COMPARATIVE ANATOMY IN FOUR CHEILANTHOID FERNS

ANATOMÍA COMPARADA EN CUATRO HELECHOS CHEILANTOIDEOS

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SUMMARY

Background and aims: Some species of Cheilanthoid ferns are characterized by show adaptations to xeric habitats. Furthermore, presents the peculiarity of the roll their blades under drought stress and when conditions are optimal they expand again. The aims of this work are provide morpho-anatomical information of the leaves and relate with the environmental conditions in which grow.

M&M: The fronds' morpho-anatomy of four species (*Adiantopsis radiata*, *Cheilanthes glauca*, *Doryopteris concolor* and *D. pentagona*) were analyzed through light microscopy. Also, the stomatal density and stomatal index were obtained.

Results: Homogenous mesophyll was observed in *D. pentagona*. *Adiantopsis radiata, C. glauca* and *D. concolor* showed dorsiventral heterogeneous mesophyll. All species studied showed hypostomatic leaf, with anomocytic stomata. The stomatal index observed was between 9.1% (*A. radiata*) and 14.1% (*D. concolor*), and the range of stomata density varied between 39.7 (*D. pentagona*) and 57.8 (*D. concolor)*.

Conclusions: The mesophyll observed in *A. radiata*, and *D. pentagona* responds to a mesophytic environment. *Doryopteris concolor* was reported as a facultative epiphyte, and its dorsiventral heterogeneous mesophyll would be an advantage in this habitat. *Cheilanthes glauca* has xeromorphic and mesomorphic features, probably resulting from water availability during the different seasons. We porpose as hypothesis that the thickening of the anticlinal walls of epidermal cells could be involved in the leaf movements.

KEY WORDS

Adiantopsis, anatomy, *Cheilanthes*, *Doryopteris,* epidermis, mesophyll.

RESUMEN

Introducción y objetivos: Algunas especies de helechos cheilanthoideos son caracterizados por presentar adaptaciones a hábitats xéricos. Además, presentan la particularidad de enrollar sus láminas frente a condiciones de stress hídrico. Sin embargo, al retornar las condiciones ambientales óptimas las láminas se vuelven a expandir. El objetivo de este trabajo es proporcionar información morfo-anatómica de las frondes y asociar estructuras anatómicas a estos movimientos foliares.

M&M: Se estudiaron mediante microscopía óptica y electrónica de barrido la morfo-anatomía de las frondes de cuatro especies: *Adiantopsis radiata*, *Cheilanthes glauca*, *Doryopteris concolor* y *D. pentagona*. Además, se analizó la densidad de los estomas y se calculó el índice estomático.

Resultados: Se observó mesófilo homogéneo en *D. pentagona*. *Adiantopsis radiata, C. glauca* y *D. concolor* presentaron mesófilo heterogéneo dorsiventral. Todas las especies mostraron hojas hipoestómaticas y estomas de tipo anomocítico. El índice estomático osciló entre 9,1% (*A. radiata*) y 14,1% (*D. concolor*) y el rango de densidad de los estomas fue de 39,7 (*D. pentagona*) y 57,8 (*D. concolor*).

Conclusiones: El mesófilo observado en *A. radiata* y *D. pentagona* refleja una especialización mesofítica. El mesófilo heterogéneo dorsiventral de *D. concolor* podría ser una ventaja para su hábito epifítico facultativo. *Cheilanthes glauca* tiene características xeromorfas y mesomorfas, probablemente como resultado de la disponibilidad de agua en el ambiente durante las diferentes estaciones. Se propone como hipótesis que el engrosamiento de las paredes anticlinales de las células epidérmicas podría estar involucrados en los movimientos de las láminas.

PALABRAS CLAVE

Adiantopsis, anatomía, *Cheilanthes, Doryopteris,* epidermis, mesófilo.

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INTRODUCTION

The relationship between plant structure and the environment has fascinated plant anatomists, and continues to be of great interest to researchers (Cutler *et al.,* 2007). In general, the plant structure of a particular species shows an anatomical pattern characteristic of its taxonomic group as well as adaptations that reflect the environmental conditions in which the plants grow. For example, xerophytes plants respond or adjust their leaf morphology, anatomy and/or physiology to variations in water availability (Cutler *et al.,* 2007). The diversified degree of structural variation among xerophytes has been the focus of several research studies, from desiccation tolerance to model studies on seed plants (Shields, 1950; Fahn & Cutler, 1992; Wang *et al.,* 2003; Cutler *et al.,* 2007; Basu *et al.,* 2016).

The vegetative tissue of xerophytes plants can to withstand extreme drought. One strategy observed in certain plants is the 'in-rolling' of leaves to protect the stomata from prevailing dry conditions, reducing water loss (Fahn & Cutler, 1992; Melvin *et al.,* 2000). As they can to survive to minimum water levels, they are called "resurrection plants" (Fahn & Cutler, 1992; Melvin *et al.,* 2000). This adaptation has been proposed to be an important feature to colonize new habitats, and has been described in many fungi, algae, lichens, bryophytes, and ferns (Mishler & Churchill, 1985; Fahn & Cutler, 1992; Melvin *et al.,* 2000).

The group of Cheilanthoid ferns comprises nearly 426 species and 23 genera (PPGI, 2016). Many taxa of this group grow in dry forests, savannas or rock outcrops; some of them show adaptations to xeric habitats and resistance to hydric stress (Hevly, 1963; Tryon, 1990; Melvin *et al.,* 2000; Hietz, 2010; Link-Pérez & Hickey, 2011). Furthermore, are characterized by present complex laminae, small segments or pinnules, abscission zones and presence of indumentum (Tryon & Tryon, 1973; Link-Pérez & Hickey, 2011)

During field collections and experimental trials was observed leaf movements in dry periods in several species of this group [e.g. *Adiantopsis radiata* (L.) Fée, *Doryopteris concolor* (Langsd. & Fisch.) Kuhn, *D. pentagona* Pic. Serm., and *Cheilanthes glauca* (Cav.) Mett.]. The blades roll up under drought stress, and unroll when environmental humidity conditions returned to optimum. These movements were observed by Kessler & Siorak (2007), who conducted desiccation and rehydration experiments on the leaves of 43 ferns and lycophytes species to assess the variability of drought adaptation strategies among these plants. These authors described these reactions as a component of the poikilohydric adaptive strategy to counter drought stress in Cheilanthoid ferns.

