

## Leaf anatomy of two *Anemia* Sw. species (Schizaeaceae-Pteridophyte) from a rocky outcrop in Niterói, Rio de Janeiro, Brazil

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**ABSTRACT** – (Leaf anatomy of two *Anemia* Sw. species (Schizaeaceae-Pteridophyte) from a rocky outcrop in Niterói, Rio de Janeiro, Brazil). The ferns *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. & Bonpl. ex Willd. are widely associated with vegetation islands on rocky outcrops in Rio de Janeiro. Both species are desiccation tolerant. The leaf anatomy of these species was examined aiming to identify morphological characteristics that would allow the establishment of these species in water-scarce environments. The plants were harvested on “Pedra de Itacoatiara” and prepared according to the usual procedures. The petiole has a uniseriate epidermis with lignified cell walls, conical stigmata, and uniseriate multicellular and glandular trichomes. In *A. villosa*, the stomata protrude in a respiratory line. Under the epidermis the cells have thick, lignified walls. The parenchyma has phenolic compounds and starch grains. The petiole vascular bundles are surrounded by endodermis with Casparian strips and the xylem is V-shaped (*A. villosa*) or arc-shaped (*A. tomentosa* var. *anthriscifolia*). The leaf blades have a uniseriate epidermis with sinuous anticlinal and convex periclinal walls, conical stigmata and chloroplasts on both surfaces. The leaf margins of *A. villosa* have lignified cells. The guard cells of the stomata on the abaxial surface are on the same level or are raised above ordinary epidermal cells. Multicellular uniseriate trichomes and glandular hairs were observed. The dorsiventral mesophyll has loosely packed chlorenchyma with arm-shaped and H-shaped cells. The vascular bundles are surrounded by endodermis with Casparian strips and with parenchymatic extensions towards the epidermis. Anatomical results were analyzed considering the interaction of these plants with abiotic factors.

Key words - ecological anatomy, monilophyte, “Pedra de Itacoatiara”

**RESUMO** – (Anatomia foliar de duas espécies de *Anemia* Sw. (Schizaeaceae-Pteridófitas) de um afloramento rochoso em Niterói, Estado do Rio de Janeiro, Brasil). As pteridófitas *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel e *Anemia villosa* Humb. & Bonpl. ex Willd. estão comumente associadas às “ilhas de vegetação” nos afloramentos rochosos do Rio de Janeiro, sendo ambas tolerantes a dessecação. Este trabalho objetivou analisar a anatomia foliar e identificar estratégias morfológicas que possibilitem o estabelecimento destas espécies num ambiente com restrição hídrica. As pteridófitas foram coletadas na Pedra de Itacoatiara e submetidas às técnicas de estudos anatômicos. Os pecíolos das espécies apresentam epiderme unisseriada com paredes lignificadas, estegmatas cônicos, tricomas tectores unisseriados e glandulares. Em *A. villosa* foram observados estômatos em protrusão formando linha respiratória. Abaixo da epiderme ocorrem camadas de células com paredes espessas e lignificadas. O parênquima possui compostos fenólicos e grãos de amido. Os feixes vasculares dos pecíolos estão envolvidos por endoderme com estrias de Caspary e o xilema tem forma de “V” (*A. villosa*) ou forma de arco (*A. tomentosa* var. *anthriscifolia*). A lâmina foliar apresenta epiderme uniestratificada com paredes anticlinais sinuosas e periclinais convexas, com estegmatas cônicos e cloroplastos nas faces adaxial e abaxial. As células epidérmicas do bordo foliar são lignificadas em *A. villosa*. Estômatos na face abaxial com células guarda no mesmo nível ou acima das demais células epidérmicas. Foram observados tricomas tectores multicelulares unisseriados e tricomas glandulares. O mesofilo é dorsiventral, com clorênquima frouxo composto por células braciiformes e em forma de “H”. Os feixes vasculares são envolvidos por endoderme com estrias de Caspary e possuem extensões de parênquima em direção à epiderme. Os resultados anatômicos foram analisados considerando a interação dessas plantas com fatores abióticos.

Palavras-chave - anatomia ecológica, monilophyta, Pedra de Itacoatiara

### Introduction

Rocky outcrops are fragile ecosystems with a unique habitat, several endemic species (Meirelles *et al.* 1999),

and vegetation that is distinctly different from that of surrounding areas (Meirelles 1990, Porembski 2002). These are called “inselbergs” (Porembski 2002) and their plant communities have received little attention from scientists and conservationists (Meirelles *et al.* 1999).

