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CONTRIBUTIONS TO THE BIOLOGY OF SECRETION IN THE BROMELIACEAE

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RESUMO

Bromeliaceae é uma família de plantas neotropicais com 82 gêneros e ca. 3,715 espécies cuja expansão e diversificação pelo Neotrópico se deu por meio de radiação adaptativa associada a características-chave, as quais permitiram a ocupação de diferentes zonas adaptativas. Representantes da família exibem diversas estratégias de vida, desde linhagens terrestres em florestas úmidas até epífitas e litófitas em ambientes xéricos. A biologia da secreção na família é pouco compreendida, embora estudos recentes tenham demonstrado função glandular em tricomas peltados do eixo reprodutivo em *Aechmea blanchetiana* (Baker) L.B. Sm (Bromelioideae), com perspectivas promissoras. Este trabalho teve como objetivo a ampliação do conhecimento relativo à biologia da secreção em Bromeliaceae, com ênfase na função glandular dos tricomas peltados. Objetivos específicos incluem: (a) averiguar se função secretora associada aos tricomas também ocorre em membros da subfamília Tillandsioideae, com descrições da estrutura, ultraestrutura e atividade secretora; (b) verificar se os tricomas peltados estão relacionados à produção e liberação de secreções lipofílicas; (c) produzir uma ampla prospecção da secreção em escamas da inflorescência em Tillandsioideae; (e) descrever a ocorrência, estrutura e aspectos da secreção dos nectários extranupciais de *Pitcairnia burchellii* Mez, com breve revisão do conhecimento a respeito dessas estruturas na família. Usando metodologia padrão para histoquímica, microscopia de luz e microscopia eletrônica, verificou-se que a presença de secreção associada às inflorescências é difundida em Tillandsioideae, com exsudatos exclusivamente hidrofílicos, ou mistos. A atividade secretora ocorre, exclusivamente, em tricomas dispostos na face adaxial das brácteas florais. Possíveis funções incluem proteção contra dessecação, herbívoros, patógenos, alta irradiação, e interação com polinizadores ou outros visitantes florais. Nectários extranupciais são descritos pela primeira vez em *Pitcairnia* e compreendem uma característica homoplástica difundida em bromélias. Considerações evolutivas e taxonômicas são apresentadas, bem como direções para pesquisa futura.

Palavras-chave: coléteres, interações planta-ambiente, mucilagem, nectários, nectários extraflorais, resina, nectários extranupciais, terpenos, Tillandsioideae.

ABSTRACT

Bromeliaceae is a Neotropical plant family with 82 genera and ca. 3,715 species whose expansion and diversification across the Neotropics occurred through adaptive radiation associated with key-features, leading to the occupation of different adaptive zones. Members of the family exhibit diverse life strategies, from terrestrial lineages in humid forests to epiphytes and lithophytes in xeric environments. The biology of secretion in the family is largely unexplored, although recent studies have demonstrated glandular function in peltate trichomes of the reproductive axis in *Aechmea blanchetiana* (Baker) L.B. Sm (Bromelioideae), with promising prospects.This work aimed to expand the knowledge regarding the biology of secretion in Bromeliaceae, with emphasis on the glandular function of peltate trichomes. Specific objectives include: (a) to investigate whether secretory function associated with trichomes also occurs in members of the subfamily Tillandsioideae, with descriptions of structure, ultrastructure and secretory activity; (b) to verify if scales are related to the production and release of lipophilic secretions; (c) produce a broad prospection of the secretion in inflorescence scales of Tillandsioideae; (e) to describe the occurrence, structure and aspects of the secretion in extranuptial nectaries of *Pitcairnia burchellii* Mez, with a brief review of the knowledge about these structures in the family. Using standard methodology for histochemistry, light microscopy and electron microscopy, the presence of secretions associated with inflorescences was found to be widespread in Tillandsioideae, with exclusively hydrophilic or mixed exudates. The secretory activity is carried out exclusively by trichomes on the adaxial face of the floral bracts. Possible functions include protection against desiccation, herbivores, pathogens, high irradiation, and interaction with pollinators or other floral visitors. Extranuptial nectaries are described for the first time in *Pitcairnia* and comprise a widespread homoplastic feature in bromeliads. Evolutionary and taxonomic considerations are presented, as well as directions for future research.

