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ECOSYSTEMS

Structural and histochemical aspects in leaves of six species of *Anemia* (Anemiaceae) occurring in rocky outcrops

PEDRO G. DE MORAES NETO, NATÂNIA P.P. DA SILVA, ANA CATARINA S. FURTADO & ANA CARLA FEIO

Abstract: Rocky outcrops are known for low humidity, rainfall and high solar radiation, factors that limit the development of some vegetables. However, some species of the genus *Anemia* occurring in these environments. Thus, understanding the anatomical characters present in these vegetables are important for botanical and biodiversity knowledge in rock fields. We described the leaf anatomy of six species of *Anemia* to identify characters adapted to rocky outcrops for ferns. Herbarium samples were rehydrated. Field-collected leaves, were also sampled, the material was subjected to standard anatomical study by light microscopy, and secretions were evaluated by histochemical of the secondary compounds, with ruthenium red, tannic acid, ferric chloride, lugol, Sudan black B, vanillin/hydrochloric acid, Dragendorff's reagent and ponceau xylidine. Histochemical tests were positive for phenolic compounds, alkaloids, polysaccharides, and proteins in *A. buniifolia*, *A. oblongifolia*, *A. presliana*, and *A. trichorhiza*. Our findings revealed that several structural and histochemical characters of *Anemia* with trichomes, conical stegmatas, phenolic compounds, mucilages and alkaloids are related to reducing water loss, providing an adaptive value to species in extreme environments, such as rocky outcrops, in addition to new data relevant to the group taxonomy, such as the presence of amphistomatic leaves in *A. trichorhiza*.

Key words: ferns, plant strategy, secretory structures, stegmata.

INTRODUCTION

Anemiaceae is a pantropical family of ferns comprising ca. 115 species in a single genus, *Anemia* Sw. (Mickel 2016). Most of its species are confined to the Neotropical region, with few reaching Africa, Madagascar, southern India, and the Indian Ocean Islands (Mickel 2016). Species of *Anemia* frequently occupying xeric environments such as dry tropical forests and savannas (Hietz 2010), from open and dry areas, to the terry environment to rocky outcrops (Mickel 2016). This habitat is characterized by exposure to severe environmental conditions determined by limiting abiotic factors, such as intense sunlight incidence, high temperatures, hydric stress, and low nutrient availability (Biedinger et al. 2000). In addition, rocky outcrops represent refuges for endemic, threatened, and geographically disjointed species, commonly being the source of new species discovery (Oliveira & Godoy 2007, Silva 2016).

Over the last two decades, few studies have focused on rocky outcrops ferns (Xavier & Barros 2003, Santos & Sylvestre 2006, Santos et al. 2006, Ribeiro et al. 2007, 2011, Silva 2016). Ribeiro et al. (2007) analyzed the leaf anatomy of *A. tomentosa* var. *anthriscifolia* and *A. villosa* Humb. & Bonpl. ex Willd., evaluating the species' adaptive strategies to hydric stress in rocky

outcrops. Despite the importance demonstrated by these studies' results, additional studies on the leaf anatomy of *Anemia* might reveal different characters adapted to hostile environmental conditions (Oliveira & Godoy 2007). Anatomical traits related to such conditions have been poorly studied for ferns (Ribeiro et al. 2007), with rare reports for plasticity on ecologically adaptative characters (Bradshaw 1965). Another few investigated field in *Anemia*'s anatomy is the diversity of secretory structures and its compounds' chemical nature, which may present an untapped potential as new sources of natural products of economic importance (Santos et al. 2006, Ribeiro et al. 2007).

Thus, in order to identify anatomical structures of *Anemia* and interpret its presence in the environment of occurrence, we intend to comparatively analyze the leaf structure of six species and the histochemistry of four species of *Anemia* occurring in rocky outcrops.

MATERIALS AND METHODS

To contemplate the largest number of *Anemia* specimens, selection considered the availability of living specimens from field expeditions and herborized specimens deposited at the MG herbaria. Fully expanded leaves of *A. buniifolia, A. oblongifolia, A. presliana*, and *A. trichorhiza* were collected from natural populations at Serra of the Martírios/Andorinhas State Park (PESAM). This integral protection area is located in the municipality of São Geraldo do Araguaia, southeastern State of Pará (06°03'00" to 06°23'00" S, 48°22'30" to 48°36'30" W). In this location, the average monthly rainfall is less than 60 mm (Martorano et al. 1993). We additionally complemented our sampling with herborized specimens of *A. elegans* and *A. phyllitidis* from the MG herbarium. All information, including species authorship and voucher specimens, is summarized in Table I.

Herbarium samples from 18 specimens (fragments from the: 1. median portion of the leaf-blade's interveinal region, 2. midrib, 3. margin, 4. petiole, and 5. rachis) of all six samples were rehydrated according to the methodology of Smith & Smith (1942) which consists of boiling the material in water for 5 min or until submerged, then placing 2% potassium hydroxide for 30 minutes, washing several times with distilled water at intervals of 30 min, dehydrate in ethylic series from 20% - 60% and at the end stored in 70% ethanol. They were then dehydrated in a decreasing ethanol series and embedded in 2–hydroxyethyl methacrylate resin for sectioning (Historesin Leica®, solutions were prepared according to manufacturer's instructions) following Meira & Martins (2003). These samples were transversely and longitudinally sectioned from 3 to 7 µm thick. Sections were stained with toluidine blue pH 4.6 (O'Brien et al. 1965), and slides were mounted in resin (Permount®, Fisher Scientific, New Jersey, USA). Some samples were also cleared with 5% sodium hydroxide and 20% hypochlorite solutions, stained with 50% ethanol-diluted fuchsin (Shobe & Lersten 1967), and mounted in glycerinated gelatin (Only samples collected Kaiser 1880).

In the field were used for histochemical tests Table III. Three fixative treatments were used for three different sets of samples. The first set was fixed in FAA formalin: acetic acid: 50% ethanol, 1:1:18 by volume, Johansen 1940) for 24 h for both structural characterizations in light microscopy and histochemical hydrophilic substances. The second set was fixed in neutral buffered formalin (NBF) for 48 h (Lillie 1965) to preserve lipophilic substances. The third was fixed in FSF ferrous sulfate in formalin (Johansen 1940) to detect total phenolic compounds. Part

Table I. Selected taxa and the respective analyzed vouchers. Herbarium acronyms in parentheses, following Thiers (2018). Notes: (TE) terrestrial; (RU) rupicolous. Material collected from herbarium specimens is indicated with an (*).

Species	Voucher	Life-form	Habitat
A. buniifolia (Gardner) T.Moore	Pereira 1067* (MG) Moraes Neto & Feio 02, 05, 06 (MG)	RU	In rock cracks, on rocky escarpments under full sun, over stony soils
A. elegans (Gardner) C.Presl	Pereira 1065* (MG) Salino 11733* (MG) Glaziou 20163* (MG)	RU	In shaded locations
A. oblongifolia (Cav.) Sw.	Pereira 1066*, 1072* (MG) Heringer 3534*, 6317*, 17702* (MG) Moraes Neto & Feio 07 (MG)	RU	Over sandstone, in shaded environments, in open locations under full sun, on rocky escarpments, in savannas, over stony soils, in dry marshes, and vegetation with sparse trees and shrubs
A. phyllitidis (L.) Sw.	Sobral 3824* (MG) Oliveira 6333* (MG) Jangoux 1544* (MG) Heringer 1025*, 3216*, 6210* (MG) Plowman 8514* (MG)	RU/TE	By the roadside over soggy soils; in upland forests; riparian forests; lowland forests over rocky slabs; on rock blocks; in forests below waterfalls, over rock blocks and alongside streams
A. presliana Prantl	Pereira 1029* (MG) Moraes Neto & Feio 01, 03, 04 (MG)	RU	In shaded locations and under full sun
A. trichorhiza Gardner ex Hooker	Pereira 1071* (MG) Moraes Neto & Feio 08 (MG)	RU	On rocky escarpments by the roadside under full sun

of each sample set was also dehydrated in a tert-butanol series (Johansen 1940), embedded in histological paraffin with DMSO (Paraplast® Embedding Medium, Oxford Lab., USA), and serially sectioned at 10–12 µm thick (Kraus & Arduin 1997).

