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An approach on anatomical structure of *Chaetostoma glaziovii* Cogn – Melastomataceae – and its successful establishment at high-altitude fields

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Abstract: *Chaetostoma glaziovii* Cogn. is an endemic species of the Brazilian flora and grows exclusively in fields at elevations above 2000 meters. It belongs to Melastomataceae family, Microlicieae tribe, which presents little variation on vegetative organs, making it difficult to identify sterile individuals. Anatomical studies, using optical and scanning electron microscopes are carried on comparing the data with the anatomical characteristics of xeromorphic plants, cited in the literature, to check its similarities. This study aims, also, highlight data that may contribute to characterization of the species due to the great similarities among sterile individuals within the tribe. Leaf and stem anatomical data of *C. glaziovii* are interpreted considering climatic factors, aiming to understand the species' successful establishment in an environment characterized by intense light exposure, strong winds and extreme temperatures. The material was collected in the Itatiaia National Park at an altitude of 2350 meters, latitude S 22º 23' 26" and longitude W 44º 40' 17". Histochemical tests were performed on unfixed samples to identify starch grains, lipids, phenolic compounds, glycides and crystals. It is noteworthy the great amount of fibers on the abaxial and marginal regions of leaf blade, forming an armor against environmental aggressions. In addition, this characteristic is valued from the taxonomic perspective because no other species of the tribe exhibits such extensive fiber development. The anatomical data revealed that some characteristics of *C. glaziovii* are like those found on xeromorphic plants and are important for its identification beyond to contribute for understanding the intricate plant-environment relationships.
Keywords: ecological anatomy, Melastomataceae, *Chaetostoma glaziovii,* Itatiaia National Park, environmental indicator.

Uma abordagem sobre a estrutura anatômica de *Chaetostoma glaziovii* Cogn. – Melastomataceae – e seu estabelecimento bem sucedido em campos de altitude

Resumo: *Chaetostoma glaziovii* Cogn. é uma espécie endêmica da flora brasileira encontrada exclusivamente em campos de altitude, acima de 2000 metros. Pertence à família Melastomataceae, tribo Microlicieae, cujos indivíduos apresentam pouca variação nos órgãos vegetativos, dificultando a identificação de indivíduos estéreis. As características anatômicas reveladas através dos microscópios óptico e eletrônico de varredura, são comparadas com os dados de plantas xeromórficas, citados na literatura, para verificar suas semelhanças. Este estudo objetivou, também, destacar os dados que possam contribuir para a caracterização da espécie, visto que há grandes semelhanças morfológicas entre os indivíduos dessa tribo. Os dados anatômicos da folha e do caule de *C. glaziovii* são interpretados considerando-se os fatores climáticos, com o objetivo de entender o sucesso do estabelecimento da espécie em um ambiente caracterizado por intensa exposição à luz, ventos fortes e temperaturas extremas. As amostras botânicas foram coletadas no Parque Nacional de Itatiaia a uma altitude de 2350 metros, latitude S 22º 23' 26"e longitude W 44º 40' 17". Testes histoquímicos foram aplicados em amostras não fixadas para identificar grãos de amido, lipídios, compostos fenólicos, glicídios e cristais. Vale ressaltar a grande quantidade de fibras nas regiões abaxial e marginal da folha, formando uma verdadeira armadura contra as agressões ambientais. Além disso, esta característica é destacada sob a perspectiva taxonômica, pois nenhuma outra espécie da tribo exibe um desenvolvimento de fibras com tal magnitude. Os dados anatômicos revelaram que várias características de *C. glaziovii* são semelhantes às de plantas xeromórficas e contribuem para a sua identificação, além de possibilitarem o aprofundamento das discussões sobre as intrincadas relações planta-ambiente.
Palavras-chave: anatomia ecológica, Melastomataceae, *Chaetostoma glaziovii,* Parque Nacional de Itatiaia, indicador ambiental.