In this context, we examined the anatomy of the above mentioned species, in order to provide morpho-anatomical information of the leaves and attempt look for possible anatomical structures associates with leaf movements.

MATERIAL AND METHODS

Analyzed species

Were taken four species of the subfamily Cheilanthoideae to study. These, were selected because of easy accessibility to be collected, choosing species from different environments: *Adiantopsis radiata*, *Doryopteris pentagona*, and *D. concolor*, representatives of mesic environments. Furthermore, this latter has been mentioned as facultative epiphyte (Meza Torres *et al.,* 2013). To other hand, we selected to *Cheilanthes glauca* as a representative of xeric environments (de la Sota *et al.*, 1998).

Leaf movements were corroborated submitting the plants to hydric stress. Which were removed from the ground and leaving them without water for three hours (blades roll up). To return the blades to original shape, was submerge the roots in water for three hours. The photos that were taken belong to same specimen, subjected to normal humidity and temperature conditions and hydric stress.

Adiantopsis radiata. ARGENTINA. Prov. Corrientes, *Depto. Ituzaingó*, Hogar Filadelfia, 27º 34' 33,3" S, 56º 29' 39" W, 75 m a.s.l., terrestrial, on the margin of flooded area along Paraná River, it grows in humid and shady environments, abundant, 6/V/2016, *Dematteis et al. 20* (CTES).

Doryopteris concolor. ARGENTINA. Prov. Chaco, *Depto. 1º de Mayo*, Colonia Benítez, Colonia Benítez Educational Natural Reserve, 27º 19' 04" S, 58º 57' 01.3" W, 64 m a.s.l., terrestrial, edge of trail, 23/XII/2014, *Dematteis et al. 13* (CTES). Prov. Corrientes, *Depto. Capital*, Riachuelo, area adjacent to Puente Pexoa, 27º 33' 34" S, 58º 43' 21" W, 82 m a.s.l., herb, approximately 15 cm in height, abundant, interior of secondary forest, 11/II/2014, *Dematteis & Meza Torres 2* (CTES).

Doryopteris pentagona. ARGENTINA. Prov. Chaco, *Depto. 1º de Mayo,* Colonia Benítez, Colonia Benítez Educational Natural Reserve, 27º 19' 03.8" S, 58º 57' 02.2" W, 47 m a.s.l., terrestrial, scarce, interior of forest but also on the trail edge, 23/XII/2014, *Dematteis et al. 11* (CTES).

Cheilanthes glauca. ARGENTINA. Prov. Neuquén, *Depto. Picunches*, Paso de Pino Hachado, National Gendarmerie post, 38º 39' 09.4" S, 70º 49' 37.2" W, 1500 m a.s.l., *Nothofagus* forest with elements of *Araucaria araucana,* among rocks, exposed to the sun and abundant, 25/II/2014, *Meza Torres 1566* (CTES).

Light Microscopy

The leaves were fixed in field using formaldehyde–acetic acid–alcohol (FAA). Transverse and paradermal sections of lamina and petiole were prepared according to the techniques of Johansen (1940). Permanent microscope slides were prepared by a series of Histological Dehydration (Biopur ®) and the pre-impregnation was conducted with Clarifying Preimpregnant (Biopur) (Gonzalez & Cristóbal, 1997). Infiltration in paraffin was performed using the technique of Johansen (1940), and the material was later embedded in 'Histoplast®' (Biopack, Buenos Aires, Argentina). Starch was determined following Johansen (1940); transverse sections of petiole were treated with potassium iodide and sublimed iodine. Sections (10–15 mm length) were cut with a rotary microtome and stained with astra blue-safranin (Luque *et al.,* 1996) before mounting with synthetic Canada Balsam (Biopur Buenos Aires, Argentina). Fronds were cleared using the method of Payne (1969) and stained with safranin to study their vascularization.

The stomatal morphology was analyzed and classified according to the nomenclature provided by Baranova (1987). Stomata were counted in semi-permanent slides; leaves of two to three specimens of each species were cleared (Payne 1969) and stained with safranin.

To estimate stomata size and density and stomatal index (Wilkinson, 1979), 10 samples were randomly taken from the adaxial and abaxial sides of the lamina. Length and width of stomata were measured using light microscope equipped with a micrometer scale. For this, we counted stomata and epidermal cells from a randomly selected 1 mm^2 area. Observations and photographs were performed with a Leica MZ6 stereomicroscope and a Leica DM LB2 binocular microscope, both equipped with a digital camera.

RESULTS

The results of the leaves movement of the species during dry periods are shown in Fig. 1 (A-F). The Table 1 summarizes the leaf morpho-anatomical data (density and size of epidermal cells; size, index and density of stomata; and thickness of mesophyll and cuticle).

Petiole anatomy

Except for *C. glauca*, all the taxa have simple trichomes on their petioles. In *A. radiata* and *D. pentagona,* trichomes usually are present on the distal part and are found more concentrated towards the base of the leaf blade (rarely all over the petiole). In *D. concolor*, the trichome are present only on the adaxial side (of the sulcate area of petiole).