The histochemical tests were only performed on field-collected samples of *A. buniifolia*, *A. oblongifolia*, *A. presliana*, and *A. trichorhiza*. All species have idioblasts dispersed in their leaf tissues, besides secretory structures and glandular trichomes (Fig. 3a-3l).

For the detection of the main classes of secondary compounds, the following histochemical tests were carried out: ruthenium red for acidic mucilage (Gregory & Baas 1989), tannic acid/ferric chloride for neutral mucilage (Pizzolato & Lillie 1973), lugol for starch (Johansen 1940), Sudan black B for total lipids (Pearse 1985), vanillin/hydrochloric acid for tannins (Mace & Howell 1974), Dragendorff's reagent for alkaloids (Furr & Mahlberg 1981), and ponceau xylidine for total proteins (O'Brien & McCully 1981). Four leaf samples of each individual of the four histochemistry species mentioned in Table I.

For standard control, a sample without reagent was made to compare the histochemical test in each reaction. Slides were mounted in glycerinated gelatin (Kaiser 1880).

Table II. Number of layers of parenchyma cells, sclerenchyma cells, endoderm and pericycle in the petiole and rachis, (-) inapplicable.

Species	Petiole				Rachis			
	Number of layers							
	Parenchyma	Sclerenchyma	Endoderm	Pericycle	Parenchyma	Sclerenchyma	Endoderm	Pericycle
A. Buniifolia	4 to 9 layers	1 to 2 layers	1 layer	1 to 2 layers	-	-	-	-
A. elegans	5 to 7 layers	-	1 layer	1 to 2 layers	-	-	-	-
A. oblongifolia	5 to 13 layers	1 to 2 layers	1 layer	1 to 2 layers	12 to 14 layers	6 to 7 layers	1 layer	1 to 2 layers
A. phyllitidis	7 to 15 layers	2 to 3 layers	1 layer	1 to 3 layers	9 to 17 layers	2 to 6 layers	1 layer	1 to 3 layers
A. presliana	7 to 11 layers	1 to 5 layers	1 layer	1 to 2 layers	7 to 16 layers	1 to 4 layers	1 layer	1 to 3 layers
A. trichorhiza	5 to 8 layers	2 to 3 layers	1 layer	1 to 2 layers	3 to 9 layers	1 to 2 layers	1 layer	1 to 2 layers

Sections were obtained with a rotary microtome (model RM 2245, Leica® Biosystems, Heidelberg, Germany) using tungsten knives (Leica® Biosystems). Observations and photographic documentation were performed with a light microscope (Axio Scope, A1, ©Carl Zeiss, Göttingen, Germany) equipped with a digital camera (AxioCam HRc, ©Carl Zeiss). Macro images were obtained using a stereomicroscope (SteREO Discovery, V8, ©Carl Zeiss) coupled to a digital camera (AxioCam ICc5, ©Carl Zeiss). Anatomical descriptions were made using the terms adopted by Brasil (2009) contour of the Petiole and leaf, Mickel & Lersten (1967) for stomata typology, by Metcalfe & Chalk (1950), Ogura (1972), and Fahn (1990) for trichomes, and by Ogura (1972) for the vascular system.

RESULTS

Petiole

The petiole contour is convex plane (Fig. 1a) in transversal section in *A. elegans* and *A. trichorhiza*, slightly square (Fig. 1b) in *A. oblongifolia* and *A. presliana*, and cordate (Fig. 1c) in *A. buniifolia* and *A. phyllitidis*. All species showed cuticle (Fig. 1f) and unistratified epidermis (Fig. 1h), with stomata low frequency observed above the epidermic cells in *A. buniifolia*, *A. oblongifolia, A. phyllitidis* and *A. presliana* forming the forming aerophore (Fig. 1d). Glandular trichomes *A. elegans, A. phyllitidis* and *A. trichorhiza* (Fig. 1e) were identified, but non-glandular trichome was observed in all species (Fig. 1f). Conic stegmata (Fig. 1g) on periclinal walls of the epidermis were only identified in *A. oblongifolia*, *A. presliana*, and *A. trichorhiza*. All species have he cortex with 1–5 layers of sclerenchyma (Fig. 1l), 5–13 layers of parenchyma (Fig. 1b), except for *A. elegans,* in which the cortex only shows parenchymatous cells. Pericycle is formed by 1–2 layers of parenchymatous cells in all species, except for