Um enfoque sobre la estrutura anatómica de *Chaetostoma glaziovii* Cogn. – Melastomataceae – y su establecimiento exitoso em campos de altitud

Resumen: *Chaetostoma glaziovii* Cogn. es uma espécie endémica de la flora brasileña encontrada exclusivamente em campos de altitude, por encima de 2000 metros. Pertenece a la família Melastomataceae, tribu Microliceeae, cuyos indivíduos presentan poca variación em los órganos vegetativos, dificultando la identificación de indivíduos estériles. Las características anatómicas reveladas a través de los microscópios óptico y electrónico de exploración, se comparan com los datos de plantas xeromórficas, citados em la literatura, para verificar sus semejanzas. Este estúdio objetivo, también, destacar los datos que puedan contribuir a la caracterización de la espécie, ya que hay grandes semejanzas morfológicas entre los indivíduos de esa tribu. Los datos anatómicos de la hoja y del caucho de *C. glaziovii* se interpretan considerando los factores climáticos, con el objetivo de entender el éxito del establecimiento de la especie em um ambiente caracterizado por intensa exposición a la luz, ventos fuertes y temperaturas extremas. Las muestras botánicas fueron recolectadas en el Parque Nacional de Itatiaia a una altitud de 2350 metros, latitud S 22º 23' 26" y longitud W 44º 40' 17". Las pruebas histoquímicas se aplicaron em muestras no fijadas para identificar granos de almidón, lípidos, compuestos fenólicos, glicídios y cristales. Es importante resaltar la gran cantidad de fibras en las regiones abaxial y marginal de la hoja, formando uma verdadeira armadura contra las agresiones ambientales. Además, esta característica es destacada bajo la perspectiva taxonómica, pues ninguna outra especie de la tribu exhibe um desarrollo de fibras com tal magnitud. Los datos anatómicos revelaron que várias características de *C. glaziovii* son similares a las de plantas xeromórficas y contribuyen a su identificación, además de possibilitar la profundización de las discusiones sobre las intrincadas relaciones planta-ambiente.
Palabras clave: anatomia ecológica, Melastomataceae, *Chaetostoma glaziovii*, Parque Nacional de Itatiaia, indicador ambiental.

INTRODUCTION

Chaetostoma glaziovii Cogn. belongs to Melastomataceae, a botanical family, distributed in pantropical regions and is one of the biggest families of Angiosperms, with about 4570 species (RENNER, 1993; CLAUSING & RENNER, 2001). Despite the easy identification of their representatives in the field, based on its opposite and crossed leaves, typical pattern of venation, acrodromous, sickle stamens and poricidal anthers, their intra-family relationships are very complex and remain poorly understood. In Brazil there are approximately 1,500 species distributed in 69 genera (BAUMGRATZ, 1985; BAUMGRATZ *et al.*, 2014) and Microlicieae is one of the biggest clades with 275-300 species, 90% endemic to the Brazilian Cerrado (FRITSCH *et al.*, 2004).

Chaetostoma is a genus that presents 11 species and only two species, *C. pungens* (PFLAUM, 1897) and *C. fastigiatum* (MENTTINK & BAAS, 1992) were analized from the anatomical viewpoint. *C. glaziovii* occurs only in southeastern Brazil and always in mountainous areas (RIBEIRO *et al.*, 2007). BRADE (1956) stated that this species was endemic of Itatiaia rock outcrops, but it was found later in Serra dos Órgãos – RJ, in Campos da Bocaina – SP and in Passa Quatro – MG. In any of these regions, the species inhabits at altitudes above 2,000 meters. The vegetation found in these places undergoes the effects of strong winds, freezing temperatures in some periods, intensive light and high ultraviolet radiation (RIBEIRO *et al.*, 2007). Environments with different characteristics require specific adaptations of flora.

These interrelationships have attracted the researchers' interest in the ecological anatomy and plant physiology areas– ecophysiology – to elucidate the intricate mechanisms involved in the survival of plants in different conditions.

ESAU (1977) states that plants show variations in leaf structure as evolutionary adaptations to the conditions of a specific habitat. However, different plants growing in the same ecologic niche overcome the adverse conditions in different ways. In a habitat deficient in water, for example, some plants develop features protecting the aerial parts from excessive loss of water, others form underground water storage organs or develop roots reaching great depths, and still others control the problem by having a short life span restricted to the time when water supply is most abundant.