Inselbergs are considered to be diversity centers of desiccation tolerant species (Porembski & Barthlott 2000). There are about 300 species of vascular plants that have some level of desiccation tolerance (Porembski &

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Barthlott 2000). Nine fern families belong to the group with this attribute, especially Selaginellaceae, Pteridaceae and Aspleniaceae (Proctor & Pence 2002).

*Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. & Bonpl. ex Willd. (Schizaeaceae) are fern species commonly associated with vegetation islands on rocky outcrops in Rio de Janeiro State (Santos & Sylvestre 2006). Both species are considered to be desiccation tolerant (Gaff 1987, Meirelles 1990).

Biochemical, physiological and morphological mechanisms are found in plants that undergo water shortage (Bohnert *et al.* 1995, Larcher 2000). Several structural characteristics may contribute to decrease in the rate of water loss, such as reduction of leaf surface, increased thickness of epidermal cell walls, increased thickness of cuticle, high trichome density and stomata located on the abaxial surface (Fahn 1990, Dickinson 2000). In addition to these features, others such as the presence of water storage tissues, high sclerenchyma density and mucilage storage result in improved mechanical conditions and/or reduced tissue damage (Dickinson 2000).

Here we examine the leaf anatomy of *A. tomentosa* var. *anthriscifolia* and *A. villosa* from a rocky outcrop in the state of Rio de Janeiro (Niterói) in order to identify morphological characteristics that would allow the establishment of these species in a water-scarce environment.

## Material and methods

Specimens of *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. & Bonpl. ex Willd. were harvested on “Pedra de Itacoatiara”, a gneiss-granitic rocky outcrop located in Niterói, Rio de Janeiro (22°58'33" - 22°59'00" S and 43°01'33" - 43°02'00" W), part of the “Parque Estadual da Serra da Tiririca”. Vouchers were deposited at the “Faculdade de Formação de Professores” of the “Universidade do Estado do Rio de Janeiro” herbarium (RFFP).

Fully expanded leaves were harvested and fixed in FAA 70% (Johansen 1940). Cross and longitudinal sections were made of the proximal and distal regions of the petiole, the median region of the pinnae and leaf margins using a stainless steel blade. Sections were cleared using commercial sodium hypochloride (50%), neutralized and stained with safranin and Astra blue (Bukatsch 1972). Sections were subsequently mounted in glycerin (50%). Histochemical tests were made on fresh leaves treated with the following reagents: Sudan III to detect lipid compounds (Jensen 1962); Lugol for starch grains (Langeron 1949); iodated Zinc chloride for cellulose (Jensen 1962). Detection of phenolic compounds was done on leaves fixed in ferrous sulfate (Johansen 1940).

Identification of vascular bundle types followed Ogura (1972), and stomata, Mickel & Lestern (1967). Results were registered on photomicrographs. The authors of the scientific names were abbreviated according to Pichi-Sermolli (1996) recommendations for pteridophytes.