In cross section of petiole, all the species studied show a uniseriate epidermis of rounded and compact cells covered with a thick cuticle and with presence of stomata (Figs. 2A; 3A; 4A; 5A). In subdermal position, there are several sclerenchyma layers with thickened walls, except in *C. glauca*, where the cells have less thickened walls. In addition, all the species show compact parenchyma composed of isodiametric cells with thin walls and wide lumen with presence of starch in *C. glauca.* The endodermis and the pericycle are located in the central region of petiole; the pericycle has up to three layers of parenchyma cells with visible nucleus. *Adiantopsis radiata* and *D. pentagona* have an endodermis formed by cells with thickenings throughout the cell wall. *Doryopteris concolor* present a layer of less thick endodermis cells, which could be the beginning of Casparian strip. In turn, the endodermis of *C. glauca* is formed by large globose cells with thickened walls and cytoplasm with dense granular content.

The vascular bundle is amphicrival, with tracheids and fibrotracheids of xylem surrounded

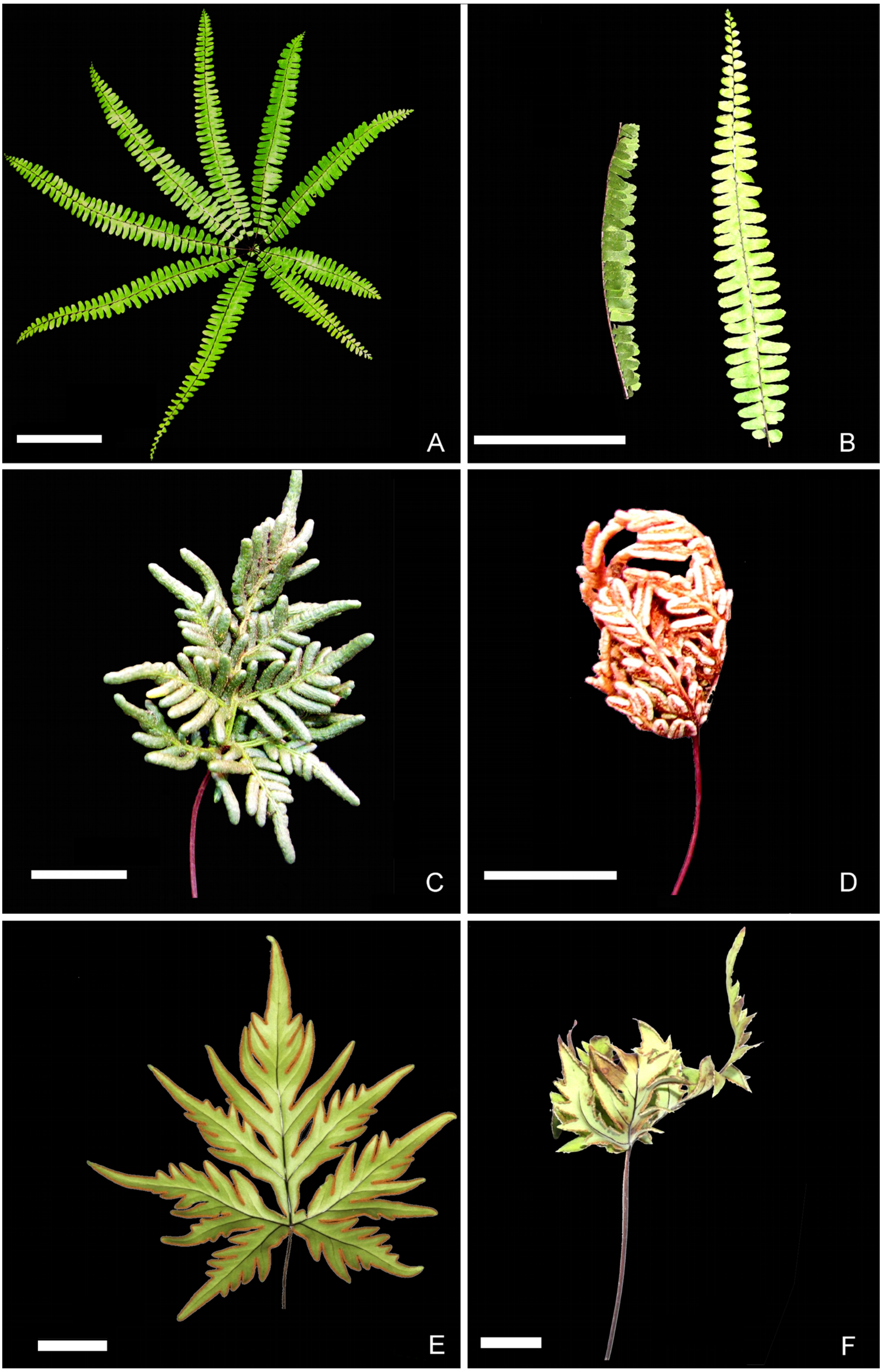

Fig. 1. Results of the blades movement. **A-B**: *Adiantopsis radiata*, **C-D**: *Cheilanthes glauca*, **E-F**: *Doryopteris pentagona*. **A**, **C**, **E**: Fronds in normal conditions of humidity and temperature. **B**, **D**, **F**: Fronds exposed to drought conditions. Scale: A, B: 5 cm; C, D: 1 cm; E, F: 2 cm.

Table 1. Environmental data and leaf anatomy of the studies species.