Keywords: colleters, extrafloral nectaries, extranuptial nectaries, mucilage, nectaries, plant-environment interactions resin, terpenes, Tillandsioideae.

SUMÁRIO

1. INTRODUCTION

1.1 *The family Bromeliaceae*

Bromeliaceae is a diverse neotropical plant family with 58 genera and ca. 3715 species (Gouda and Butcher, *cont. updated*) distributed along various ecosystems of the southern North America, Central and South America (Smith and Downs, 1974, 1977, 1979; Givnish, 2007, 2011, 2014). Long-distance dispersal is believed to have spread the family also to West Africa, where a single species occurs (Givnish, 2014). Adaptive radiation and pulses of speciation played important roles during the diversification of the family from the Guyana Shield to other portions of tropical and subtropical America (beginning at ca. 15.4 Ma; Givnish, 2007; Givnish, 2014). This remarkable diversification was triggered by the acquisition of several key innovations (e.g., absorbing trichomes, avian pollination, CAM photosynthesis, leaf impounding tanks) and the invasion of key-landscapes, such as moist, fertile mountainous regions, and dry, seasonal areas of South and Central America (Givnish, 2014). Not surprisingly, bromeliads exhibit various lifeforms and can be found occupying many distinct niches, from terrestrial plants in the ground of tropical forests, to small or massive herbs living as rheophytes, lithophytes, and epiphytes (Smith and Downs, 1974, 1977, 1979; Saraiva and Forzza, 2020). The epiphytic habit is especially diverse, ranging from large plants with leaf impounding rosettes to extremely reduced, tankless epiphytes often referred to as "atmospheric" bromeliads (Benzing, 2000; Givnish, 2014).

Phylogenetic analyses of the family have recognized eight subfamilies: Brocchinioideae, Lindmanioideae, Tillandsioideae, Hechtioideae, Navioideae, Pitcairnoideae, Puyoideae and Bromelioideae (Givnish, 2007, 2011; Fig. 1). The monotypic Brocchinioideae is the sister group to all other bromeliads, and is limited to the hiperhumid infertile locations of the Guyanas (Givnish, 2011). Lindmanioideae is also restricted to the Guyana Shield, while the other subfamilies have spread throughout the Neotropics, with centers of diversification in Central America, the Andes, Amazonia and the Brazilian Shield (Givnish, 2011, 2014). It is important to note, however, that the current treatment of Bromeliaceae subfamilies is challenged by some studies, which have generated doubts regarding the number of subfamilies and the status of Puyoideae and Pitcairnioideae (Schütz et al., 2016; Yardeni et al., 2021)

The diversification and geographical expansion of Bromeliaceae is not only associated with key traits and landscapes, but also with a certain degree of correlated and contingent evolution between these key-factors (Givnish, 2014). A remarkable example is the independent origin of epyphitism and impounding tanks in Tillandsioideae and Bromelioideae, both favored by the rise of moist montane habitats during the uplifting of the northern Andes (Tillandsioideae) and the Brazilian Highlands (Bromelioideae; Givnish, 2014).