## Results

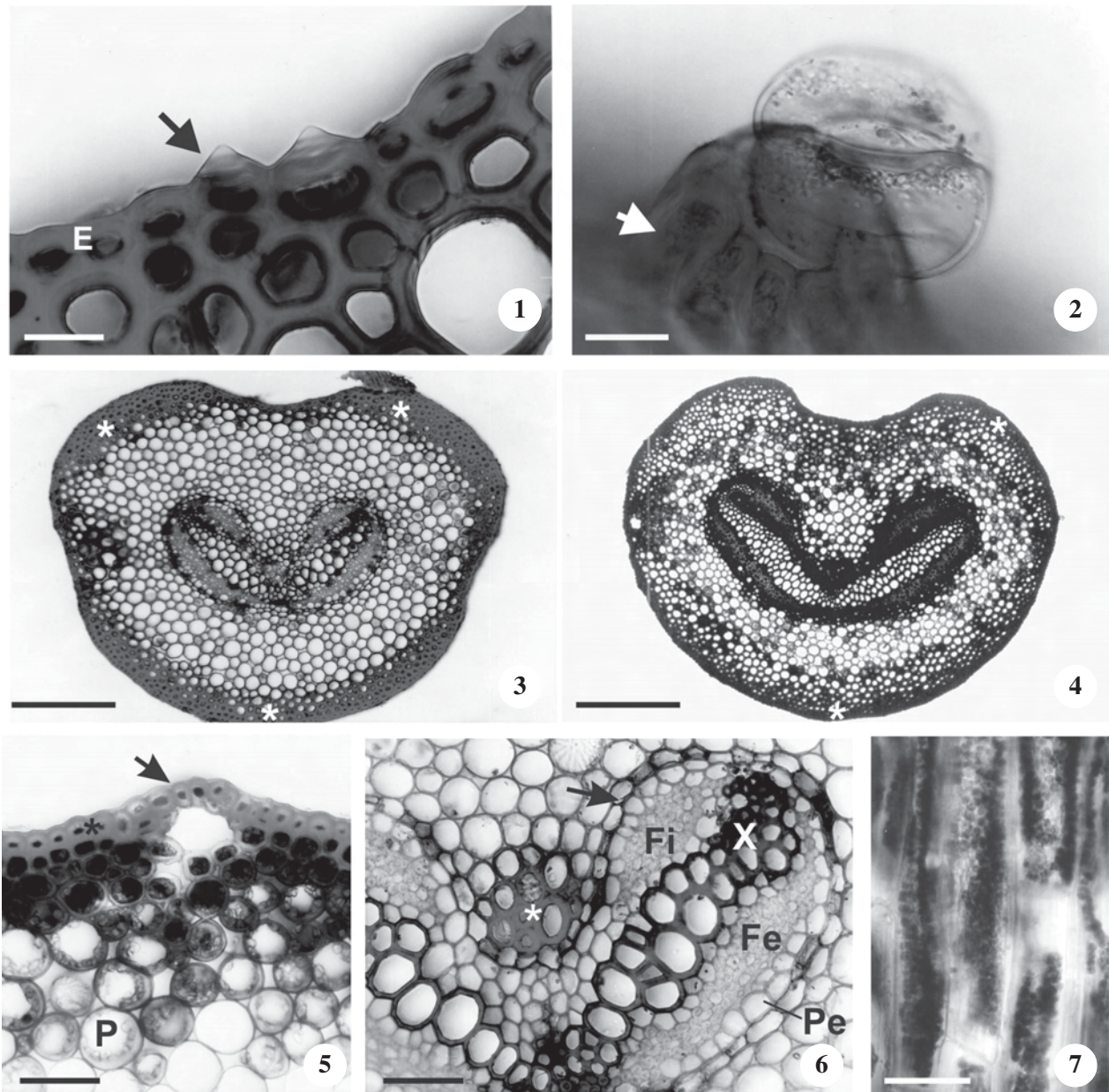
The anatomical features of *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. & Bonpl. ex Willd. leaves are summarized in table 1. The petioles of both species have a uniseriate epidermis, with thick, lignified cell walls, and conical stegmata (figure 1, table 1). Multicellular uniseriate trichomes and glandular hairs are present in both species. Stomata with protruding guard cells suspended by lignified epidermal cells were observed in *A. villosa*, forming a respiratory line (figures 2, 5).

Cells below the epidermis have thick, lignified walls, arranged in two to six layers (figures 3, 4). The outermost cells have thicker walls (figure 1) when compared to the innermost cells, which may also contain phenolic compounds. Parenchyma cells of both species are filled with starch grains, mainly on the distal petiole (figures 5, 7), and contain phenolic compounds.

The vascular bundle is arched in *A. villosa*, which has three in the proximal and one in the distal petiole (figure 3, table 1). *A. tomentosa* var. *anthriscifolia* has one vascular bundle along the whole petiole (figure 4, table 1). In both species, the vascular bundle is surrounded by endodermis with Casparian strips (figure 6). The xylem is V-shaped in *A. villosa* and arc-shaped in *A. tomentosa* var. *anthriscifolia* (table 1); xylem margins are curved in both species. The vascular bundle is concentric, with phloem surrounding xylem, and is sometimes discontinuous, giving rise to an inner and outer phloem (figure 6). The parenchyma associated with vascular tissues, including the pericycle, has starch grains and phenolic compounds.