Parameters				***A. radiata***	***D. concolor***	***D. pentagona***	***C. glauca***
Environment				Shadow	Shadow	Shadow	Sunny
Lamina	Cuticle thickness (µm)		Adaxial	1.7	2.4	1.5	4.5
			Abaxial	1.5	2	1.6	5.1
	Epidermal cells	Cross section * (µm)	Adaxial	34.7×16.4	23.6×30.7	21.5×31.2	26.1×39.7
			Abaxial	35×17.3	19.7×30.6	22.4×35.5	19.7×33.8
		Surface view * (µm)	Adaxial	52.7×25.4	75.2×36.5	75.5×38.1	82.7×41.7
			Abaxial	76.2×30.6	72.8×37.9	73×37.6	94.2×24.6
	Stomata		Type	Anomocytic			
			Size *(µm)	44.3×28.2	38.8×30.2	47.1×36.7	45×34.3
			Index	9.1%	14.1%	9.3%	-
			Density (N°/mm^2)	44.1	57.8	39.7	-
	Mesophyll	Type		Heterogeneous	Heterogeneous	Homogeneous	Heterogeneous
		thickness (µm)		152.3	275.2	287.3	416
	Indumentum			Glabrous	Simple trichomes	Simple trichomes	Simple trichomes and paraphyses
Petiole	Indumentum			Simple trichomes	Simple trichomes (only on adaxial side)	Simple trichomes	Glabrous

*Length × width

by sieve cells of phloem and arranged in a "U" shape in *C. glauca* (Fig. 5A) or an elongate "V" shape in *A. radiata, D. pentagona* and *D. concolor* (Fig. 2A; 3A; 4A).

Venation pattern

The species studied are characterized by presenting an open pinnate venation (Figs. 2B-C; 3B-C; 5B). However, *D. pentagona* exhibit closed pinnate venation (Fig. 4B). *Adiantopsis radiata* and *C. glauca* have a principal vein that ended slightly bifurcated at the apex of the pinnule and many secondary veins that ended near its margins (Figs. 2B; 5B). In *D. pentagona*, the secondary veins that arose from the principal vein became dichotomous near the pinnule margins (Fig. 4B).

Leaf anatomy

Adaxial epidermis: In surface view, the analyzed species exhibit epidermal cells of irregular form, sinuous walls of varied thickness, dense cytoplasm and absence of stomata and indumentum (Figs. 2D; 3D; 4C; 5C). In cross section, the species present uniseriate epidermis and compact epidermal cells arranged linearly. *Cheilanthes glauca* show uniseriate epidermis, bigger epidermal cells, with thickened anticlinal walls (Fig. 5D) compared with the others, which presented thinner walls. *Doryopteris concolor* has the smallest epidermal cell diameter of the group, whereas *D. pentagona* has the greatest. A thin cuticle covers the epidermis of *A. radiata, D. concolor* and *D. pentagona,* whereas *C. glauca* has a much thicker cuticle (Table 1).

Mesophyll: In cross section, *Doryopteris pentagona* present a homogeneous mesophyll characterized by cells that delimited large intercellular spaces and a chlorophyll parenchyma with cells which present lateral projections. These, are connected to the adjacent cells and are mostly concentrated in the upper epidermis (Figs. 4D-E). In *A. radiata* and *D. concolor*, the mesophyll is slightly dorsiventral and heterogeneous, with a single layer of palisade parenchyma and various layers of spongy parenchyma, with clearly differentiated intercellular spaces (Figs. 2E-F; 3E). *Cheilanthes glauca* show a dorsiventral heterogeneous mesophyll with up to three layers of chlorophyll palisade parenchyma