The Itatiaia National Park was the first national park established in Brazil and has an area of 30,000 hectares. It is located in the Itatatiaia Massif in Mantiqueira Mountains, between the states of Rio de Janeiro, Minas Gerais and São Paulo, in the municipality of Itatiaia – RJ. It extends to the south of Minas Gerais, in the municipalities of Itamonte, Alagoa and Bocaina de Minas. Is geographically located between the parallels 22º 19' and 22º 45' of south latitude and between the medians 44º 15' and 44º 50' of west longitude. The origin of the name "Itatiatia" is from language Tupy and means "full of tips cliff". Has mountains above 2,000 meters high and maintains a highly diverse flora and fauna due to the varying altitude and climate. The park is divided into two distinct environments: lower part (the park headquarters) and the high part (plateau). Thus, the vegetation is structured according to the altitudinal gradient, as described by BRADE (1956): subtropical forests hygrophilous (below 1,100 meters), transition forest (1,200 to 1,800 meters), Araucaria region (1,600 to 2,300 meters), high-fields (2,000 to 2,400 meters) and cliff and rock vegetation (above 2,400 meters).

AXIMOFF & RIBEIRO (2012) state that different species, found in an altitude above 2,000 meters, might present many similarities in the morphology and disposition of its leaves, helping them protect against climate effects, as high incidence of radiation. *C. glaziovii*, for example, inhabits in rocky crevices, usually in the presence of small Bromeliaceae around it. Both kind of plants present very small leaves, which are, also, upright and partially imbricated.

The environment influences not only the plant morphology, but also its anatomical structure. Ecological anatomy has contributed with their data to understand the interrelationships of plants with the environment around them.

Considering this scenario, the aim of this study was contribute genus natural characterization through the presentation of its anatomy, besides evidencing if the anatomical characteristics of *C. glaziovii* may be like those found in xeromorphic plants.

MATERIAL AND METHODS

Itatiaia National Park, where C. glaziovii inhabits (figures 1, 2), present mesothermal climate with seasons well marked with cold, dry winters and very humid summers. The average temperature is about 14º C and lower temperatures drop below -10º C in approximately 56 freezing nights per year. The annual rainfall is about 2,400 mm, concentrated in the summer months (RIBEIRO et al., 2007).

Figure 1 – Itatiaia National Park altitude fields, visualizing the Agulhas Negras peak, in the background.
Figure 2 – *Chaetostoma glaziovii* (brown color, arrow) next to bromeliads (light green) in the track to Rebouças shelter.
Source: authors.

Figure 3 – Detail of the cylindrical branches and imbricated leaves.
Source: authors.

Chaetostoma glaziovii Cogn. is a sub shrub with green, upright leaves (figure 3). Its flowers are showy, with pink corolla and yellow stamens, relatively large compared to the size of their clumps. Flowering occurs from November to July. Besides Itatiaia, it can be found at Serra dos Órgãos – RJ, Campos da Bocaina – SP and Passa Quatro – MG. The samples of leaves and stems analyzed in this work are from Itatiaia National Park, collected at 2,530 meters high, 22º 23' 26" latitude S and 44º 40' 17" longitude W (licence: IBAMA – SISBIO 16688-1, 05/07/2008). Vouchers were registered at Universidade do Estado do Rio de Janeiro (Rio de Janeiro State University) Herbarium, which received the number H-RJ – 11.116. The identification of material was made based on literature (KOSCHNITZKE, 1997) and by comparison with exsiccated samples from Jardim Botânico do Rio de Janeiro (Rio de Janeiro Botanical Garden). Anatomical study was carried out on fixed and fresh leaves and stems samples. The fresh ones were used for histochemical tests. The material was fixed with glutaraldehyde 2,5% and CRAF III (KRAUS & ARDUIN, 1997). Histological sections were obtained by freehand and treated according to the usual techniques in Plant Anatomy Anatomy to prepare semipermanent slides. These procedures comprise clarifying the histological sections with 50% sodium hypochlorite in water, washing three times with distilled water, neutralizing with acetic water 1: 500, washing again and finally coloring (JOHANSEN, 1940; JENSEN, 1962; KRAUS & ARDUIN, 1997). The dyes used were Astrablue 1% and Safranin 1%, the ratio of 9:1 (BUKATSCH, 1972). Part of the material was infiltrated with paraffin (JOHANSEN, 1940; JENSEN, 1962) and seccionated in rotative microtome with 10-15 µm thick for permanent slides. The epidermis was highlighted with Jeffrey solution and the fragments dyed with safranin. Histochemical tests were performed on unfixed sections to identify starch grains, lipids, phenolic compounds and sugars, using, respectively, lugol, Sudan III, ferrous sulfate and Benedicts' reagent (KRAUS & ARDUIN, 1997). The chemical nature of the crystals was analyzed by its solubility in acids (HOWARTH & WARNE, 1959). The slides were analyzed with optical microscopy (OM) and the photographic documentation was obtained with the equipment Axiostar Plus, Zeiss, coupled to a computer, equipped with Axiovision 4,5 software. Samples of leaves and stems, fixed in glutaraldehyde, were dried to the critical point and coated with gold palladium, using the sputter Emitech K575X to be examined by scanning electron microscopy (SEM) were made with microscopes LEO 1450VP and EVO 40, Zeiss. Samples of dried material, with no previous treatment, were also metalized for SEM analysis.

