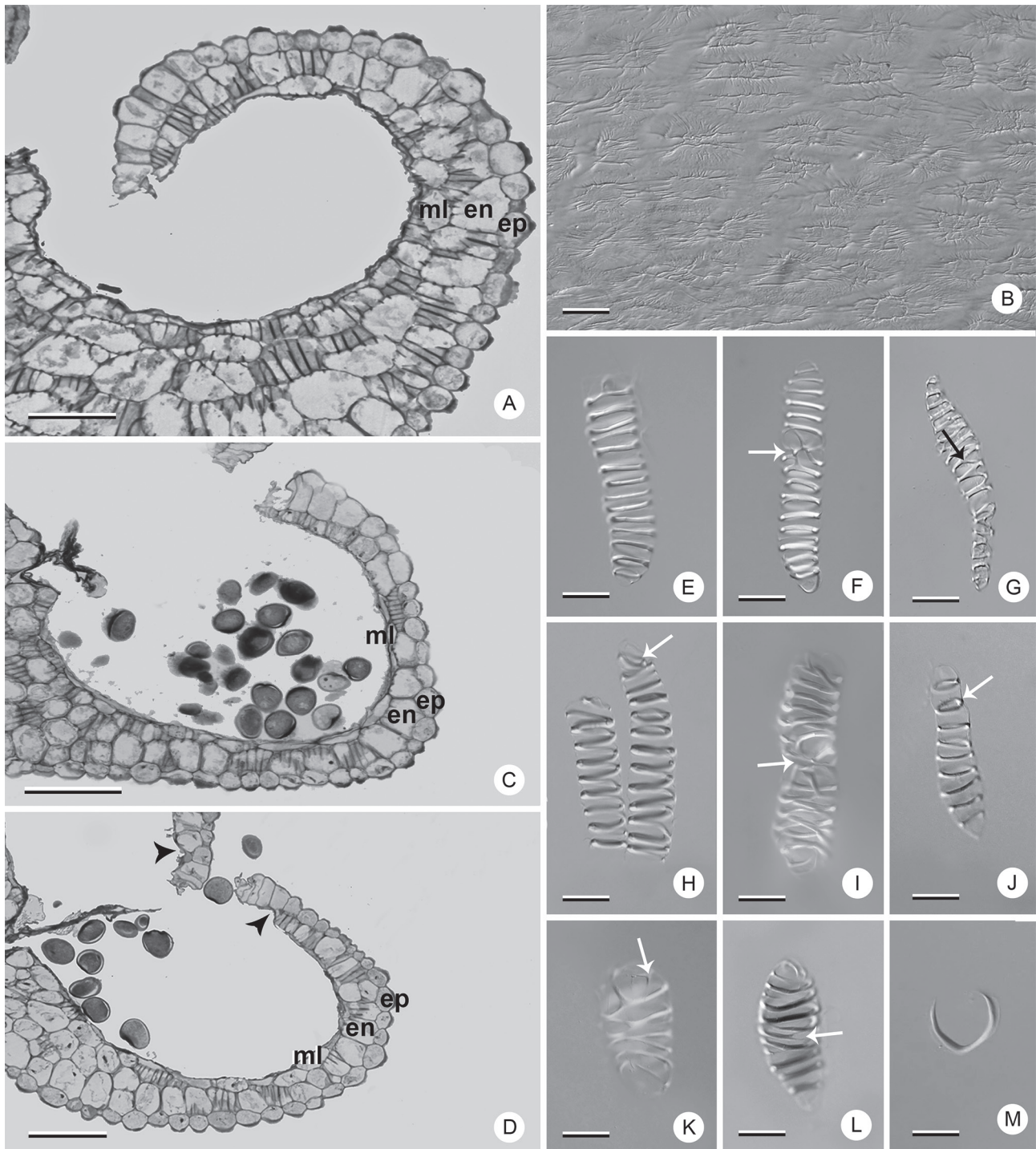
Figure 3. Sporangia and fibrous thickenings in *Dyckia* species. A, B, E-G, M: *D. ibicuiensis*. C, H-J: *D. racinae*. D, K, L: *D. polyclada*. A, C, D: sporangia in median cross section under bright field microscopy. B, E-M: Differential Interference Contrast microscopy (DIC). B: sporangial surface detail. E-M: endothecium cells showing different patterns of fibrous thickening. Arrows: bifurcations or rings; arrow heads: region with absent endothecium; **en**: endothecium; **ep**: epidermis; **ml**: middle layer. Bars: A, C, D: 100 μm; B, E-M: 10 μm.