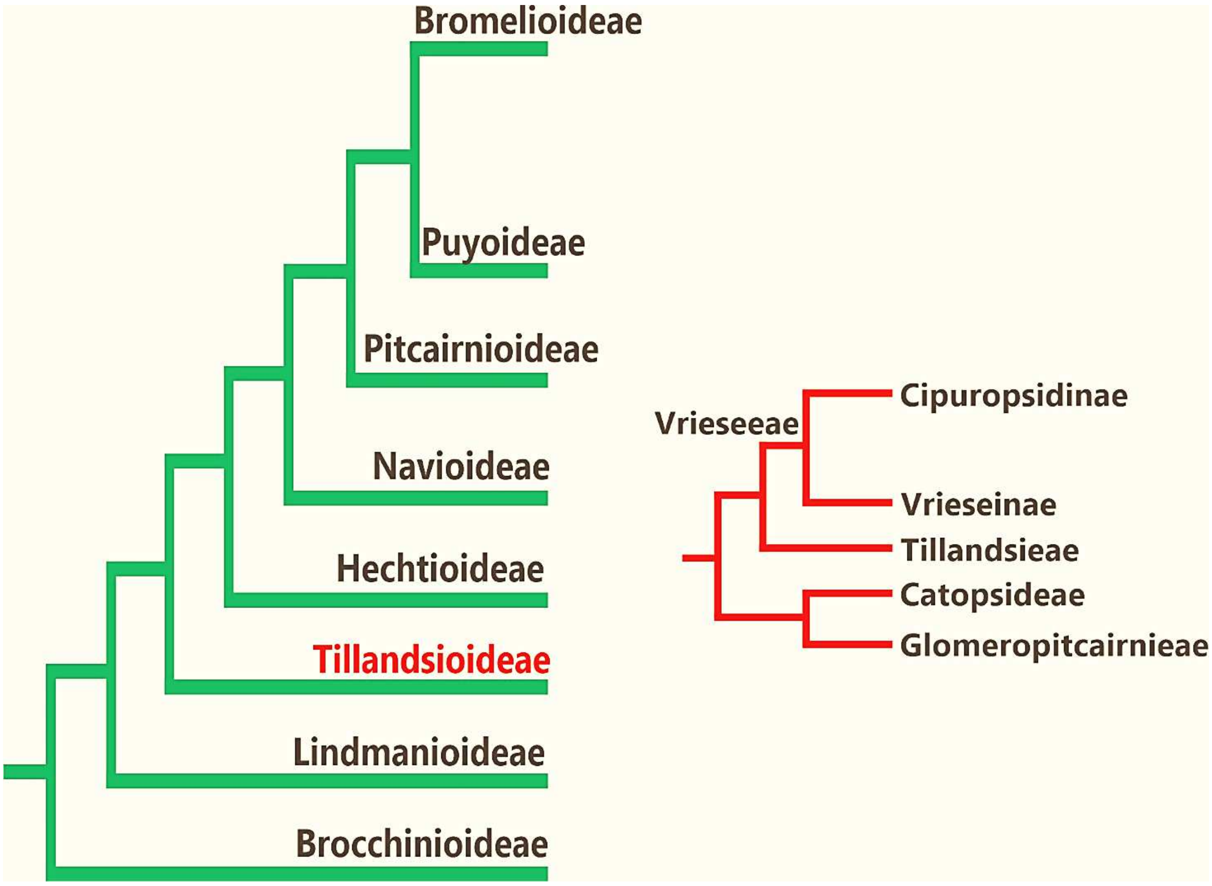

Figure 1. Proposed relationships among bromeliad subfamilies (on the left), and the major lineages of Tillandsioideae (on the right) based on Givnish et al. (2011) and Barfuss et al. (2016).

The systematics of Bromeliaceae beyond the level of subfamilies is an ongoing enterprise (Schulte et al., 2005, 2009; B arfuss et al., 2005, 2016; Gomes-da-Silva e Souza-Chies, 2018; Gomes -da-Silva et al., 2019; Machado et al., 2020). The relatively recent divergence of many genera, coupled with extensive morphological variation and low rates of molecular evolution poses great challenges in this direction (Givnish et al., 2011; Machado et al., 2020). Nonetheless, recent advancements with broad samples of taxa are gradually improving our understanding of the relationships between extant bromeliads (Barfuss et al., 2016; Machado et al., 2020).

Of special interest to this work are the recent improvements in the evolution and classification of subfamily Tillandsioideae (Barfuss et al., 2016; Gomes-da-Silva and Souza-Chies, 2018; Machado et al., 2020). This group is the largest of the eight bromeliad subfamilies, with more than 1,500 species (Gouda and Butcher, *cont. updated*). It is mostly comprised of epiphytes, appearing in both mesic and xeric environments (Smith and Downs, 1977; Barfuss et al., 2016). Tillandsioid bromeliads have been recently reviewed by Barfuss et al. (2016), with considerable changes in the traditional taxonomic classification, including the description of several new genera. While that revision has received some criticism (Gomes-da-Silva e Souza-Chies, 2018), it seems to be well supported by molecular phylogenetic analysis using comprehensive datasets (Machado et al., 2020). Accordingly, Tillandsioideae is currently divided into four distinctive tribes (Tillandsieae, Vrieseeae, Catopsideae and Glomeropticairnieae; Barfuss, 2005, 2016; Gomes-da-Silva and Souza-Chies, 2018; Machado et al., 2020; Fig. 1). Tribe Tillandsieae and Vrieseeae comprise the core group, with the majority of species. Vrieseeae, in turn, splits into two major lineages: subtribe Vrieseinae (usually ascribed to eastern Brazil, but see Machado et al., 2020) and subtribe Cipuropsidineae (ascribed to the Andes, Central America and Caribbean; Barfuss, et al. 2016; Fig. 1).