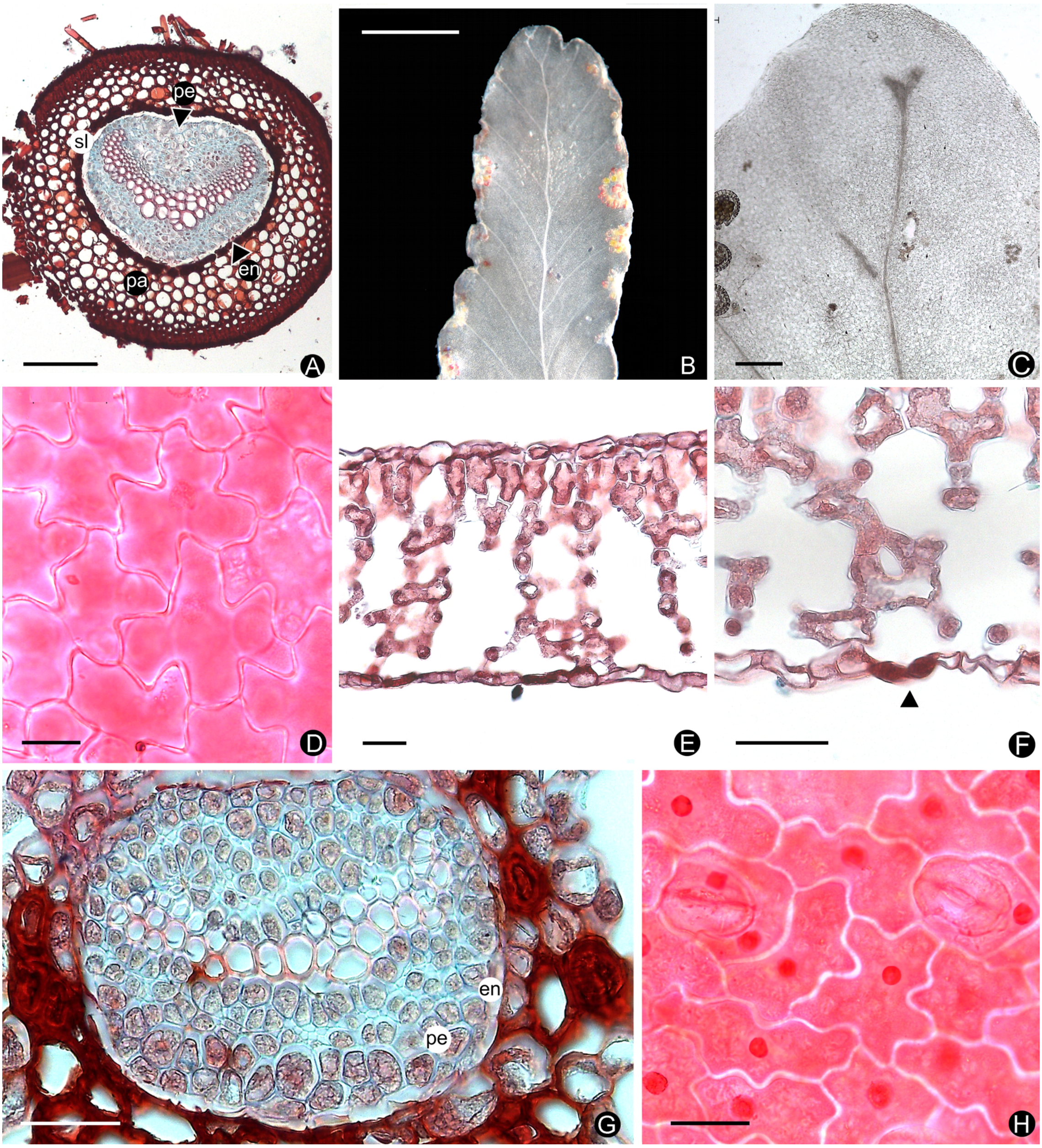

Fig. 2. *Adiantopsis radiata*. **A**: Cross section of petiole. **B**: Segment of pinnule showing open pinnate venation, is noted a principal vein and secondary veins. **C**: Detail of pinnule apex showing the slight bifurcation of the principal vein. **D**: Adaxial epidermis showing the epidermal cells. **E**: Cross section of a pinnule, note the adaxial epidermis, the dorsiventral heterogeneous mesophyll and the abaxial epidermis. **F**: Detail of spongy parenchyma of mesophyll and closed stoma (arrowhead). **G**: Detail of vascular bundle in a cross section of pinnule **H**: Abaxial epidermis showing the anomocytic stomata and epidermal cells with sinuous walls. (en) endodermis; (pa) parenchyma; (pe) pericycle; (sl) sclerenchyma layer. Scale: A, E: 100 µm; H, D, F, G: 30 µm; B: 2 mm; C: 300 µm.