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thickenings present in this layer, which generate differential tensions with typical dehydration during anthesis, aiding to open the sporangia (Keijzer 1987), by means of hygroscopic mechanisms. The greater structural diversity of the endothecium cells and the conservation of these types in different groups of plants (Manning 1996) justify its utilization in phylogenetic and taxonomic analyses. In the present study, in the same anther, different types of fibrous thickenings in endothecial cells were observed, in addition to types exclusive to the species (Fig. 3E-M).

In the species analyzed here, the endothecium consists of a layer of cells that is continuous until the stomial region, with the exception of *D. polyclada*, in which the endothecium cells do not occur in that region (Fig. 3D). In all the species, there is a predominance of helical thickening, combined or not with rings at the extremities or with bifurcations (Fig. 3E-I, K-L). In addition, cells with annular thickenings may also be observed (Fig. 3J). *D. ibicuiensis* and *D. racinae* also present U-shaped thickenings (Fig. 3M), not observed in *D. polyclada*. The predominant types in the three species are variations of the helical type described by Manning (1996), which, according to the author, is one of the most important types from a phylogenetic point of view, in addition to the U-shapes. Manning & Linder (1990) consider U-shaped thickenings as a derived type in Poales families. According to them, this type emerged from the helical type in this group, a hypothesis that may justify future studies in *Dyckia*, due to the variability in fibrous thickenings found in the three species analyzed. Besides the absence of U-shaped thickenings, *D. polyclada* is also characterized by the absence of the endothecium near the stomial region.

The middle layer, in the three species, consists of a non-uniform layer of cells that also presents fibrous thickenings (Fig. 3A, C-D). This parietal layer is conspicuous only in *D. ibicuiensis* (Fig. 3A). The structure of the middle layer in the mature anther proved to be of interest in the comparison among the species since, by remaining in the anthers, its cells also developed fibrous thickenings. The permanence of the middle layer with thickenings in the mature anter is common and has been reported in other monocot families, such as Rapateaceae (Oriani & Scatena 2013) and Commelinaceae (Hardy *et al.* 2000), despite being unidentified in *Dyckia* and other Bromeliaceae. It probably aids in anther dehiscence together with the endothecium, due to the presence of fibrous thickening.

Some studies investigating the anther anatomy in *Dyckia* have shown the location and types of vascular bundles (Sajo *et al.* 2005; Mendes *et al.* 2012; Dorneles *et al.* 2014). Likewise, a number of studies have recognized the phylogenetic and taxonomic importance of anther vasculature in other taxonomic groups (Hufford 1980; French 1986; Dettke & Santos 2011). In this study, *D. ibicuiensis* and *D. racinae* present a bifurcation of the vascular bundle in the anter, extending from the insertion point of the filament to the basal region of both theca (Fig. 4A-B), unlike *D. polyclada*, which presents vascular bundles without ramifications (Fig. 4C). *D. ibicuiensis* presents a small bifurcation at the apex of the antepetalous stamens, corresponding to the rostral lobes (Fig. 4A), which does not occur in the other two species. The vascularization patterns described are easily observed anatomically along the anther (Fig. 4 D-I), due to the occurrence not only of conducting elements, but also the vascular sheaths. In *D. polyclada* this tissue is also present along the entire length of the anther, although in the basal region only the vascular sheaths occur (Fig. 4F-G). In the basal region of the theca of some anthers of this species there are projections of non-vascular tissue (Fig. 1K), with epidermis and parenchymatous content (Fig. 4J). Relationships between anther volume and vascular branching in the connective have been proposed previously (Dettke & Santos 2011) and in the present study, it is possible to observe a simplification of the vascular tissues in *D. polyclada*, possibly associated with the smaller size of the anther. In the other species, the vascular branching possibly occurs due to the larger size of the anthers and to the distance from the site of entry of vascularization in the connective.