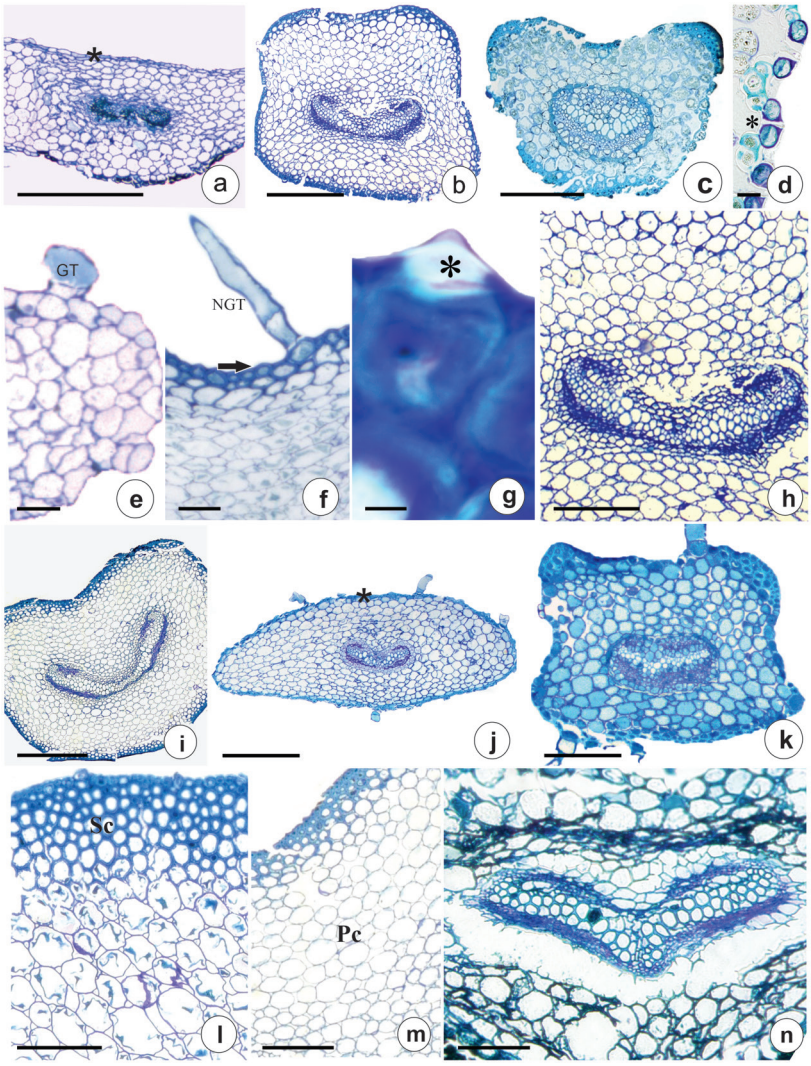

Figure 1a-1n. ***Anemia buniifolia*** **(c, d),** ***Anemia elegans*** **(a, e),** ***Anemia oblongifolia*** **(b, g, h, i, j, l, m),** ***Anemia presliana*** **(f) and** ***Anemia trichorhiza*** **(k, n). a-h) Petiole cross-sections of** ***Anemia*** **species, i-n) Rachis cross-sections of** ***Anemia*** **species. a) Convex plane, with unistratified epidermis (*), b) Slightly square, c) Cordate, d) Stomata forming the respiratory line (Asterisk), e) Glandular trichomes (GH), f) Non-Glandular trichomes (NGH) and slender cuticles (arrow), g) Conical stegmata on the epidermis (Asterisk), h) Arch-shaped amphicribral vascular bundles, i) Cordate, cortical region composed of sclerenchyma and parenchyma, j) Convex plane, with unistratified epidermis (*), k) Slightly square, l) Sclerenchymatous cells (SC), m) Parenchyma cells (PC) and n) V-shaped amphicribral vascular bundle. Bars: 20 µm (k, l), 50 µm (a, b, f, g, h, n) 300 µm (c, d, e, i, j, m).**

A. phyllitidis, in which it may have up to 3 cell layers. The vascular system is amphicribal (Fig. 1h), arranged in an arch in all species.

Rachis

The leaves of *A. buniifolia* and *A. elegans* lack a rachis. In contrast, the rachis of *A. phyllitidis* was cordate (Fig. 1i) in transversal section, cylindrical (Fig. 1j) in *A. oblongifolia* and *A. trichorhiza*, and slightly square (Fig. 1k) in *A. presliana*. All species present slender cuticle (Fig. 1k), unistratified epidermis (Fig. 1j), and glandular trichome (Fig. 1e), except for *A. trichorhiza* that trichomes. Additionally, all species have a cortex of 1–7 layers of sclerenchyma (Fig. 1l), followed by 3–17 layers of parenchyma (Fig. 1m) (Table II). The pericycle has 1–2 cell layers in *A. oblongifolia* and *A. trichorhiza*, while in *A. phyllitidis* and *A. presliana* it may have up to 3 layers (Table II). The vascular system was amphicribal and arranged in an arch, except for *A. trichorhiza*, which was arranged in a "V".

Leaf-blade

All species have both surfaces of the epidermis (Fig. 2a-b) with sinuous anticlinal walls in frontal view. Non-glandular (Fig. 2c) and glandular (Fig. 2d) trichomes occur in all species, except for

Table III. Results of histochemical tests applied to the secretion of leaf structures present in *Anemia* species. Notes: (+) positive result; (-) negative result.

Test		Species			
		A. buniifolia	***A. oblongifolia***	***A. presliana***	***A. trichorhiza***
Total lipids	Sudan Black B	-	-	-	-
Total phenolic compounds	Formalin with ferrous sulphate (Fig. 3a-3c)	+	+	+	+
Tannin	Vanillin-hydrochloric acid	-	-	-	-
Alkaloid	Dragendorff's reagent (Fig. 3d-3e)	+	+	+	+
Acid mucilage	Ruthenium red (Fig. 3f-3h)	+	+	+	+
Neutral mucilage	Tannic acid-ferric chloride	-	-	-	-
Starch	Lugol (Fig. 3i-3j)	+	+	+	+
Total proteins	Ponceau Xylidine (Fig. 3k-3l)	+	+	+	+

A. buniifolia that lacks glandular trichomes. Floating stomata (in surface view, they appear surrounded by an annular subsidiary cell, without coming into contact with any of its anticlinal walls (Mickel 1962)) (Fig. 2e) are present only on the abaxial surface of leaves of *A. buniifolia, A. oblongifolia, A. phyllitidis*, and *A. presliana*. Alternatively, in *A. elegans*, they only occur on the adaxial surface, and in *A. trichorhiza*, they appear on both surfaces (Fig. 2l) (amphistomatic leaves). Suspended stomata (presents intruded wall of surrounding epidermal cell (Mickel & Lersten 1967)) (Fig. 2f) were also observed on the abaxial surface only of *A. buniifolia*.

The leaf-blade of all species have epidermal characters similar to petioles, with conical stegmata observed only in *A. oblongifolia*, *A. phyllitidis*, *A. presliana*, and *A. trichorhiza.* The mesophyll of *A. elegans* is homogeneous with a lacunose parenchyma. The other species, dorsiventral mesophyll. The midvein is concave-convex (Fig. 2k) with the vascular system comprising amphicribal bundles in *A. buniifolia*, *A. phyllitidis*, and *A. trichorhiza*. Even though a midvein was absent in *A. elegans*, *A. oblongifolia*, and *A. presliana*, their vascular bundles are also amphicribal (Fig. 2l). A sheath extension made of parenchymatous cells with thickened walls was only recorded in *A. elegans*. Alternatively, *A. phyllitidis* and *A. trichorhiza* have a sheath extension made of parenchymatous cells with thickened walls plus sclerenchymatous cells near the epidermis (Fig. 2m).

Secretory structures

During the collection expedition, no secretion was observed macroscopically on the leaf surfaces of the species. Histochemical tests were