The leaf blades of both species have a uniseriate epidermis, whose cells have mainly sinuous anticlinal walls (figures 8-10) and convex outer periclinal walls, usually with conical stegmata (figures 11, 12, table 1). Chloroplasts are present in the adaxial and abaxial epidermis. Near the veins, epidermal cells are elongate with thicker walls, mainly on the adaxial surface. The cells below this layer have similar characteristics (figures 11, 12).

Stomata are found only on the abaxial surface. Guard cells are located at the same level or above the epidermal cells (figure 11). Most stomata are applied in both species (figure 8), but they differ as regards the additional type found. *A. villosa* has stomata suspended by a projection



Figures 1-7. Petiole anatomy of *Anemia* species: 1. Cross section of *A. tomentosa* var. *anthriscifolia* petiole showing epidermis with conical stigmata (arrow) and layers of lignified cells below the epidermis. 2. *A. villosa* stomata in protrusion supported by lignified cells (arrow). 3. *A. villosa* distal petiole; layers of cells with lignified walls (\*); arched vascular bundle with V-shaped xylem. 4. *A. tomentosa* var. *anthriscifolia* distal petiole; layers of cells with lignified walls (\*); arched vascular bundle with arc-shaped xylem. 5. *A. villosa* with lignified epidermis (\*); cortex parenchyma storing starch grains; elevated epidermal cells (arrow). 6. Detail of *A. villosa* vascular bundle showing lignified cells in the inner arc (\*); endodermis with Casparian strips (arrow). 7. *A. tomentosa* var. *anthriscifolia* petiole in longitudinal section showing starch grains stained with lugol inside parenchyma cells. (E = epidermis; Fe = outer phloem; Fi = inner phloem; P = parenchyma; Pe = pericycle; X= xylem). Bars = 20µm (1, 2, 7), 50 µm (5, 6), 300 µm (3,4).

of the anticlinal wall (figure 8) while *A. tomentosa* var. *anthriscifolia* has floating stomata. Trichomes are glandular and multicellular uniseriate (figure 9, 10).

The dorsiventral mesophyll has loosely packed chlorenchyma composed of arm-shaped cells (figures 11, 12). On the adaxial surface, these cells are elongate

and H-shaped (figures 13, 14). The collateral vascular bundles are surrounded by endodermis with Casparian strips (figure 15). Parenchyma forms bundle-sheath extensions. Leaf margins have lignified cells in *A. villosa* (figure 16) but not in *A. tomentosa* var. *anthriscifolia* (figure 17).

Table 1. Leaf characteristics of *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. & Bonpl. ex Willd. from “Pedra de Itacoatiara”, Niterói, Rio de Janeiro State, Brazil.

Characteristics	<i>A. tomentosa</i>	<i>A. villosa</i>
<b>Petiole</b>		
Stomata in protrusion	–	+
Glandular hairs	+	+
Multicellular uniseriate trichomes	+	+
Epidermal cells with lignified walls	+	+
Conical stegmata	+	+
Cortex parenchyma containing starch grains	+	+
Vascular bundle with V-shaped xylem	–	+
Vascular bundle with arc-shaped xylem	+	–
Vascular bundle number at proximal region	1	3
Vascular bundle number at distal region	1	1
<b>Leaf blade</b>		
Hypostomatic leaf	+	+
Floating stomata	+	–
Stomata suspended by a projection of anticlinal wall	–	+
Applied stomata	+	+
Glandular hairs	+	+
Multicellular uniseriate trichomes	+	+
Epidermal cells with sinuous walls	+	+
Conical stegmata	+	+
Mesophyll with arm-shaped cells of chlorenchyma	+	+
Collateral vascular bundle	+	+
Lignified cells on leaf margins	–	+