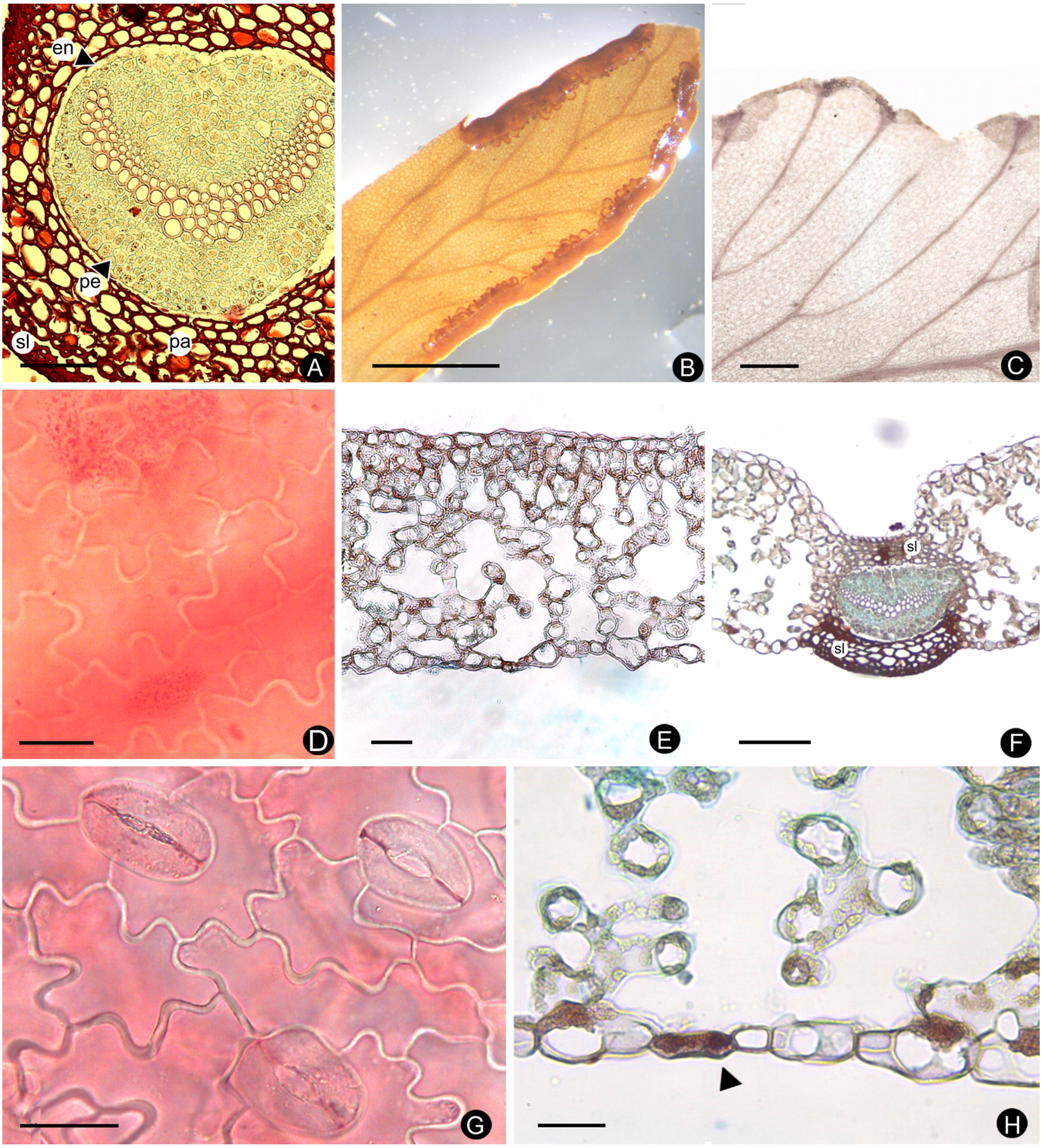

Fig. 3. *Doryopteris concolor*. **A**: Cross section of petiole. **B**: Segment of fertile pinnule showing open pinnate venation. **C**: Detail of dichotomous traces with open venation in sterile pinnule. **D**: Adaxial epidermis showing the epidermal cells. **E**: Detail of dorsiventral heterogeneous mesophyll showing two layers of palisade parenchyma and several layers of spongy parenchyma. **F**: Cross section of pinnule, is observed the main vein formed by an amphicrival vascular bundle. **G**: Abaxial epidermis showing the anomocytic stomata and epidermal cells with sinuous walls. **H**: Abaxial epidermis with closed stoma located to same level of epidermal cells (arrowhead). (en) endodermis; (pa) parenchyma; (pe) pericycle; (sl) sclerenchyma layer. Scale: A: 100 μm; B: 2 mm; C: 300 μm; D, E, F, G, H: 30 μm.

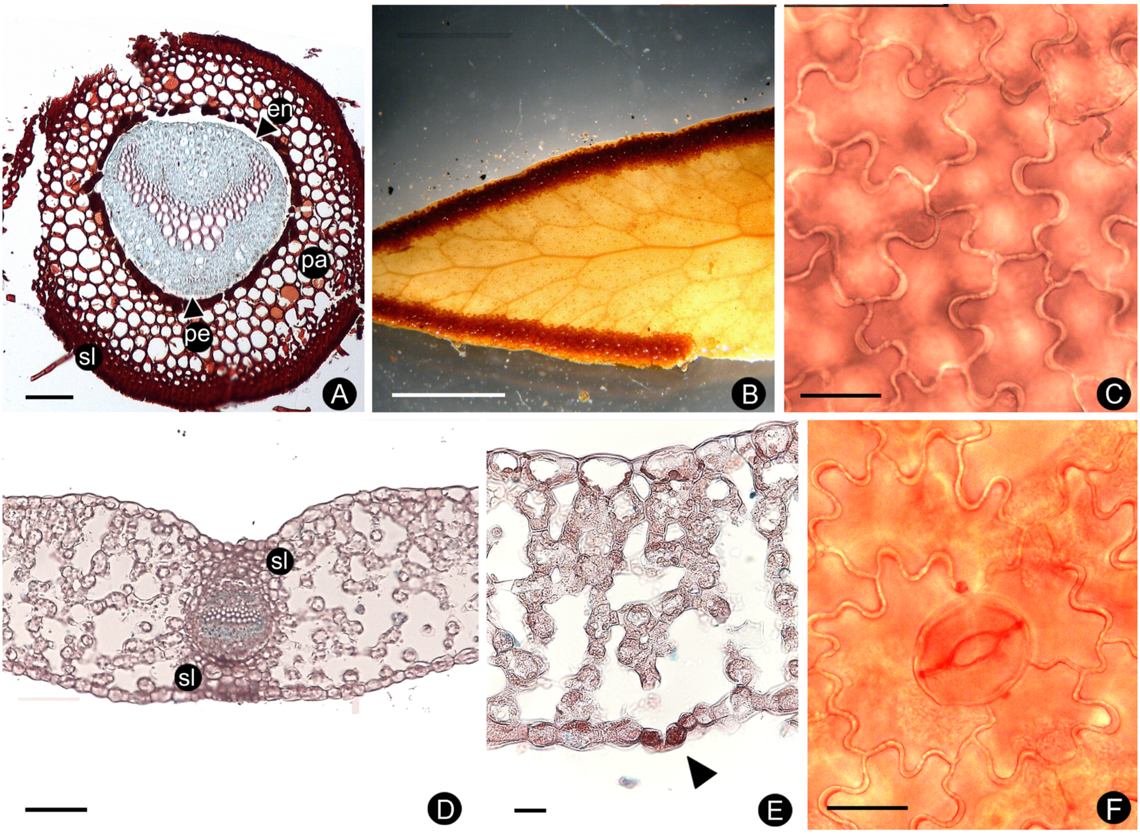

Fig. 4. *Doryopteris pentagona.* **A**: Cross section of petiole. **B**: Pinnule segment showing the closed pinnate venation. **C**: Adaxial epidermis showing the epidermal cells. **D**: Cross section of pinnule, note the main vein formed by a periphloematic vascular bundle. **E**: Detail of abaxial epidermis with open stoma (arrowhead). **F**: Abaxial epidermis showing the anomocytic stomata and epidermal cells with sinuous walls. (en) endodermis; (pa) parenchyma; (pe) pericycle; (sl) sclerenchyma layer. Scale: A, D: 100 µm; B: 3 mm; F, C, E: 30 µm.

and a reduced portion of spongy parenchyma (Fig. 5D). Mesophyll varies in thickness among species, ranging from 152.3 µm in *A. radiata* (Fig. 2E) to 416 µm in *C. glauca* (Fig. 5D) (Table 1). For all species, the principal vein is composed of an amphicrival bundle surrounded by one to two layers of pericycle and one of endodermis (Figs. 2G; 3F; 4D; 5D). The latter present a conspicuous thickening in its walls and sclerenchyma bands that extended to the both epidermis, which are absent in *C. glauca* (Figs. 3F; 4D).

Abaxial epidermis: In surface view, all the studied species had epidermal cells with sinuous to gradually sinuous anticlinal walls. The stomata present kidney-shaped and absence of annex cells, which are classified as anomocytic stomata (Figs. 2H; 3G; 4F; 5E). In all studied taxa, the stomata size ranging from 38.8×30.2 µm (*D. concolor*) to 47.1×36.7 µm (*D. pentagona*). The highest stomatal index (SI) and the highest stomatal density (SD) were recorded in *D. concolor* (Table 1). These parameters were not determined in *C. glauca* due to the dense indumentum on the leaves.