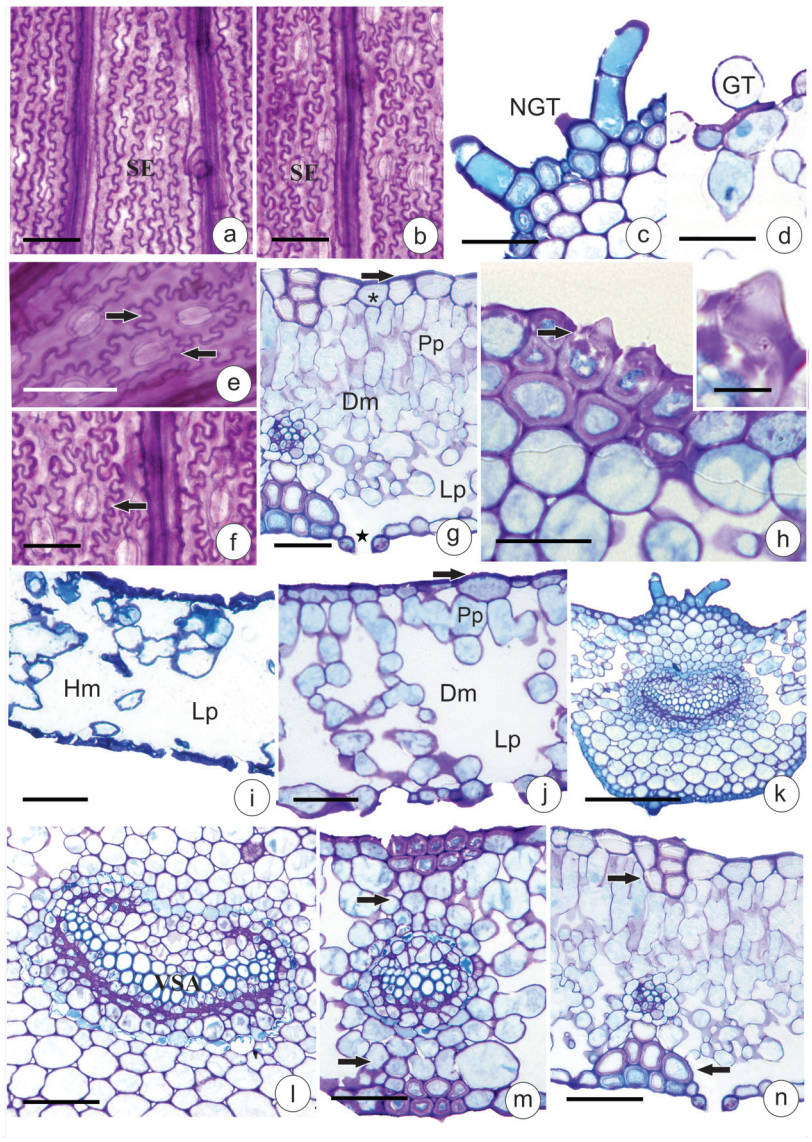

Figure 2a-2n. ***Anemia buniifolia*** **(a, b, e, f),** ***Anemia elegans*** **(i),** ***Anemia oblongifolia*** **(g, n) and** ***Anemia phyllitidis*** **(c, d, h, j, k, l, m). Leaf blade of** ***Anemia*** **species. a) Frontal view of the adaxial epidermis (SE) Sinuous epidermis, b) Frontal view of the abaxial epidermis (SE) Sinuous epidermis, c) (NGH) Non-glandular trichomes, d) (GH) Glandular trichomes, e) Floating-type stomata (arrow), f) Suspended-type stomata (arrow), g) Slender cuticles (arrow), Unistratified epidermis (Asterisk), homogeneous mesophyll (Hm) and Lacunose parenchyma (Lp) and stomata above the level of ordinary epidermal cells (Star), h) Epidermis with conical stegmata (arrows), i) Homogenous mesophyll (Hm) Lacunose parenchyma (Lp), j) Slender cuticles (arrow) and dorsiventral mesophyll (Dm), Lacunose parenchyma (Lp), k) Concave-convex midrib, l) Midrib vascular system with amphicribral bundles (VSA) Vascular system amphicribral, m) Parenchymatous sheath extension (arrows) and n) Sclerenchymatous cells (arrow). (Bars: 20 µm (d, e, m), 50 µm (a, b, c, f, g, h, i, j, k, l, n).**

performed on petioles, rachis, and leaf-blades, with positive results for phenolic compounds (Fig. 3a-c.), alkaloids (Fig. 3d-e), acidic mucilage (Fig. 3f-h), starch, and total proteins (Fig. 3k-l) in *A. buniifolia, A. oblongifolia, A. presliana,* and *A. trichorhiza*. The same species showed negative results for total lipids, tannins, and neutral mucilage.

DISCUSSION

Ferns may exhibit morphological patterns that indicate ecological plasticity in response to heterogeneous environments (Arens 1997). This plasticity might explain their adaptive success to diverse conditions (Bradshaw 2006), such as the limited supply of water and nutrients, high irradiation of sunlight, and wide temperature variations, as observed in rocky outcrops (Burke et al. 2002).

All six studied species of *Anemia* showed common leaf epidermal cells with sinuous anticlinal walls, corroborating a pattern already reported to rocky outcrop species (Pant & Khare 1972, Ribeiro et al. 2007, 2011). The sinuosity of anticlinal epidermal cell walls may be related

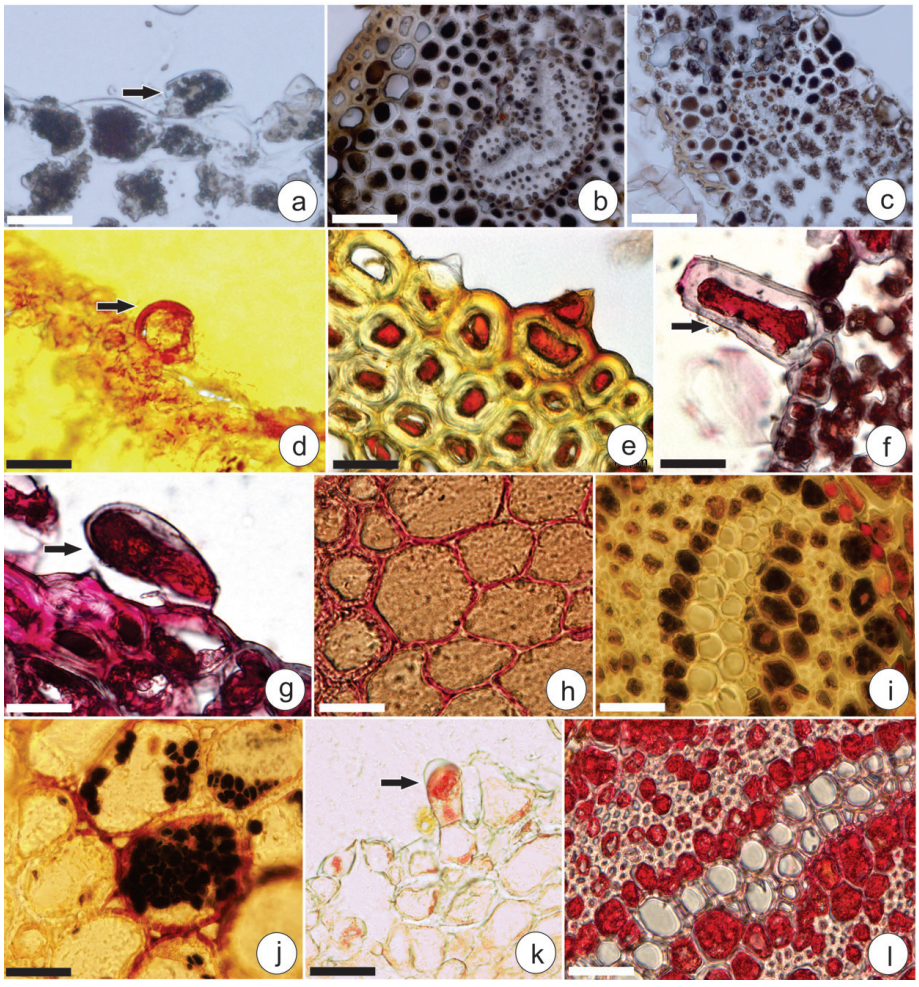

Figure 3a-3l. ***Anemia oblongifolia*** **(g, j),** ***Anemia presliana*** **(a, h) and** ***Anemia trichorhiza*** **(b, c, d, e, f, i, k, l). Petiole (b, d, h, i, j, l) and leaf-blade (a, c, e, f, g, k). Results of histochemical tests applied to the secretory product of the foliar secretory structures of** ***Anemia*** **species, a-c) Formalin with ferrous sulphate: Total phenolic compounds, notice the secretory product in the glandular trichomes (arrow), d-e) Dragendorff's reagent: Alkaloids (arrow), f-h) Ruthenium red: Acid mucilage, notice the secretory product in the glandular trichomes (arrow), i-j) Lugol: Starch grains, k-l) Ponceau Xylidine: Total proteins, notice the secretory product in the glandular trichomes. Bars: 30 µm (a, d, g, j, k), 50 µm (b, c, h, i, l).**

not only to a high incidence of solar radiation (Wilkinson 1979, Graçano et al. 2001) but also to low water availability. This sinuosity is associated with leaf expansion/contraction due to water inflow/outflow, which may lead to the development of mechanical adaptations that help prevent the collapse of the organ (Krauss 1949), such as rocky outcrops species of *Anemia*. The presence of sinuous walls confirms that environmental factors influence this character's plasticity, the primary way plants react to their habitat's heterogeneity (Bradshaw 1965, Barboza et al. 2006, Valladares et al. 2007).