## Discussion

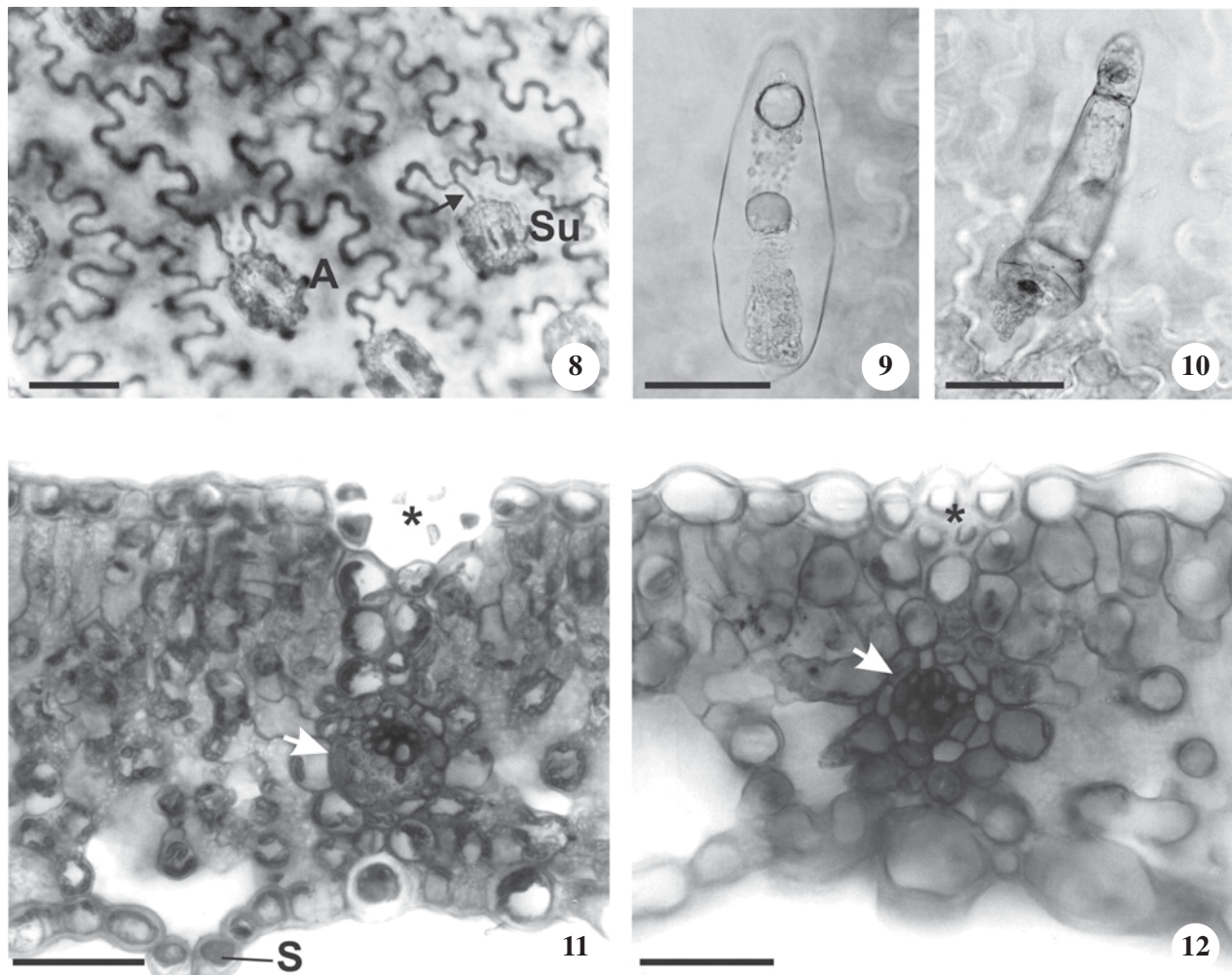
Studies on the anatomy of plants that live in dry, nutrient-poor habitats have shown that these species have xeromorphic characteristics which may prevent water loss (Castro & Menezes 1995). Pteridophytes are outstanding

in their capacity to occupy marginal environments under extreme conditions (Page 2002). This author also emphasizes that most Schizaeaceae species grow vigorously in nutrient-poor environments. However the anatomy of pteridophytes living under these conditions has been underexploited. Several epidermal cell characteristics have been linked to the avoidance of excessive water loss and high light levels; these include cuticle and external wall thickness, relative stomata position and trichome density (Fahn 1990, Dickinson 2000).

In this study we observed anatomical peculiarities of the petiole and leaf blade epidermis of *Anemia tomentosa* (Sav.) Sw. var. *anthriscifolia* (Schrad.) Mickel and *Anemia villosa* Humb. & Bonpl. ex Willd. The presence of stegmata on the periclinal walls of epidermal cells deserves attention. According to Ogura (1972), this is a common characteristic of the genus. These structures are composed of silica, which can form crystals inside the cell or become incrustated on the cell wall, as was observed for the species studied here. The presence of stegmata seems to be restricted to certain plant groups like the Orchidaceae, Musaceae, Poaceae and Pteridophytes. Furthermore, these structures have specific shapes and locations, which lead Prychid *et al.* (2004) to stress the potential use of stegmata for the systematics of the groups in which they occur (see Møller & Rasmussen 1984).