Despite this growing knowledge, several issues remain (Barfuss, et al. 2016; Machado et al., 2020) and only through the accumulation of morphological and molecular data we will be able to achieve a more comprehensive understanding of this truly fascinating group of plants (see Gomes-da-Silva et al, 2012).

In the present work, Chapter I, II and III bring some contributions to the biology of Tillandsioideae species, while Chapter IV focus on a pitcairnioid species, with considerations for other groups in the family.

1.2 *Trichomes in bromeliads*

The great botanist and ecologist David H. Benzing once wrote that "*about the only trait shared by all bromeliad trichomes is multicellularity*" (Benzing, 2000). Perhaps surprising to many, this statement clearly identifies the diversity of the indumentum in bromeliads. Beyond the typical peltate trichomes, often referred to as "scales", Bromeliaceae display a wide variety of epidermal appendages, including filiform, capitate and stellate trichomes (Tomlinson, 1969; Benzing, 2000; Figure 2).

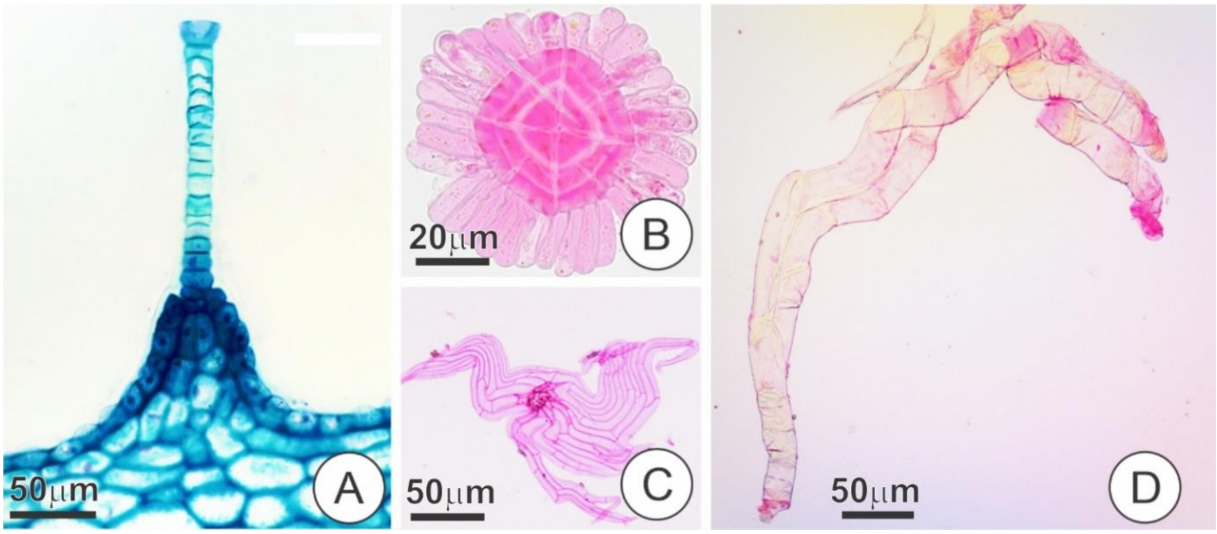

Figure 2. Diversity of trichomes in bromeliads. **A.** Capitate trichomes in a species of *Aechmea*. **B-C**. Peltate trichomes with distinct structures in *Vriesea procera* and *Canistrum aurantiacum,* respectively. **D.** Irregular trichome in the inflorescence axis of *Brocchinia reducta*.