The amphistomatic leaf-blade found in *A. trichorhiza* is considered a rare trait among ferns (Kramer 1990). This character state is recorded in this study for the first time for *Anemia*. Stomata occurrence on both leaf surfaces is considered a xeromorphic feature (Parkhurst 1978) wich allows for higher carbon dioxide conduction and increased photosynthetic capacity, both of which provide adaptive advantages to plants living in environments subjected to high sunlight incidence, establishing itself as pioneers in the process of ecological succession (Mott et al. 1982). Amphistomatic leaves are also observed in Angiosperms, being common mainly in Asteraceae that occur in open areas, of high light intensity (Silva et al. 2019, Vieira-Neto et al. 2020), so this is an important feature for kind of open environments (Liesenfeld et al. 2019). However, studies suggest that photosynthetic efficiency is

related to a regular gradient of carbon dioxide in the leaf and the presence of stomata on both leaf surfaces is not favorable to this gradient. Thus, stomata on both leaf surfaces do not contribute to the efficiency of photoassimilate production in arid environments (Mcelwain et al. 2005).

The epistomatic leaf-blade, which we found in *A. elegans*, is an uncommon trait in *Anemia*. Hypostomatic leaves, on the other hand, such as those found in *A. buniifolia*, *A. oblongifolia*, *A. phyllitidis*, and *A. presliana*, are more common (Ogura 1972, Ribeiro et al. 2007).

Although floating stomata have been initially described based on *A. phyllitidis* (Link 1841), they are not widely distributed in *Anemia*, being absent in *A. adiantifolia* (L.) Sw. and *A. villosa* Humb. & Bonpl. ex Willd. (Mickel & Lersten 1967). On the other hand, floating stomata are not exclusive to *Anemia*, having also been reported to genera of Polypodiaceae and Salviniaceae, such as *Lemmaphyllum* C.Presl (Kondo 1962), *Pyrrosia* Mirb. (Mickel & Lersten 1967), *Pleopeltis* Humb., and *Azolla* Lam. (Inamdar et al. 1971). From an evolutionary perspective, floating stomata are possibly specialized structures (Mickel 1962), yet no specific functional or ecological role has ever been attributed to them (Mickel & Lestern 1967, Inamdar et al. 1971, Sen & De 1992). In xerophyte plants, stomata are typically sunk in epidermal depressions, which protect the plant against excessive water loss (Beck 2010), in the opposite hand, the *Anemia* analyzed presents stomata exposed to edaphyte factors, making them more susceptible to water stress, on the other hand, it can be assumed that different biochemical and physiological mechanisms are found in plants that undergo water scarcity (Bohnert et al. 1995), as observed in *Drymoglossum piloselloides* (L.) Presl and *Pyrrosia longifolia* (Burm.) Morton, in which biochemical studies confirmed the presence of CAM metabolism (Wong & Hew 1976), reinforcing the presence of this type of metabolism in some ferns (Evert & Eichhorn 2014), responsible for increasing water use efficiency (Bohnert et al. 1992). Therefore, we infer a probable relationship that ferns with floating stomata, among them *Anemia*, present CAM metabolism, as an adaptive strategy to water stress, since no morphological adaptations were observed strong enough to withstand long periods of drought. Further in-depth analyses of their ecophysiological role, especially in extreme environments, are needed.

Glandular and non-glandular trichomes are common among species of *Anemia* (Roux et al. 1992, Ribeiro et al. 2007, 2011). Both are considered xeromorphic characters, as they play a significant role in preventing water loss (Gibson 1996) by regulating the temperature and reflecting the excess radiation (Larcher 2000). It also optimizes the absorption of atmospheric humidity by improving water retention on the leaf surface (Hietz & Briones 1998). Additionally, Manetas (2003) proved that trichome can protect tissues from uv-b damage. These characteristics reinforce the probable role of reducing the sweating played by trichome (Barros & Soares 2013).

In terms of plant-animal interactions, trichomes also contribute to reducing herbivory (Dickison 2000). Calo et al. (2006) cited that trichomes or other external leaf structures act as a physical barrier against herbivore attacks. In contrast to this statement, Eisner et al. (1998) state that this type of protection depends on this structure's morphology, such as the hooked trichomes of *Mentzelia pumila* var. *pumila* (Nutt.) Torr. & Gray, capable of trapping and killing some arthropod species that come into contact with the leaf. In that sense, it should be noted that no visitor was seen during field collections. A more extensive study on the existence of possible plant-animal interactions would be worthwhile.

Stegmata are characterized by a thickened wall adjacent to the underlying sclerenchyma cells, with progressively thinner lateral walls and thin outer walls with silica bodies inside,are observed in families of monocotyledons Orchidaceae, Arecaceae, Bromeliaceae and Cyperaceae (Prychid et al. 2004), and apparently typical in Trichomanes L. (Mettenius 1865).

Conical stegmata, found on petioles and external periclinal walls of common epidermal cells in *Anemia*, are variably shaped (i.e., conical, elliptical, or spherical) and genetically regulated, being little influenced by environmental factors (Møller & Rasmussen 1984). These structures are restricted to some plant groups, such as *Anemia*, in which its occurrence on the leaf-blade epidermis might be related to the reduction of water loss (Campos & Labouriau 1969, Ribeiro et al. 2007), representing a xeromorphic trait associated with the efficiency of water intake (Zanenga-Godoy & Costa 2003). In animal-plant interactions, stegmata also contribute to the defense against microorganisms and smaller herbivores, blocking the latter's urinary trait (Vicari & Bazely 1993).

Some types of anatomical characters in the mesophyll can optimize the photosynthetic process due to environmental variations (Erbano & Duarte 2010). The most accepted hypothesis is that mesophyll architecture is determined by light intensity, being a highly plastic feature (Arens 1997), hypothesis corroborated by our findings, where the type of mesophyll varied according to light intensity. The homogenous-type mesophyll with spongy parenchyma found in *A. elegans* commonly occurs in species growing in shaded environments (Graçano et al. 2001), as it allows for optimized use of solar radiation (Larcher 2000), increasing photosynthetic efficiency (DeLucia et al. 1996). On the other hand, the dorsiventral mesophyll usually occurs in species of *Anemia* from rocky outcrops (Ribeiro et al. 2007, 2011). The occurrence of the dorsiventral mesophyll in ferns is closely related to environments subjected to high sunlight incidence (Arens 1997), with the plicate parenchyma favoring increased photosynthetic rates due to its high concentration of chloroplasts (Queiroz-Voltan et al. 2011).

The synthesis of secondary metabolites in plants represents a necessary physiological process that might their effect intensified when these compounds interact, hindering development and resistance in the event of simultaneous attacks by herbivores and microbes (Wink 2010). These properties make secondary metabolites more efficient in biologically defending plants (Wink 2008). It is noteworthy that intrinsically correlated abiotic factors may jointly affect the secondary metabolism of the plant. Examples of such factors may include seasonality, rainfall, temperature, and altitude (Gobbo-Neto & Lopes 2007).

Phenolic compounds are usually related to different adaptive strategies like protection against drying and attack by animals (Feio et al. 2013), being commonly found in ferns (Ogura 1972) and xerophytes from other plant groups (Pyykkö 1966). In both groups, their production increases under unfavorable conditions, therefore acting as a stress bioindicator (Siddiqui & Arif-uz-Zama 2004, Achakzai et al. 2009). Phenols are essential for the adaptation of plants to terrestrial environments, acting in the defense not only against fungal proliferation (Croteau et al. 2000, Taiz & Zeiger 2006) but also against herbivory by providing plants with unpleasant tastes and smells that prevent them from being attacked (Strack 1997). Furthermore, phenols have also been reported to play a significant role in protecting plants against drought and high sunlight intensity (Waterman & Mole 1994).