With respect to the possible functions carried out by these structures, Prychid *et al.* (2004) mention that silica may help to maintain rigidity in organs with stegmata, and it also may be related to reducing herbivory, grazing and infection by microorganisms. When present in the blade epidermis, they may reduce transpiration, thus improving water-use efficiency. In the species studied here, stegmata were observed in the petiole epidermis, close to lignified cells, and also above the vascular bundles in the leaf blade. They may therefore act in frond support. Some authors found a tendency for the presence of stegmata in the epidermis to be associated with xeromorphic characteristics, as exemplified by the orchid *Cattleya araguaiensis* Pabst (Zanenga-Godoy & Costa 2003) and other epiphytes. They are absent in mesomorphic orchids (Møller & Rasmussen 1984).

Another prominent characteristic is the distinct, diversified stomata types in pteridophytes (Sen & De 1992). In some species, the guard cells of the stomata are entirely surrounded by one epidermal cell. This condition, described as “floating stomata” or adetostomy (Mickel 1962), is common in *Anemia* and can also be found in *Pyrrosia* Mirb. and in some *Drymoglossum* C. Presl, *Lemmaphyllum* C. Presl and *Campyloneurum* C.



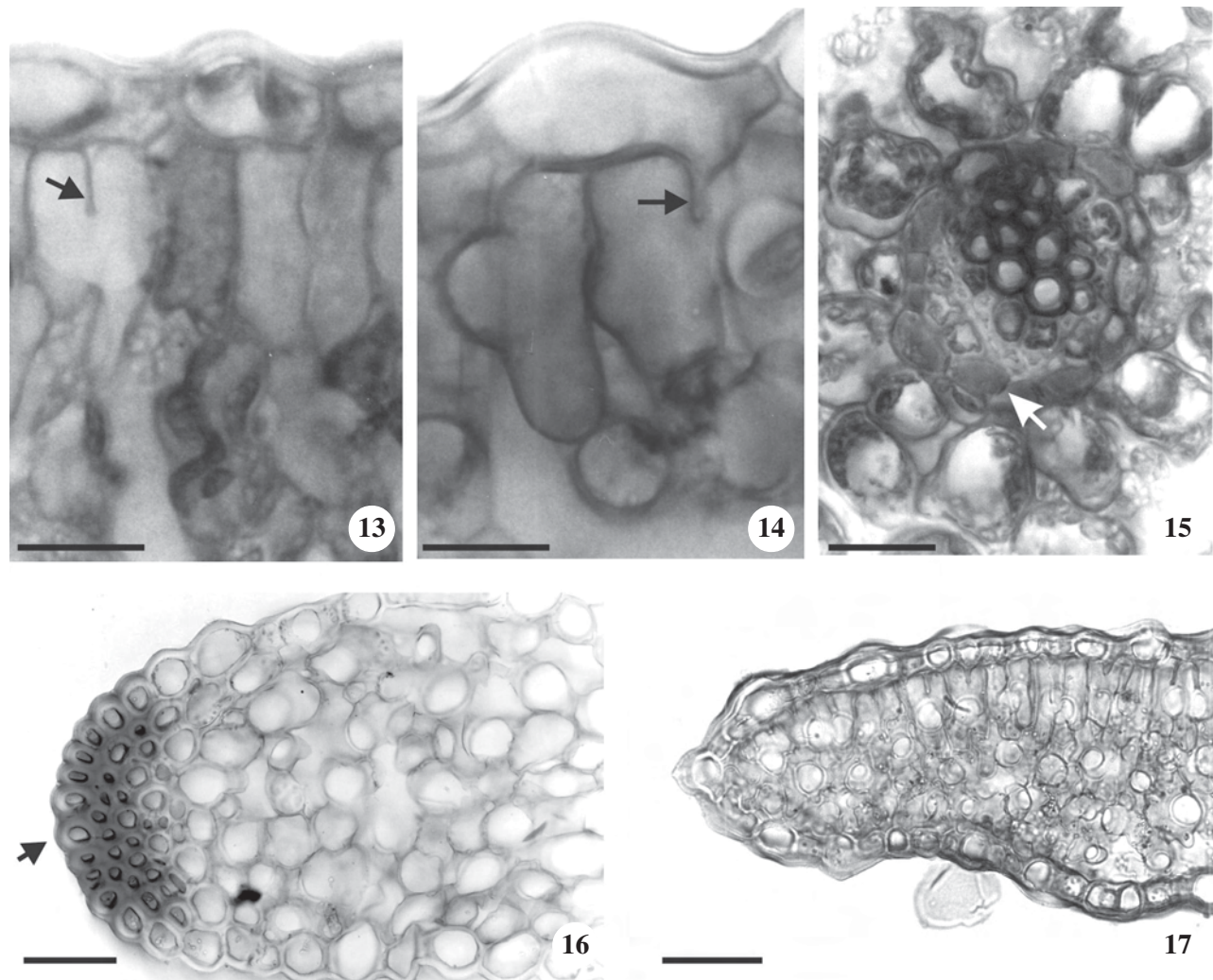
Figures 8-12. Leaf blade anatomy of *Anemia* species: 8. Abaxial surface of *A. villosa* epidermis, with applied stomata (A) and suspended (Su) by a projection of the anticlinal wall (arrow). 9. Apical cell of glandular hair on *A. villosa* epidermis. 10. Glandular hair with 3 cells on *A. villosa* epidermis. 11. Cross section of *A. tomentosa* var. *anthriscifolia* leaf blade showing stegmata (\*), parenchyma surrounding the vascular bundle (arrow) and stomata in protrusion (S). 12. Cross section of *A. villosa* leaf blade showing stegmata (\*) and parenchyma surrounding the vascular bundle (arrow). Bars = 20  $\mu\text{m}$  (9, 10), 50  $\mu\text{m}$  (8, 11, 12).