RESULTS

Leaf

Leaf dimensions are 2.0-7.0 mm length and 0.7-1.8 mm wide, leathery, triangular, with acuminate and pungent apex, entire margin or serrated with hardened texture.

Anatomically, the adaxial epidermis shows, in surface view, rare glandular trichomes, consisting of a pedicel and a highly developed glandular head (figure 4). Anomocytic stomata occur, on a parallel disposition (figures 5, 6) spreaded all over this surface (figure 7) except in the apex and in the margins, which contains, exclusively, epidermal cells and fibers (figure 13). Epidermis cells have very thick walls (figures 5, 7, 8) and the anticlinal ones have very tiny ripples (figure 5). The cuticle is striated (figures 6, 7). Histochemical test for identifying phenolic compounds revealed strong positive reaction (figures 5, 10, 11). The abaxial epidermis is glabrous (figure 8) and presents stomata only in the basal region of the leaf.

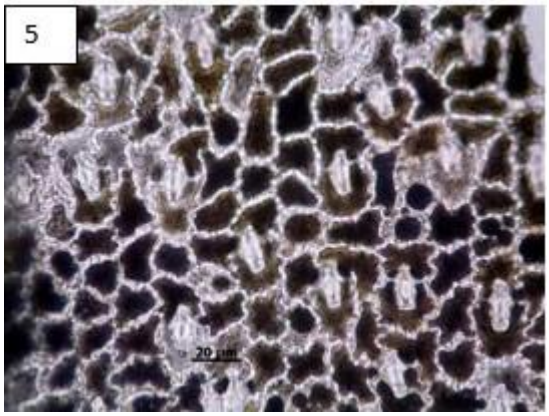
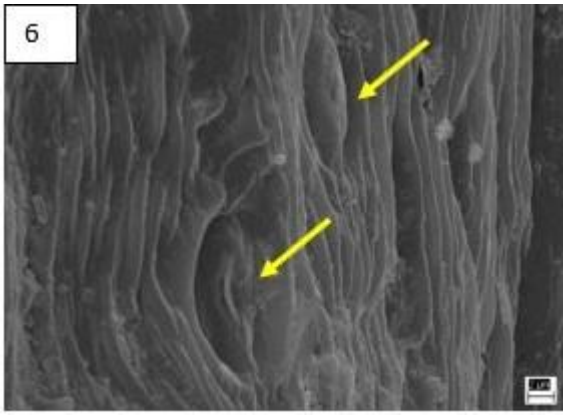


Figure 4 – Surface view of the upper side of the leaf, showing a glandular trichome (arrow). **Figure 5** – Surface view of the adaxial epidermis, treated with ferrous sulfate to identify phenolic compounds (black), showing anomocytic stomata.
Source: authors.