The presence of alkaloids, which we found in all species analyzed, has been under studied

among seedless plants. However, such a rarity could be due to the scarcity of fern studies that include histochemical analyses on this metabolic class (Evans 1996, Watson et al. 2001, Feio et al. 2013). Aside from reducing palatability, alkaloids are also related to the defense against herbivores and parasites (Vicari & Bazely 1993, Facchini 2001). They also play an allelopathic role (Robinson 1974), frequently found in increased amounts on plants under some kind of stress (Vicari & Bazely 1993). They represent a more economical strategy than defoliation in nutrient-poor environments (Gershezon 1983), such as rocky outcrops.

Mucilages are natural constituents of the plant body, being more common in organs with water-retention function (Simões et al. 2000). Due to their physical characteristics, they might aid in leaf extension, increasing the water supply during hydric stress (Thadeo et al. 2009). These mixed-nature compounds are pivotal for protecting developing organs, acting in the defense against herbivores and tolerance against desiccation (Gregory & Baas 1989). Furthermore, proteins, in addition to being crucial components in the metabolism and structure of plants, (Thadeo et al. 2009) can also act together with tannins against agents in protection against herbivores and pathogens (Klein et al. 2004, Markham et al. 2006, Miguel et al. 2006). Its presence in these structures can (Thadeo et al. 2009).

Although naturally found in the plant cell protoplast, starch has been suggested to have a storage function in the leaves of *A. villosa* (Ribeiro et al. 2007). Additionally, starch may also be related to the prevention of mechanical damage to the cell membrane during hydric stress (Vicré et al. 1999) since it occurs in tissues of desiccation-tolerant seeds. Feio et al. (2013) indicated that in species of *Elaphoglossum* Schott ex Smith, the starch grains found on the leaf-blade might be transiently stored in chloroplasts during the day and degraded at night to maintain the metabolism in plants under stress**.** The stage of development, hydric stress, and leaf loss may alter the amount and regions where starch is found in plant structures. This polysaccharide was observed in the rhizome of the morphological and histochemical study of *Adiantum latifolium* Lam. (Pteridaceae) (Da Cruz et al. 2019).

Histochemistry in representatives of Anemiaceae or closely-related groups is still incipient. The absence of studies focusing on these groups means that the discussions are new and often based on unrelated groups. However, taxonomic studies can be useful for a preliminary analysis of the identification of secretions in secretory structures (Vizzotto et al. 2010).

Despite the environmental restrictions imposed by the rocky outcrop habitat, anemia species are well adapted to this type of environment, adaptive success is related to the presence of anatomical characters with trichomes and conical stegmatas and histochemical characters such as phenolic, mucilages and alkaloids, which in addition to reducing water loss, protect against herbivory, which suggests the development of adaptive strategies for this type of environment. The present study also provides new data relevant to the taxonomy of the group, such as the presence of ampyrtomatic leaves in *A. trichorhiza* that increase knowledge about the leaf anatomy of *Anemia*.

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REFERENCES

ACHAKZAI AKK, ACHAKZAI P, MASOOD A, KAYANI SA & TAREEN RB. 2009. Response of plant parts and age on the distribution of secondary metabolites on plants found in quetta. Pak J Bot 41: 2129-2135.

ARENS NC. 1997. Responses of leaf anatomy to light environment in the tree fern Cyatheta caracasana (Cyatheaceae) and its application to some ancient seed fern. Palaios 12: 84-94.

BARBOZA SBSC, GRACIANO-RIBEIRO D, TEIXEIRA JB, PORTES TA & SOUZA LAC. 2006. Anatomia foliar de plantas micropropagadas de abacaxi. Pesqui Agropecu Bras 41: 185-194.

BARROS IO & SOARES AA. 2013. Anatomical adaptations in leaves of the quince and velame of the Brazilian caatinga. Rev Cienc Agron 44: 192-198.

BECK CB. 2010. The leaf: Perspective: evolution of the leaf. In: BECK CB (Ed), An introduction to plant structure and development: Cambridge University Press, New York, United States of America, p. 324-360.

BIEDINGER N, POREMBSKI S & BARTHLOTT W. 2000. Vascular plants on inselbergs: vegetative and reproductive strategies. In: POREMBSKI S & BARTHLOTT W (Eds), Inselbergs: biotic diversity of isolated rock outcrops in tropical and temperate regions. Berlin: Springer-Verlag, Germany, p. 117-142.

BOHNERT HJ, NELSON DE & JENSEN RG. 1995. Adaptations to Environmental Stresses. Plant Cell 7: 1099-1111.

BOHNERT HJ, VERNON DM, DEROCHER EJ, MICHALOWSKI CB & CUSHMAN JC. 1992. Biochemistry and molecular biology of CAM. Society for Experimental Biology Seminar Series 49: 113-137.

BRADSHAW AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13: 115-151.

BRADSHAW AD. 2006. Unravelling phenotypic plasticity – why should we bother. New Phytol 170: 644-648.

BRASIL. 2009. Ministério da Agricultura, Pecuária e Abastecimento: Glossário ilustrado de morfologia / Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. – Brasília: Mapa/ACS, p. 13-406.

BURKE JM, GARDNER KA & RIESEBERG LH. 2002. The potential for gene flow between cultivated and wild sunflower (Helianthus annuus) in the United States. Am J Bot 89: 1550-1552.

CALO L, GARCÍA I, GOTOR C & ROMERO LC. 2006. Leaf hairs influence phytopathogenic fungus infection and confer an increased resistance when expressing a Trichoderma α-1, 3-glucanase. J Exp Bot 57: 3911-3920.

CAMPOS AC & LABOURIAU LG. 1969. Corpos silicosos de gramíneas dos cerrados. II. Pesqui Agropecu Bras 4: 143-151.

CROTEAU R, KUTCHAN TM & LEWIS NG. 2000. Natural products (secondary metabolites). In: BUCHANAN B, GRUISSEM W & SONES R (Eds), Biochemistry and molecular biology of plants, Rockville: American Society of Plant Physiologists, Maryland, USA, p. 1250-1318.

DA CRUZ MB, CHAVES ALF, CONCEIÇÃO AO, OLIVEIRA LA, VILLELA JS, FRANÇA JP & FRANÇA LP. 2019. Estudos morfológicos e histoquímico de Adiantum latifolium LAM. (Pteridaceae, Pteridophyta) Ocorrente no Campus da Universidade Estadual De Santa Cruz – Uesc –Ilhéus – Ba. In: JUNIOR JMBO & CALVÃO LB (Eds), Ciências Biológicas: Campo Promissor em Pesquisa-3, Ponta Grossa: Atena Editora, Paraná, Brasil, p. 180-190.

DELUCIA EH, NELSON K, VOGELMANN TC & SMITH WK. 1996. Contribution of intercellular reflectance to photosynthesis in shade leaves. Plant Cell Environ 19: 159-170.

DICKISON WC. 2000. Integrative Plant Anatomy, San Diego: Academic Press, California, USA, 557 p.

EISNER T, EISNER M & HOEBEKE ER. 1998. When defense backfires: detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. Proc Natl Acad Sci USA 95: 4410-4414.

ERBANO M & DUARTE RD. 2010. Morfoanatomia de folha e caule de Genipa americana L., Rubiaceae. Braz J Pharmacog 20: 825-832.

EVANS WC. 1996. Trease and Evans Pharmacognosy, London: WB Saunders, UK, 612 p.

EVERT RF & EICHHORN SE. 2014. Fotossíntese, luz e vida. In: EVERT RF & EICHHORN SE (Eds), Raven - Biologia vegetal: Editora Guanabara Koogan LTDA, Rio de Janeiro, Brasil, p. 250-297.

FACCHINI PJ. 2001. Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation and metabolic engineering applications. Annu Rev of Plant Physiol Plant Mol Biol 52: 29-66.

FAHN A. 1990. Plant anatomy, New York: Pergamon Press, New York, USA, 588 p.