Presl species (Mickel & Lersten 1967). Applied stomata are the main type found here. The guard cells have lateral contact with more than one epidermal cell; however, the species differ in that *A. tomentosa* var. *anthriscifolia* has floating stomata, while *A. villosa* has stomata suspended by a projection of the anticlinal wall. Guard cells in protrusion are present in these species, a feature found mostly in species where water is abundant. Ogura (1972) mentions this condition in *Anemia* Sw. and *Schizaea* Sm.

In a study focusing on the ontogeny of fern stomata, Sen & De (1992) used different names for the types describe above. Applied stomata are polocytic, the most widely distributed type in ferns. Suspended stomata are desmocyctic and floating stomata are pericyctic. These

authors demonstrated ontogenetic relationships between these types of stomata and proposed that polocytic is the basal form of other types, including desmocyctic and polocytic. The same study reports the presence of four other types of stomata in *A. villosa*, but they were not observed in this study. No functional or ecological relationship of the referred stomata was proposed by Mickel & Lestern (1967) and Sen & De (1992).

Besides the special types found in the leaf blade, stomata in protrusion were found along the petiole in *A. villosa*. According to Ogura (1972) this arrangement constitutes a respiratory line, which probably provides aeration since the hypodermis layer is interrupted under this line. In this respect, Haberlandt (1928) mentioned that stomata in protrusion are common in species densely



Figures 13-17. Leaf blade anatomy of *Anemia* species: 13. *A. tomentosa* var. *anthriscifolia*: detail of H-shaped parenchyma (arrow). 14. *A. villosa*: detail of H-shaped parenchyma (arrow). 15. Collateral bundle in *A. tomentosa* var. *anthriscifolia* showing endodermis with Casparian strips (arrow). 16. Cross section of *A. villosa* leaf margin showing cells with lignified walls (arrow). 17. Cross section of *A. tomentosa* var. *anthriscifolia* leaf margin. Bars = 20  $\mu$ m (13-15), 50  $\mu$ m (16, 17).

covered by trichomes. These stomata differ from those described by Haberlandt (1928) and Metcalfe & Chalk (1979) by having lignified cells distinct from the other epidermal cells. Their elongate shape projects them outwards, while the stomata described by the above-mentioned authors have similar epidermal cells projected not due to their shape, but to their distinct disposition along the tissue.