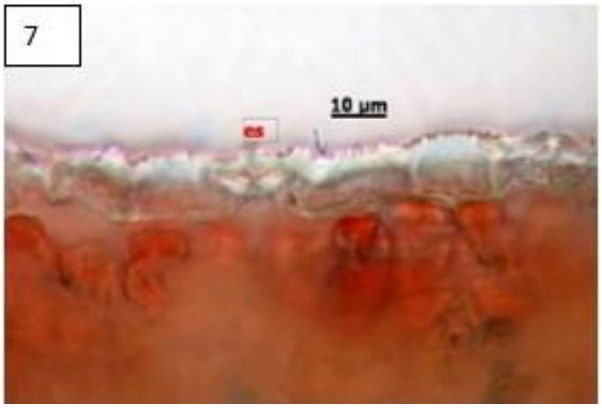
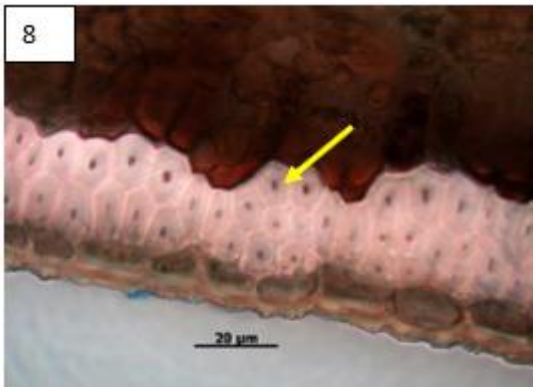


Figure 6 – SEM of adaxial surface, showing two stomata (arrows) and striated cuticle.
Figure 7- Detail of transection of adaxial epidermis of the leaf of *Chaetostoma glaziovii*,
Source: authors.

The mesophyll is isobilateral, that is, with palisade parenchyma on both sides of the leaf (figure 9). The abaxial region of the leaf shows, under the epidermis, a well-developed sclerenchyma, composed by fibers, forming an armor (figures 9, 10, 11). In the basal region of the leaf blade, however, chlorenchyma interrupt these fibers and the epidermis contain stomata. Spongy parenchyma occurs in the middle region of the mesophyll and the cells near the midrib, present bigger intercellular spaces (figure 12).



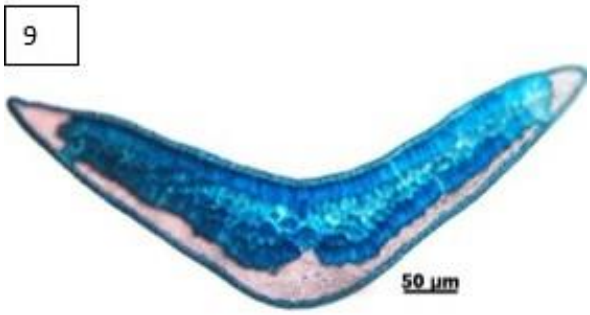


Figure 8 – Cross section of the abaxial epidermis of the leaf of *C. glaziovii*. Fibers (arrow) form a protector shield.
Figure 9 – Cross section of the leaf showing isobilateral organization and great amount of fibers (clearer areas).
Source: authors.

Lipids, starch grains, sugars and phenolic compounds are present in parenchyma cells. Vascular bundles are collateral and are involved with parenchymatous sheaths. In the middle third of the leaf blade there are 11 vascular bundles that fuse as they approach the apex,

without reach the distal portion, which is formed only by fibers and epidermis (figure 13), making this region hard and pungent. In the basal region of the leaf, there are many druses in contrast with the other regions.

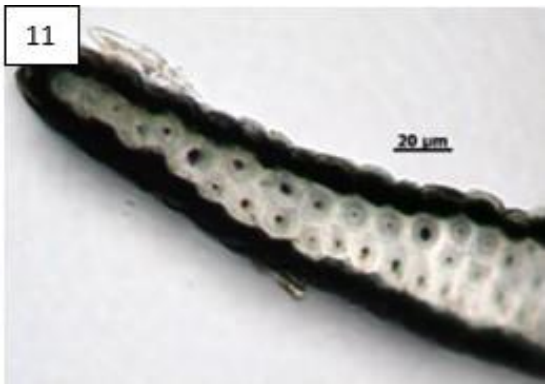
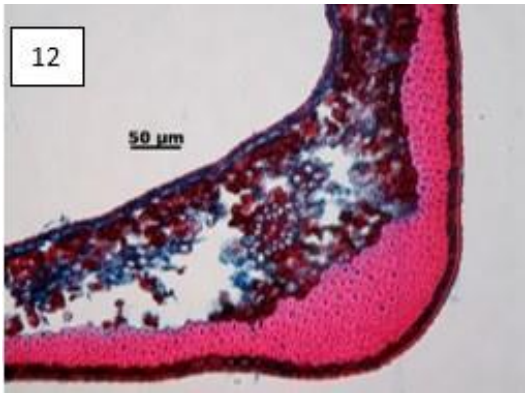