FEIO AC, AGUIAR-DIAS ACA & POTIGUARA RCV. 2013. Elaphoglossum (Dryopteridaceae-Fern) of Amazon Rainforest in Brazil: Anatomic Characterization and Adaptativeg VFGG Strategies. Am J Plant Sci 4: 1863-1871.

FURR M & MAHLBERG PG. 1981. Histochemical analyses of laticifers and glandular trichomes in Cannabis sativa. J Nat Prod 44: 153-159.

GERSHEZON J. 1983. Changes in the levels of plant secondary metabolites under water and nutrient stress. Recent Adv Phytochem 18: 273-320.

GIBSON AC. 1996. Structure-function relations of warm desert plants, Heidelberg: Springer, Germany, 216 p.

GOBBO-NETO L & LOPES NP. 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quím Nova 30: 374-381.

GRAÇANO D, AZEVEDO AA & PRADO J. 2001. Anatomia foliar das espécies de Pteridaceae do Parque Estadual do Rio Doce (PERD) – MG. Braz J Bot 24: 333-347.

GREGORY M & BAAS P.1989. A survey of mucilage cells in vegetative organs of the dicotyledons. Israel J Bot 38: 125-174.

HIETZ P. 2010. Fern adaptations to xeric environments. In: MEHLTRETER K, WALKER L & SHARPE JM (Eds), Fern ecology, New York: Cambridge University Press, New York, USA, p. 140-170.

HIETZ P & BRIONES O. 1998. Correlation between water relations and within canopy distribution of epiphytic ferns in a Mexican cloud forest. Oecologia 114: 305-316.

INAMDAR JA, PATEL RC & BHATT DC. 1971. Structure and development of stomata in some Leptosporangiate Ferns. Ann Bot 35: 643-651.

KAISER E. 1880. Verfahren zur herstellung einer tadellosen glycerin-gelatine. Botanisch Zentralb 180: 25-26.

KLEIN DE, GOMES VM, SILVA-NETO SJ & DA-CUNHA M. 2004. The structure of colleters in several species of Simira (Rubiaceae). Ann Bot 94: 733-740.

KONDO T. 1962. A contribution to the study of the fern stomata. Res Bull Fac Educ Shizuoka Univ 13: 239-267.

KRAMER KU. 1990. Davalliaceae. In: KRAMER KU & GREEN PS (Eds), The Families and Genera of Vascular Plants. Volume I. Pteridophytes and Gymnosperms, Berlin: Springer-Verlag, Germany, p. 74-80.

KRAUS JE & ARDUIN M. 1997. Manual básico de métodos em morfologia vegetal. Edur, Seropédica, 198 p.

KRAUSS BH. 1949. Anatomy of the vegetative organs of the pineapple Ananas comosus (L.) Merr.: II. The leaf. Bot Gaz 110: 333-404.

LARCHER W. 2000. Ecofisiologia Vegetal, São Carlos: Rima, São Paulo, 531 p.

LIESENFELD V, GENTZA P, FREITAS EM & MARTINS S. 2019. Leaf morphology and anatomy of Asteraceae of the Pampas biome (sandfields). Flora 258: 1-13.

LILLIE RD. 1965. Histopathologic technic and practical histochemistry, New York: McGraw-Hill Book Company, New York, USA, 715 p.

LINK HF. 1841. Icones selectae anatomico-botanicae. Ausgewahlte anatomisch-botanische abbildungen, Heft III, Taf. IV, Fig. 8. Berlin: Haude and Spenersche, Germany, Fasc. I-IV.

MACE ME & HOWELL CR. 1974. Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings. Can J Bot 52: 2423-2426.

MANETAS Y. 2003. The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of Verbascum speciosum are due to solar UV-B radiation. New Phytol 158: 503-508.

MARKHAM K, CHALK T & STEWART-JR CN. 2006. Evaluation of fern and moss protein based defenses against phytophagous insects. Int J Plant Sci 167: 111-117.

MARTORANO LG, PEREIRA LC & NECHET D. 1993. Tipologia climática do Estado do Pará – Adaptação do Método de KOPPEN. Bol Geogr Teor 23: 307-312.

MCELWAIN JC, WILLIS KJ & LUPIA R. 2005. Cretaceous CO2 decline and radiation and diversification of angiosperms. In: BALDWIN IT ET AL. (Eds), A history of atmospheric CO2 and its effects on plants, animals and ecosystems. New York: Springer, New York, USA, p. 133-165.

MEIRA RMSA & MARTINS FM. 2003. Inclusão de material herborizado em metacrilato para estudos de anatomia vegetal. Rev Árvore 27: 109-112.

METCALFE CR & CHALK L. 1950. Anatomy of the Dicotyledons: Leaves, Stems and Wood in Relation to Taxonomy with Notes on Economic Uses, Volume II, Oxford: Clarendon Press, UK, p. 11-724.

METTENIUS G. 1865. Über die Hymenophyllaceae. Abhand. d. math.-physik. Classe d. König. Sächs. Ges. d. Wissenseh. Leipzig 11: 402-504.

MICKEL JT. 1962. A monographic study of the fern genus *Anemia*, subgenus *Coptophyllum*. Iowa State J Res 36: 349-482.

MICKEL JT. 2016. *Anemia* (Anemiaceae). Flora Neotrop 118: 1-181.

MICKEL JT & LERSTEN NR. 1967. Floating stomates (adetostomy) in ferns: distribution and ontogeny. Am J Bot 54: 1181-1185.

MIGUEL EC, GOMES VM, OLIVEIRA MA & CUNHA M. 2006. Colleters in *Bathysa nicholsonii* K. Schum. (Rubiaceae): ultrastructure, secretion protein composition, and antifungal activity. Plant Biol 8: 715-722.

MØLLER JD & RASMUSSEN H. 1984. Stegmata in Orchidales: character state distribution and polarity. Bot J Linn Soc 80: 53-76.

MOTT KA, GIBSON AC & O'LEARY JW. 1982. The adaptive significance of amphistomatic leaves. Plant Cell Environ 5: 455-460.

O'BRIEN TP, FEDER N & MCCULLY ME. 1965. Polychromatyc staining of plant cells walls by toluidine blue O. Protoplasma 59: 368-373.

O'BRIEN TP & MCCULLY ME. 1981. The study of plant structure: principles and selected method, Melbourne: Termarcarphi, Australia, 357 p.

OGURA Y. 1972. Comparative anatomy of vegetative organs of the Pteridophytes, Berlin: Gebr. Borntraeger, Germany, 502 p.

OLIVEIRA RB & GODOY SAP. 2007. Composição florística dos afloramentos rochosos do Morro do Forno, Altinópolis, São Paulo. Biota Neotrop 7: 37-47.

PANT DD & KHARE PK. 1972. Epidermal Structure and Stomatal Ontogeny of *Anemia* Spp. Ann Bot 36: 809-821.

PARKHURST DF. 1978. The adaptive significance of stomatal occurrence on one or both surfaces of leaves. J Ecol 66: 367-384.

PEARSE AGE. 1985. Volume 2, Analytical technology. In: PEARSE AGE (Ed), Histochemistry: theoretical and applied, Edinburgh: Churchill Livingstone, UK, p. 442-1055.

PIZZOLATO TD & LILLIE RD. 1973. Mayer's tannic acid-ferric chloride stain for mucins. J Histochem Cytochem 21: 56-64.

PRYCHID CJ, RUDALL PJ & GREGORY M. 2004. Silica Bodies in Monocotyledons. Bot Rev The 4: 377-440.

PYYKKÖ M. 1966. The leaf anatomy of East Patagonian xeromorphic plants. Ann Bot Fenn 3: 453-622.