More common, the peltate trichome also display a great diversity of forms, especially in regard to shield morphology, which can vary from loosely arranged caps to extremely sophisticated structures (Benzing, 1976; Benzing et al., 1985). Due to their capacity for water absorption, these sophisticated scales have received the attention of botanists for more than a century (Schimper, 1888; Mez, 1904; Tietze, 1906; Wetzel, 1924; Dolzmann, 1964, 1965; Tomlinson, 1969; Benzing, 1976). In fact, such trichomes are considered a key-trait in the diversification of bromeliads (Givnish, 2011; 2014), and together with the presence of water impounding tanks and CAM photosynthesis, comprise an important strategy of water-use in bromeliads (Males, 2016). Absorbing scales may also play a role in nutrient supply, in some cases associated to nutrition strategies not so common in plants, such as carnivory (Givnish et al. 1984).

Structurally, the absorbing scales of bromeliads have been described in detail by Tomlinson (1969) and Benzing (1976). They usually comprise two basal cells continuous with the ordinary epidermis, a uniseriate stalk and a distal flattened shield parallel to the epidermis. The uppermost cell of the stalk is sometimes distinct, appearing inflated and with a dome-like shape. The shield is divided in a group of central cells (usually four), which are surrounded by one or more concentric rings of quadrangular cells. The cells of the outmost ring are usually elongated radially, forming a broad, flat “wing” portion. Cuticularization occurs along the anticlinal walls of the stalk, forming annular projections toward the periclinal plane. Irregular thickening of the cell walls is usually associated with more specialized trichomes that engage in a pump-like mechanism.

Unfortunately, the anatomy and the ecological significance of less specialized and non-peltate trichomes is largely unexplored. In general, the functional roles of these structures are explained in regard to the supposed functions of most trichomes in other angiosperms, (see Benzing, 2000). Glandular function has been described for trichomes in *Tillandsia usneoides* (L.) L. (Tillandsioideae) and *Aechmea blanchetiana* (Baker) L.B. Sm. (Bromelioideae). In the former, secretory activity is related to a discrete intracellular secretion of pectic compounds in the stalk cells, possibly associated with water-absorbing efficiency (Brighigna et al., 1988.). In *A. blanchetiana*, trichomes in the inflorescence axis were shown to produce an abundant mucilaginous secretion that cover young portions of the inflorescence (Ballego-Campos and Paiva, 2018a). In that same species, secreting trichomes were also described for the vegetative axis (Ballego-Campos and Paiva, 2018b). In addition, a unique glandular function associated with secretion of digestive enzymes has been proposed by Givnish et al. (1984) in foliar trichomes of *Brocchinia reducta* Baker.

Beyond these observations, glandular functions for trichomes in bromeliads are largely unexplored. This might be due to a neglection of the indumentum in the reproductive axis, that has received far less attention from anatomists, taxonomists (see Carvalho et al., 2017) and ecologists.

Chapter I and II of this work bring in-dept analyses of structural and secretory aspects of scales in members of the Tillandsioideae, including some notes on their functions.

1.3 *Secretory structures in bromeliads*

Secretion plays a variety of roles in the life of many – perhaps most – plants (see Fahn, 1979; Thomas, 1991), from pollination and protection, to osmotic balance and digestion (Fahn, 1979). Additional plant-environment interactions mediated by plant exudates include dispersal, seed germination, dormancy and seedling development (Yang et al., 2012; De-Paula et al., 2015). Secretory products of plants are produced by a great diversity of specialized structures and can be either liberated inside the plant body (endogenous), or directly to the outside (exogenous, Fahn, 1979).

Some secretions are also accumulated inside the secretory cell itself (intracellular). Despite this diversity, secretory tissues are generally characterized by cells with small vacuoles and dense, organelle-rich cytoplasm (Fahn, 2000).

Nectaries are perhaps the most well-known of all secretory structures, with whole volumes and compilations dedicated to them (Bentley et al., 1983; Nicolson et al., 2007, Weber and Keeler, 2013). Functionally, these structures can either mediate pollination (nuptial nectaries; *sensu* Delpino, 1875), or attract ants and other arthropods that provide defense against herbivores (extranuptial nectaries; *sensu* Delpino, 1875).