Figure 10 – Cross section of the leaf with ferrous sulfate to identify phenolic compounds (black color).
Figure 11 – Detail showing the leaf margin formed, exclusively, by epidermis and fibers.
Source: authors.



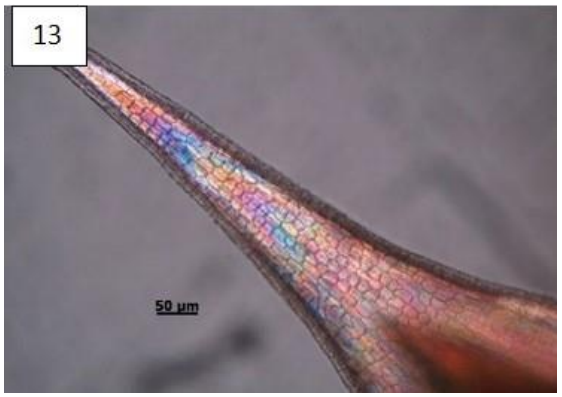


Figure 12 – Detail of the middle region of the leaf with remarkable amount of sub epidermal fibers (pink) on the abaxial side.
Figure 13 – Leaf apex showing the region formed, exclusively by epidermis and fibers, lightened by polarizing filters.
Source: authors.

Stem

Cylindrical branches internodes measure 1-2 mm in length, and enlarged nodes have glandular trichomes (figures 14, 15, 16).

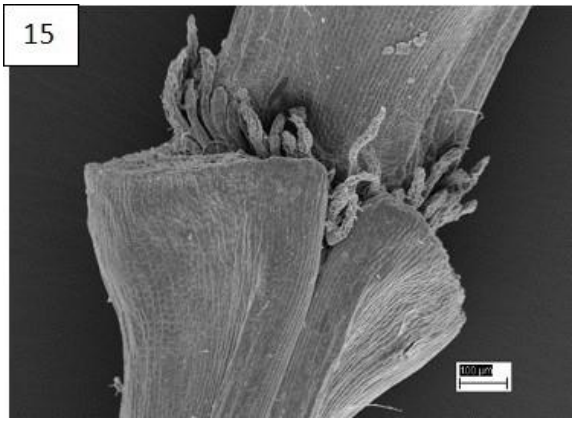
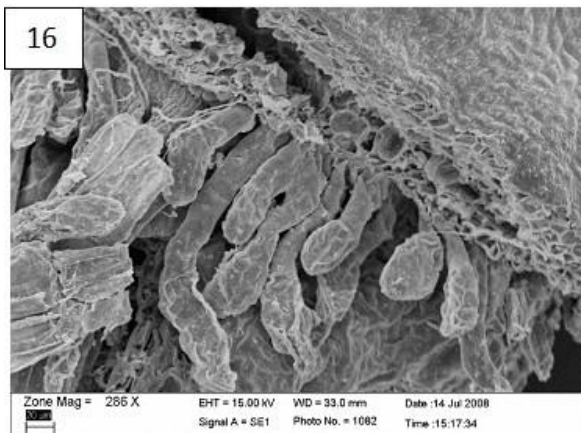


Figure 14 – Connection between leaf and stem, with glandular trichomes and leaf primordes (arrow).
Figure 15 – SEM of the node region of the stem.
Source: authors.

Anatomically, the stem of *C. glaziovii* displays rounded transverse section with lateral recess (figure 17), where occur glandular trichomes (figure18) with positive reaction to lipid test (figure 18). Simple epidermis, with very thick periclinal walls, with stomata in the recesses region. Sub epidermal fibers occur except in the recesses of the stem. From there, the parenchyma extends to the endodermis. In the central cylinder, the vascular system, which has internal phloem, surrounds a parenchymatous pith (figure 17).