In addition to stomata types, the epidermis of the blade has sinuous anticlinal walls. Haberlandt (1928) proposed that this feature increases contact between adjacent cells, and may help to maintain leaf structure under mechanical stress. “Pedra de Itacoatiara” is located near the sea and is exposed to strong winds. The vegetation is exposed to substrate limitations causing leaf blades to roll up when water is scarce.

Similarly, trichomes are referred to as plant protection features and both species have glandular hairs containing oil. The essential oil of *A. tomentosa* var. *anthriscifolia* is sesquiterpene isoafrikanol (Santos *et al.* 2003). *A. villosa* essential oil was also extracted, but the component has yet to be identified (Santos & Sylvestre 2006). Such substances may act as allelochemicals and may be helpful in chemotaxonomy. Several sesquiterpenes have been correlated with plant protection and monoterpenes (*e.g.* gossypol) show insecticide activity (Croteau *et al.* 2000).

As regards the fundamental tissues, attention should be drawn to the arm- and H-shaped mesophyll cells. This type of cell is generally found in plants from wet or aquatic habitats (Fahn 1990), conferring ample intercellular space for gas diffusion. Leaf structure was reported as the most important factor in leaf assimilation

capacity (Niinemets 1999). Haberlandt (1928) reports that H-shaped cells, such as observed in these *Anemia* species, permit a higher exposure surface for chloroplasts. Moreover, the connections between cells create an efficient route for substance conduction towards the vascular bundle. During photosynthetic events, such as light capture and distribution, oxygen production, carbon dioxide diffusion and fixation, leaf tissues cooperate and their structure greatly contributes to the entire process (Vogelman *et al.* 1996). The species studied here have chloroplasts inside the epidermal cells, suggesting a role for this tissue beyond optimization of light absorption during photosynthesis.

Plants growing in habitats with contrasting environmental conditions of water availability may show distinct mesophyll architecture. Leaves of xeromorphic species tend to be more compact. Slaton & Smith (2002) mention that there is up to 95% contact surface between cells and little contact with intercellular spaces. The tendency to have relatively large intercellular spaces has already been observed in xeromorphic species with dorsiventral mesophyll and abaxial surface densely covered by trichomes (Burrows 2001). The leaf blades of the species studied here have this configuration, although they lack the dense covering of trichomes.

Starch grains were observed in the petiole parenchyma, indicating a storage function for this leaf part, especially in *A. villosa*, that has a reduced rhizome. Nevertheless this characteristic may be associated with the prevention of mechanical damages in plasmalema during reduced water availability, as suggested by Vicré *et al.* (1999) for tissues of desiccation tolerant seeds.

Another factor associated with the protection of these species is the presence of phenolic compounds located mainly in the supporting tissues and close to the parenchyma of the bundles. These compounds are commonly found in ferns (Ogura 1972), as related by Graçano *et al.* (2001) for Pteridaceae species. According to Croteau *et al.* (2000) these compounds were important for plant adaptation to the terrestrial environment, as much as structural component, as in several defense processes to biotic and abiotic factors. Waterman & Mole (1994) emphasize their importance for plant protection in circumstances, such as drought, high light intensity and other soil conditions. However it is unclear how these substances behave and how they function in response to drought. Gershezon (1983) suggested that increased substance levels may be the result of reduction in growth rates and synthesis of lignin precursors.

Another characteristic that deserves attention is the presence of endodermal cells girdled by Casparian strips

around the vascular bundles of the petiole and leaf blade, a common feature of ferns (Ogura 1972). In Angiosperms this feature is always found in roots, frequently in underground or submerged stems, and seldom in leaves (Lestern 1997). Its function has been related to solute retention in the stele and sap flow canalization, thus forming closed, defined apoplastic routes (Lestern 1997, Yokoyama & Karahara 2001). It is also essential for the protection of aerenchyma chambers in submerged plants (Dalla Vecchia *et al.* 1999). In the species studied here, the endodermis may insure greater efficiency in sap flow to the leaf blades. On the other hand, xeromorphic features of the petiole suggest it is a structural adjustment to water shortage.

In spite of the environmental restrictions imposed by the rocky outcrop habitat, *Anemia* species from "Pedra de Itacoatiara" have leaf blades with mesomorphic characteristics which contrast with the xeromorphic petiole. This raises some questions about survival strategies in these environments such as: Are mesomorphic leaf characteristics conservative or are they adaptations to microclimatic variations? What mechanisms regulate photosynthesis, water balance and plant development?

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