The antesepalous and antepetalous filaments (Fig. 5), in a same flower, are distinct in *D. ibicuiensis* and *D. racinae* (Fig. 5A-B, D, G-H). In these species, the filaments of the antesepalous stamens are narrow and possess a concave-convex section, while those of the antepetalous stamens are broader and flattened dorsi-ventrally. In *D. polyclada*, the antesepalous and antepetalous filaments are similar and may show a small dimorphism (Fig. 5C, E-F). In the middle cross-section, the filaments present a single-layered epidermis and hypodermis followed by typical parenchymatous tissue and one or more collateral vascular bundles (Fig. 5D-H). In this region, it is possible to note a tissue continuity in *D. ibicuiensis* (Fig. 5D). All the species present a vascular bundle in the central portion of the filament, which generally possesses two parallel xylem poles (Fig. 5A-C). In *D. racinae*, in most of the flowers, there are filaments with one or two vascular bundles of smaller size (Fig. 5B, G-H), occurring in a lateralized position, between the central vascular tissue and the epidermis. These bundles remain separate from the central vascular bundle from the base of the stamens, uniting into a single bundle in the area of narrowing and insertion of the anther. The filament anatomy of the species examined is quite similar to that documented for other Bromeliaceae (Sajo *et al.* 2004; Mendes *et al.* 2012). However, the vasculature of this organ is unusual, both in terms of the common configuration of the bundles or the variants found. The conformation of vascular bundles with two parallel xylem poles in *D. ibicuiensis* and *D. racinae* can be interpreted as a consequence of the increased volume of this organ in these species, when compared to *D. polyclada*, a species with relatively narrow filaments. The patterns found in *D. racinae* are rare in most angiosperms, which present a single unbranched vascular bundle in the filament, despite ramification in the connective being common (Greyson 1994). As Puri (1951) points out, the vascular tissue in the
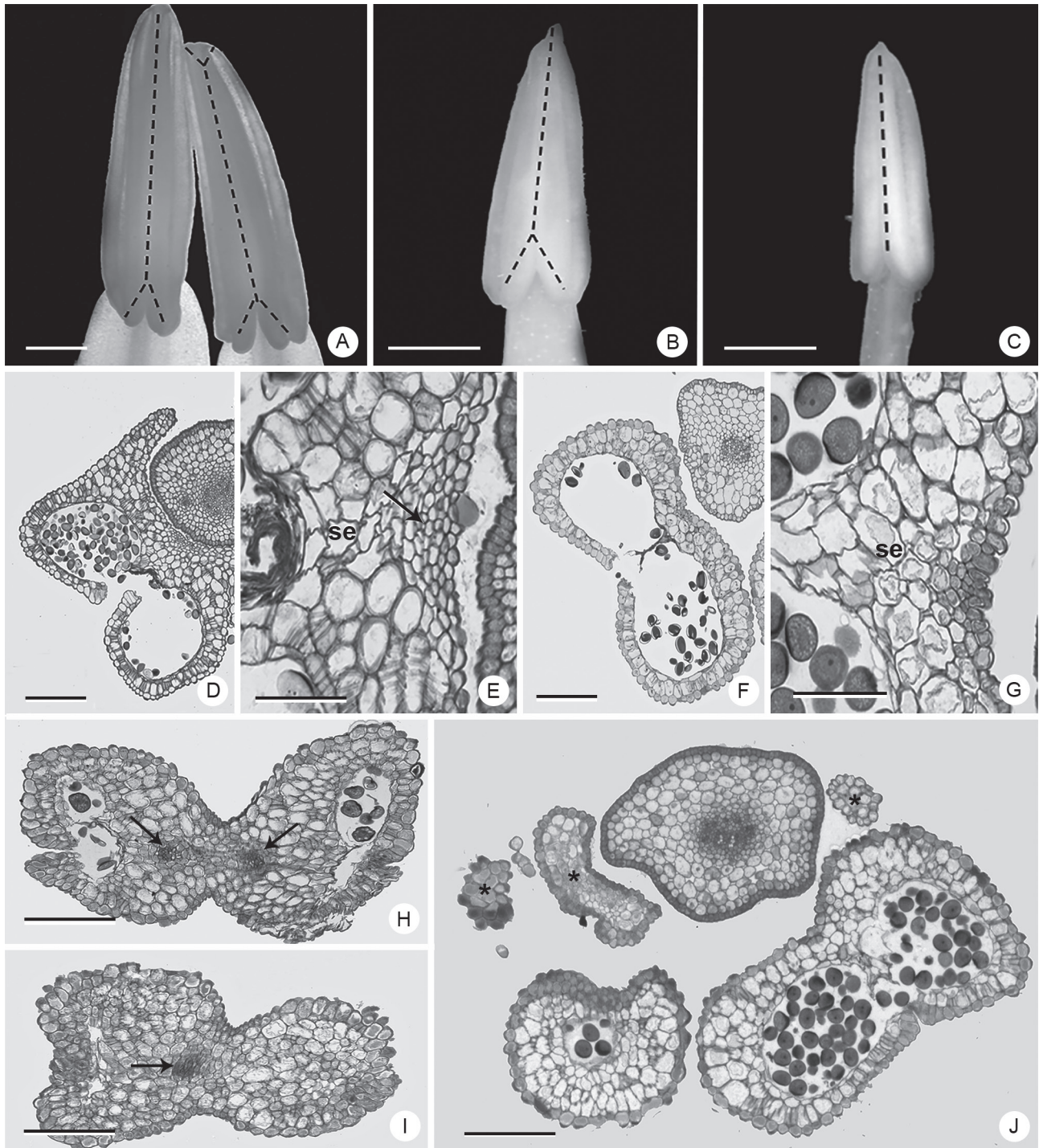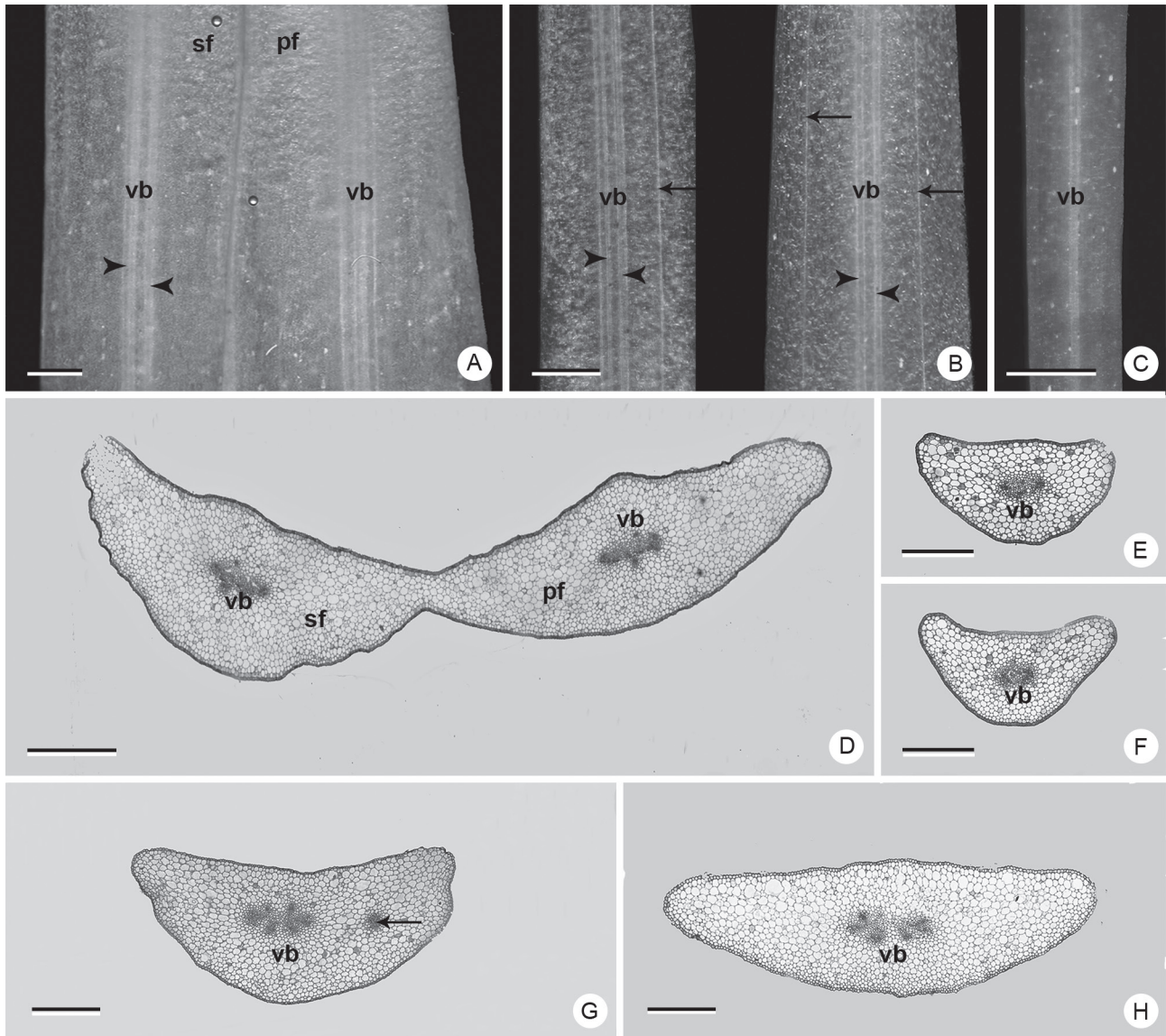
Figure 4. Vascularization of the anthers in *Dyckia* species. A, H, I: *D. ibicuiensis*. B, D, E: *D. racinae*. C, F, G, J: *D. polyclada*. A-C: images of stereomicroscopy. D-J: bright field microscopy. A-C: dashed line indicates vascularization of the anthers. D-G, J: basal portion of the anther. H, I: apices of anthers of antepetalous and antesepalous stamens, respectively; Arrows: vascular bundles; asterisk: projections of non-vascular parenchymatous tissue; **se**: bundle sheath extension. Bars: A-C: 1 mm; D, F, J: 200 µm. E, G, H, I: 50 µm.