Concerning the histochemical tests, positive reactions occurred to lipids in epidermis and endodermis, while phenolic compounds and sugars were positive in epidermis, trichomes, parenchyma and vascular system.



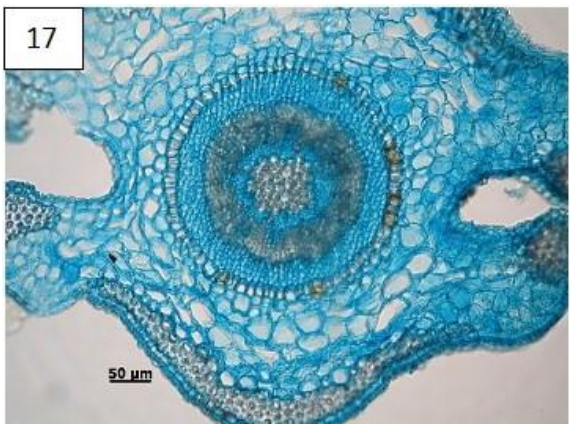


Figure 16 – SEM of the node region showing the glandular trichomes.
Figure 17- Cross section of the stem with lateral recesses and the central cylinder surrounded by an endodermis.
Source: authors.

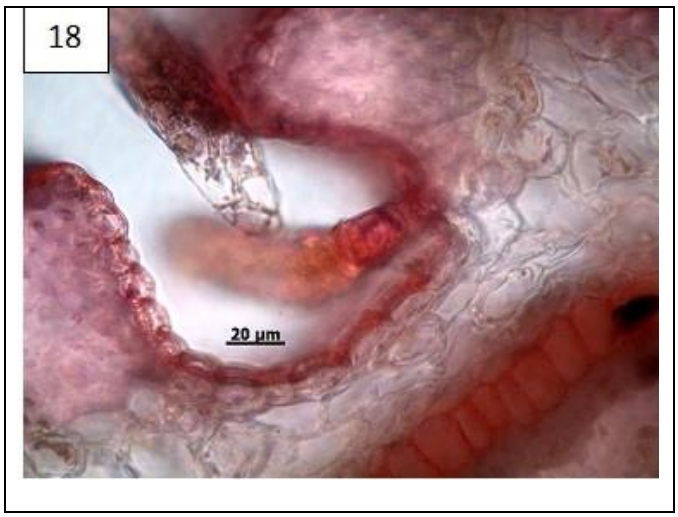


Figure 18 – Lateral recess of the stem, showing glandular trichomes, treated with Sudan to identify lipids (red color).
Source: authors.

DISCUSSION

The morphological and anatomical data of *C. glaziovii* analyzed in this work corroborate the hypothesis of similarity between this plant and the anatomical characteristics of the xeromorphic plants mentioned by ESAU (1977), FAHN (1990), CUTTER (1987) and APPEZZATO-DA-GLÓRIA & CARMELLO-GUERREIRO (2012). For example, it can be highlight that *C. glaziovii* presents high volume/surface ratio, that is, small and compact leaves, leathery texture, thick mesophyll, palisade parenchyma related to both leaf surfaces, small cells, abundant sclerenchyma, thick cell walls, especially in the epidermis and well-developed cuticle.

All these characteristics are listed by the cited authors as typical of xeromorphic plants. Besides the hard texture, the leaves of *C. glaziovii* are pungent due to the anatomical structure of its apex, formed exclusively by epidermis and fibers. Worthy of attention is the fact that the larger amount of stomata occur on the adaxial side of the leaf epidermis rather than on the abaxial, as expected in amphistomatic leaves. But the upper side is the most protected because the leaves are partially overlapped and positioned vertically. On the abaxial surface, stomata occur only in the lower portion of the leaves because this region is hidden by the leaves of the lower node, which act as a protective shield. Stomata on the abaxial surface of the leaves, near the apex, would be harmful to the plant because they would undergo highest intensity of environmental factors, and gas exchange would not be performed

successfully. MENTINK & BASS (1992) state that, considering the leaf epidermis in the Melastomataceae Family, it is more common the occurrence of stomata and trichomes on the abaxial surface, which are the opposite features found in *C. glaziovii*.