QUEIROZ-VOLTAN RB, ROLIM RS, PEDRO-JÚNIOR MJ & HERNANDES JL. 2011. Variações na anatomia foliar de videira Niagara em diferentes sistemas de condução. Bragantia 70: 488-493.

RIBEIRO MLRC, SANTOS MG, BARROS CF & COSTA CG. 2011. Intraspecific Variation in Four Distinct Populations of *Anemia villosa* Humb. & Bonpl. ex Willd. (Anemiaceae) Occurring in Rio de Janeiro, Brazil. Am Fern J 101: 70-80.

RIBEIRO MLRC, SANTOS MG & MORAES MG. 2007. Leaf anatomy of two *Anemia* Sw. species (Schizaeaceae-Pteridophyte) from a rocky outcrop in Niterói, Rio de Janeiro, Brasil. Braz J Bot 30: 695-702.

ROBINSON T. 1974. Metabolism and function of alkaloids in plants. Science 184: 430-435.

ROUX JP, VAN-DER-WALT JJA & VAN-DER-MERWE AB. 1992. Systematic studies in the genus *Mohria* (Pteridophyta: Anemiaceae) I. Comparative morphology and anatomy of the rhizome and frond. S Afr J Bot 58: 83-89.

SANTOS MG, ROCHA LM, CARVALHO ES & KELECOM A. 2006. Isoafricanol, um sesquiterpeno incomum encontrado na pteridófita *Anemia tomentosa var. anthriscifolia*. Rev Bras Pl Med 8: 71-75.

SANTOS MG & SYLVESTRE LDS. 2006. Aspectos florísticos e econômicos das pteridófitas de um afloramento rochoso do Estado do Rio de Janeiro, Brasil. Acta Bot Bras 20: 115-124.

SEN U & DE B. 1992. Structure and ontogeny of stomata in ferns. Blumea 37: 239-261.

SHOBE WR & LERSTEN NR. 1967. A technique for clearing and staining Gymnosperm leaves. Bot Gaz 128: 150-152.

SIDDIQUI Z & ARIF-UZ-ZAMA S. 2004. Effects of benlate systemic fungicide on seed germination, seedling growth, biomass and phenolic contents in two cultivars of *Zea mays* L. Pak J Bot 36: 577-582.

SILVA JB. 2016. Panorama sobre a vegetação em afloramentos rochosos do Brasil. Oecol Aus 20: 451-463.

SILVA PMF, SILVA EO, RÊGO MSC, CASTRO LMR & SIQUEIRA-SILVA AI. 2019. Anatomical and histochemical characterization of Dipteryx odorata and Taralea oppositifolia, two native Amazonian species. Rev Bras Farmacogn 29: 425-433.

SIMÕES CMO, SCHENKEL EP, GOSMANN G, MELLO JCP, MENTZ LA & PETROVIHK PR. 2000. Farmacognosia: da planta ao medicamento, Florianopólis: Editora da UFSC, Santa Catarina, Brasil, 821 p.

SMITH FH & SMITH EC. 1942. Anatomy of the inferior ovary of *Darbya*. Am J Bot 29: 464-471.

STRACK D. 1997. Phenolic Metabolism. In: DEY PM & HARBONE JB (Eds), Plant Biochemistry, London: Academic Press, UK, p. 387-390.

TAIZ L & ZEIGER E. 2006. Secondary Metabolites and Plant Defense. In: TAIZ L & ZEIGER E (Eds), Plant Physiology, Sunderland: Sinauer Associates, USA, p. 283-308.

THADEO M, MEIRA RS, AZEVEDO AA & ARAÚJO JM. 2009. Anatomy and histochemistry of the secretory structures

of the leaf of *Casearia decandra* Jacq. (Salicaceae). Braz J Bot 32: 329-338.

THIERS B. 2018. (continuously updated). Index Herbariorum: A global directory of public herbaria and associated staff, Available in: http://sweetgum.nybg.org/ih/.

VALLADARES F, GIANOLI E & GÓMEZ JM. 2007. Ecological limits to plant phenotypic plasticity. New Phytol 176: 749-763.

VICARI M & BAZELY DR. 1993. Do grasses fight back? The case for antiherbivore defences. Trends Ecol Vol 8: 137-141.

VICRÉ M, SHERWIN HW, DRIOUICH A, JAFFER MA & FARRANT JM. 1999. Cell wall characteristics and structure of hydrated and dry leaves of the resurrection plant *Craterostigma wilmsii*, a microscopical study. J Plant Physiol 155: 719-726.

VIEIRA-NETO H, ABDALLA DF & MORAES M. 2020. Stomatal plasticity in leaves of Ichthyothere terminalis (Spreng) Blake (Asteraceae) at different seasons. Res Soc Dev 9: 1-18.

VIZZOTTO M, KROLOW AC & WEBER GEB. 2010. Metabólitos secundários encontrados em plantas e sua importância, Pelotas: Embrapa Clima Temperado, Documento 316, 16 p.

WATERMAN PG & MOLE S. 1994. Analysis of phenolic plant metabolites, Oxford: Blackwell Scientific Publications, UK, 238 p.

WATSON AA, FLEET GW, ASANO N, MOLYNEUX RJ & NASH RJ. 2001. Polyhydroxylated alkaloids—natural occurrence and therapeutic applications. Phytochemistry 56: 265-295.

WILKINSON HP. 1979. The plant surface (mainly leaf. In: METCALFE CR & CHALK L (Eds), Anatomy of the dicotyledons, Oxford: Clarendon, UK, p. 97-162.

WINK M. 2008. Plant secondary metabolism: diversity, function and its evolution. Nat Prod Commun 3: 1205-1216.

WINK M. 2010. Introduction: Biochemistry, Physiology and Ecological functions of secondary metabolism. In: WINK M (Eds), Biochemistry of plant secondary metabolism, Oxford: Blackwell Publishing Limited, UK, p. 1-19.

WONG SC & HEW CS. 1976. Diffusive Resistance, Titratable Acidity, and C02 Fixation in Two Tropical Epiphytic Ferns. Am Fern J 4: 121-124.

XAVIER SRS & BARROS ICL. 2003. Pteridófitas ocorrentes em fragmentos de Floresta Serrana no estado de Pernambuco, Brasil. Rodriguésia 54: 13-21.

ZANENGA-GODOY R & COSTA CG. 2003. Anatomia foliar de quatro espécies do gênero *Cattleya* Lindl. (Orchidaceae) do planalto central brasileiro. Acta Bot Bras 17: 101-118.

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PEDRO G. DE MORAES NETO[1]
https://orcid.org/0000-0002-5045-8509

NATÂNIA P.P. DA SILVA[1]
https://orcid.org/0000-0003-2839-8367

ANA CATARINA S. FURTADO[1]
https://orcid.org/0000-0002-0584-585X

ANA CARLA FEIO[1,2]
https://orcid.org/0000-0003-4330-8294

[1]Programa de Pós-Graduação em Ciências Biológicas - Botânica Tropical, Universidade Federal Rural da Amazônia, Museu Paraense Emílio Goeldi, Avenida Perimetral, 1901, 66077-830 Belém, PA, Brazil

[2]Universidade Federal Rural da Amazônia, Coordenação de Ciências Biológicas, Campus Tomé-açu, Rodovia PA-140, 68680-000 Tomé-Açu, PA, Brazil

Correspondence to: **Ana Carla Feio**
E-mail: anacarlafeio@gmail.com

Author contributions

Pedro G. de Moraes Neto: Writing – original draft, proofreading & editing. Natânia P.P. da Silva and Ana Catarina S. Furtado: Review, Methodology, proofreading and Editing. Ana Carla Feio: Planning, Conceptualization, Writing – original draft, review and editing, Supervision.