flowers remains relatively well-conserved, being important for evolutionary inferences, especially related to fusions and structural losses. Therefore, the variations found in *D. racinae* are an important record of the variability in stamen vasculature, demonstrating the need for studies

of flower development in order to verify the origin of these configurations and their possible utilization in species characterization.

Finally, when *D. polyclada* is compared with other species of the genus, it presents similarities with species of the

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Figure 5. Vascularization and histology of filaments in *Dyckia* species. A, D: *D. ibicuiensis*. B, G-H: *D. racinae*. C, E-F: *D. polyclada*. A-C: filaments clarified under stereomicroscopy. D-H: median cross-section of filaments under bright field microscopy. C, E, G: filaments of antesepalous stamens. F, H: filaments of antepetalous stamens. Arrow: eccentric vascular bundle; arrow heads: xylem poles; **pf**: antepetalous filament; **sf**: antesepalous filament; **vb**: vascular bundle. Bars: A-C: 500 μm; D-H: 200 μm.

"maritima complex" (Strehl & Beheregaray 2006), a group that has been neglected, but which shares various floral characteristics, including the morphology of the androecium observed in this study. According to HM Büneker *et al*. (unpubl. res.) several species of *Dyckia* from southern Brazil are part of this group, including *D. myriostachya*, *D. retroflexa*, *D. rigida*, *D. maritima*, *D. selloa*, *D. alba*, *D. agudensis*, *D. nigrospinulata*, *D. tomentosa*, *D. domfelicianensis*, *D. hebdingii*, *D. delicata*, as well as *D. polyclada*. Apparently, the androecium in all these species is quite similar, with slightly connate or free stamens, often exserted, with a short common tube with the petals, similar and narrow antesepalous and antepetalous filaments and small anthers not distributed radially around the gynoecium, in contrast

to *D. racinae* and *D. ibicuiensis*, which are likely related to other dyckias of a second group (Strehl & Beheregaray 2006), apparently heterogeneous. It is important to note that there are significant overlaps in taxonomic descriptions, especially in floral characterization of the "maritima complex" species, for example, the demarcation between *D. polyclada* and *D. maritima* (Smith & Downs 1974; Reitz 1983), whose few diagnostic characteristics overlap markedly. This points to the need for an analysis that includes a greater number of floral features, especially those related to the androecium, which, according to the data of this study, may be relevant.

This study aims to expand the characterization of the androecium in *Dyckia* because of its reproductive and taxonomic importance. In addition, it contains promising

unpublished data for future studies of the genus. For comparative purposes, a number of characteristics can be highlighted, including the connective with thickened cells, the curvature of the anthers, the types of fibrous thickenings found in the endothecium, the particularities of each stamen whorl and stamen vasculature. Finally, the stamen morphanatomy in *Dyckia* was found to be important in the characterization of three rare and endangered species of this diverse genus. We emphasize that the stamen features present taxonomic potential both for revisions of already described species and for characterization of new *Dyckia* species.

Acknowledgements

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