According to PFLAUM (1897), *Chaetostoma pungens* has large bundles of fibers, independent of the ribs and parallel to them, also occurring in the leaf margins. MENTINK & BASS (1992) indicate the presence of many sclereids in the mesophyll of *Stenodon suberosus* and SOUSA (1997) comments on fibers that occur nearby the midrib in some species of *Lavoisiera*. The presence of subepidermic fibers in *C. glaziovii* is responsible for considering ribs as inconspicuous, from the morphological viewpoint. In fact, in the middle region of the leaves, occur up to 11 parallel veins. Thus, it can be assumed that other species described as having inconspicuous ribs possibly present these fibers, just as occurs in *C. glaziovii*. According to KOSCHNITKE (1997), *C. cupressinum* and and *C. scoparium* are the only species of the genus with inconspicuous ribs. In relation to *Chaetostoma glaziovii*, this author also considers that the individuals from Itatiaia National Park have inconspicuous ribs while that ones inhabiting Serra dos Órgãos may have 5-7 ribs evident morphologically. Given this information, it can be inferred that the production of these fibers is not as intense in the individuals of Serra dos Órgãos. This variation in the amount of fibers in individuals of these two locations may be due to the difference in altitude and, in consequence, to the different expositions to ultraviolet radiation. *C. glaziovii* of Itatiaia occurs in higher altitudes in comparison with Serra dos Órgãos. The taxonomic significance of this characteristic should be considered because in no other species of tribe Microlicia, the fibers are so developed as in *C. glaziovii*. Even considering other characteristics of the genus and species, such the presence of a crown of trichomes on the hypanthium apex, herbarium specimens of sterile individuals are very difficult to identify (KOSCHNITZKE, 1997). Thus, this foliar anatomical feature, that is, the presence of fibers forming a continuous layer margin to margin, could contribute to the identify specimens.

Another weighty characteristic is the great amount of phenolic compounds in the species here analyzed. According to LARCHER (2000), many plants of arid areas produce these substances as allelopathic strategy and have antimicrobial activity besides to prevent herbivory.

DICKSON (2000) refers that anatomical structure of alpine plants is like that found in plants from arid environment due to high intensity of light and winds. The xeromorphic features of *C. glaziovii*, in this way, may be associated to climate factors rather than a lack of

water. This is emphasized by the existence of hydrophytes in the neighborhood of *C. glaziovii*. SOUSA (1997) states that in Serra do Cipó hills, another rock outcrop environment, where he studied species of *Lavoisiera*, there are many places with much water in the soil, despite the arid appearance of the region.

CONCLUSION

By analyzing the obtained data it can be concluded that the anatomical characteristics of *C. glaziovii* are similar to those of xeromorphic plants.

The other objective of this work was to contribute to the genus natural characterization through the presentation of the anatomy of *C. glaziovii*.The systematic approach of this anatomical study revealed be worthwhile. As mentioned in the Introduction of this work, only two species, *C. pungens* (PFLAUM, 1897) and *C. fastigiatum* (MENTINK & BAAS, 1992) were analyzed from the anatomical viewpoint, in a universe of eleven species. SOUSA (1997) analyzed 15 species of *Lavoisiera* and BRINA (1988) undertook an anatomical study with five species of *Microlicia*, typical of high-fields. Comparing these anatomical data, one concludes that there are great similarities between these three genera, *Lavoisiera*, *Microlicia* and *Chaetostoma*, making the identification of sterile individuals a very hard task. Studies that bring to light more data from anatomy may contribute to characterize and identify the species belonging to this taxonomic group. Finally, considering that *C. glaziovii* was never found in any other kind of environment, except in high fields, a question is posed and deserve future investigations: would *C. glaziovii* be a tolerant species in relation to the adverse climatic factors or would these conditions be important for its survival?

In this connection, GUREVITCH *et al.* (2009) states that much endemic species occur in soils that are poor in several nutrients, which, frequently, contain heavy metals. Such plants are excluded from the around region because of competition with other species. These ones, of the surrounding areas, by the other way, are unable to support poor and toxic soils.

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