In cross section, the uniseriate epidermis has smaller sub–round cells. The cuticle is smooth and of similar thickness in *A. radiata* and *Doryopteris*

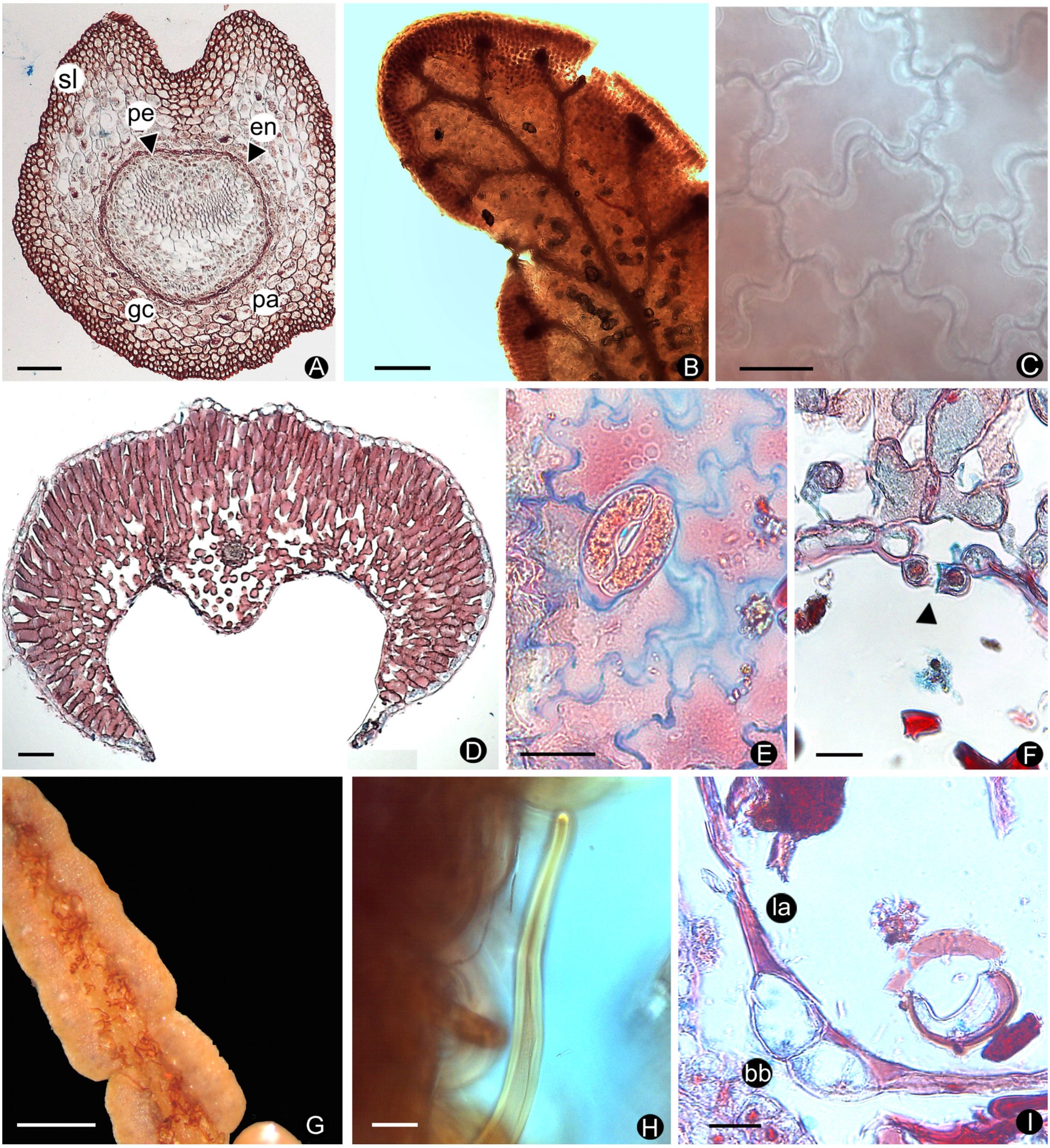

Fig. 5. *Cheilanthes glauca*. **A**: Cross section of petiole. **B**: Pinnule apex, note the open pinnate venation. **C**: Adaxial epidermis showing the epidermal cells. **D**: Cross section of pinnule and the dorsiventral heterogeneous mesophyll. **E**: Abaxial epidermis showing the anomocytic stoma. **F**: Detail of open stoma (arrowhead), slightly raised of epidermal cells. **G**: Abaxial face of pinnule and revolute margins. **H**: Unicellular hair simple in the abaxial epidermis. **I**: Paraphysis in the under surface of a pinnule. (bb) bulbous base; (la) lateral appendages; (en) endodermis; (pa) parenchyma; (pe) pericycle; (sl) sclerenchyma layer. Scale: A: 50 µm; B: 250 µm; C, D, E, F, H, I: 30 µm; G: 1 mm.

species, whereas in *C. glauca,* it is much thicker (Table 1). The stomata are at the same level of epidermal cells in *A. radiata* and the *Doryopteris* species (Figs. 2F; 3H; 4E)*,* and slightly elevated in *C. glauca* (Fig. 5F). Indumentum is observed on the abaxial side of the lamina for most species; however, *A. radiata* present glabrous laminas on both sides. The *Doryopteris* species present simple trichomes, whereas in *C. glauca* simple unicellular trichomes and paraphyses are observed (Figs. 5G-H). The latter is formed by two long lateral appendages with bulbous base and are found cover with a thick cuticle (Fig. 5I).

DISCUSSION AND CONCLUSIONS

The analyses of the species *Doryopteris pentagona* reveal anatomical features, such as uniseriate epidermis and homogeneous mesophyll with abundant spongy tissue, that are usually related to a mesophilous environment (Fahn, 1990), where a medium to high degree of humidity is constant. These species are usually found in this type of environments; therefore, the results obtained might reflect their specialization to mesomorphy.

Unlike other *Doryopteris* species, *D. concolor* presented a dorsiventral heterogeneous mesophyll. Xerophytes features were also reported for epiphyte species (Shield, 1950; Hevly, 1963; Hietz, 2010). In addition, species that present this characteristic have been described as presenting palisade parenchyma. Meza Torres *et al.,* (2013) mentioned *D. concolor* as a facultative epiphyte; this characteristic might explain the presence of a palisade-like structure, which gives this species an advantage for overcoming recurrent droughts (in the 'epiphytic environment'), as well as dry habitats. Indeed, *D. concolor* has the widest distribution range of the studied species, usually occurring mostly in the borders of vegetation, and/or fully exposed to sunlight on disturbed habitats.

Stomata size observed in this study for *D. concolor* is in agreement with data reported by Terán *et al.,* (2009) and Hernández *et al.,* (2010). In addition, in the present study anomocytic stomata were observed in the studied species. Besides this stomatical type, Terán *et al*., (2009) and Hernández *et al*., (2010) reported polocytic and diacytic stomata for *D. concolor.* On the other hand, the epidermal cell density showed differences between species of the genus *Doryopteris*, with *D. concolor* having a lower density (348/mm^2) than that of *D. pentagona* (378.7/mm^2).

The data about the anatomy of *A. radiata* are scarce; only the morphological aspects have been discussed by Ranal (1991) and Link-Pérez & Hickey (2011). Therefore, the results reported here provide the first record about the leaf anatomical morphology of this species. It is characterized by hypostomatic leaves, anomocytic stomata, smooth cuticle and dorsiventral heterogeneous mesophyll with up to two strata of palisade parenchyma and about two to six strata of spongy parenchyma. Also, the present study provides the first background about of the anatomy of leaves in *C. glauca*. These are hypostomatic and the stomata are protected by revolute margins. Furthermore, the results evidenced that this species shares xerophytes features with *C. pilosa* Goldm.*, C. buchtieni* (Rosenst.) R.M. Tryon, *C. notholaenoides* (Desv.) Maxon ex Weath, and *C. bonariensis* (Willd.) Proctor, such as division of blades, leaf indumentum, stomata density and stomata size (Hernández *et al.,* 2011).

The anatomical study evidenced that, of all the species analyzed, *C. glauca* presented features most associated with xeromorphic environments, such as epidermal cells with thickened walls, a smooth and thick cuticle and the indumentum of the lower leaf surface. According to Fahn (1990), 'sun leaves' would have a more developed palisade tissue and a larger mesophyll surface area per unit leaf area and they would be thicker than shade leaves. Regarding *A. radiata* and *Doryopteris* species*,* the mesophyll and cuticle sizes are much thin and smaller, respectively, than those of *C. glauca.* Moreover, *C. glauca* has slightly elevated stomata on the epidermal surface. It is noteworthy that the stomata raised are a character associated to plants from damp localities (Metcalfe & Chalk, 1979).

The species studied showed petioles with subepidermal layers of sclerenchyma; a tendency of the living vascular tissue to separate from the cortical tissue was also observed. Overall, all the species presented similar morpho-anatomical features. The impregnation of the cell walls of the epidermis and cortex with suberin and lignin, particularly in the rhizome and petiole, are mentioned by Hevly (1963) as a modification to control water loss in cheilanthoid ferns. These specialized cell layers probably play the role to prevent water loss through their impermeability to water (Hevly, 1963). These specialized cell layers with lignin impregnation was descripted to *sclerophyllous* plants (Eames & MacDaniels, 1947), but *Doryopteris* species showed the sclerophyllous petiole in parallel with stomata size and density, thickness of the mesophyll and the cuticle in measures that fit to characters of mesophyll plants.

Hevly (1963) proposes that the most common adaptation among xerophytes species is reduced foliar size and division of blades, with their last segments being generally reduced. These morphological characters, added to pinnules abscission during the dry season, have been observed in *A. radiata* (Link-Pérez & Hickey, 2011). In this way, the segments abscission in the dry season represents an adaptation strategy mentioned as “Drought-Deciduous” by Kessler & Siorak (2007).

Cheilanthes glauca shows a complex combination of anatomical characters, which could be explained by the environmental fluctuations to which it is exposed. Indeed, this taxon occurs in a habitat characterized by snowfall as the principal form of precipitation during autumn and winter months, and freezing temperatures. Such conditions have been mentioned to produce similar physiological responses to those induced by desiccating environments (Knight & Knight, 2001). *Cheilanthes glauca* has abundant water during the early spring due of the thaw, but at the end of this season, rainfalls are scarce, and the species undergo dry conditions during the summer months. The combination of features (xerophytes-mesophytes) of *C. glauca* would be a response to extreme changes in water availability (abundance-scarcity) of the environment where it grows.

Fahn and Cutler (1992) explained that in hydrochastic plants, the process of organs curving (e.g. fruit opening), the epidermal cells with walls thick and imbibition and cohesion mechanisms are involved. Due this work was done in an exploratory way, no conclusive evidence was found to associate the leaf movements to any specific anatomical structure. Nevertheless, the sinuous thickened anticlinal walls of the adaxial epidermis in *C. glauca* (species studied here with the stronger leaf curling) could explain these movements through imbibition and cohesion mechanisms, in analogous form to mechanism described for the hydrochastic plants. A comparative study of mesophyll and the epidermis in different states (blade roll up vs expanded blade) in several cheilanthoid ferns species might data interesting insights about the aforementioned hypothesis.

CONTRIBUTION OF THE AUTHORS

All authors have participated in the data collection, interpretation and writing of the manuscript.

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