Also very common in plants are colleters. These are secretory structures traditionally characterized as emergences of the plant body that secrete viscous exudates of hydrophilic, lipophilic, or mixed nature (Thomas, 1991). Although somewhat diverse in their structure (see Fahn, 1971; Thomas, 1991), colleters are usually associated with protection of young portions of the plant body against desiccation, herbivores and pathogens (Thomas, 1991; Tresmondi et al 2015). Other functions might include promotion of symbiotic bacterial growth (Lersten, 1874) or lubrication during the expansion of congested organs (Leitão and Cortelazzo, 2008). The distribution of colleters was once largely restricted to the eudicotyledons (Thomas, 1991; Mayer et al., 2011), but the last decade has seen an increase in the number of records for colleters in monocotyledons and other plant groups, including ferns (Leitão and Cortelazzo, 2008; Mayer et al., 2011; Oriani and Scatena, 2013; Cardoso-Gustavson et al., 2014; Ferrari and Oriani, 2017; Oliveira et al., 2017; Ballego-Campos and Paiva, 2018a, 2018b; Cassola et al., 2019).

In Bromeliaceae, studies regarding secretory processes generally make reference to nuptial nectaries. The presence of septal nectaries in the family was studied in an evolutionary context by Rudall (2002) and Sajo et al. (2004) and structural descriptions have been provided by several authors (e. g., Fiordi and Palandri, 1991; Bernardello et al. 1991; Mosti et al., 2013). Likewise, nectar composition and ecology have been the subject of several studies (e. g., Krömer et al., 2008; Göttlinger et al., 2019). Extranuptial nectaries have been investigated with some degree of detail by a few authors (Galleto and Bernadello, 1992; Schimid et al., 2010), but records of their presence in certain pitcairnioid bromeliads are common (see Gomes-da-Silva et al., 2019).

Nonetheless, beyond these nectaries, our knowledge of secretory structures in bromeliads is fairly scarce. Recently, Ballego-Campos and Paiva (2018a, 2018b) described the presence of mucilage-secreting scales in the inflorescence axis of *A. blanchetiana*, as well as a distinct secreting trichomes in the vegetative axis of this species. Both structures potentially perform colleter-like functions (Ballego-Campos and Paiva 2018a, 2018b). Presence of capitate trichomes and floral scents also have been recorded in the family (Tomlinson, 1969; Benzing, 2000; Milet-Pinheiro et al. 2021), but many aspects of these secretory systems are still unknown. In fact, much remains to be understood regarding the biology of secretion within Bromeliaceae (Ballego Campos and Paiva, 2018a, 2018b), as detailed accounts on the source and secretory activity of bromeliad glands are still scarce (Bernadello et al., 1991; Vesprini et al. 2003; Mosti et al., 2013; Ballego Campos and Paiva, 2018a, 2018b).

Interestingly, in writing about the ecological anatomy of bromeliads, Tomlinson (1969) stated that "*the tendency for Bromeliaceae to develop mucilaginous substances requires investigation.* […] *The key to epiphytism in the family may be biochemical.*" More than fifty years later, Chapter I, II and III of the present work explore a widespread secretion of mucilage and lipophilic substances in members of the Tillandsioideae. Chapter IV brings some considerations regarding extranuptial nectaries in bromeliads.

2. RESEARCH AIM AND OBJECTIVES

In light of the scarce knowledge regarding the biology of secretion in bromeliads, as well as the promising prospective brought by recent studies (Ballego-Campos and Paiva, 2018a), the aim of this work was to expand our current knowledge of secretory structures in Bromeliaceae, with emphasis in the secretory function of peltate trichomes. We specifically intended to:

(a) Investigate whether secretory scales also occur in members of Tillandsioideae, presenting a detailed account of their structure, ultrastructure and secretory activity.

(b) Verify whether secretory scales may also be related to the production and release of lipophilic secretions.

(d) Produce a broad prospection of secretion associated with scales in inflorescences of Tillandsioideae.

(e) Describe the occurrence, structure and secretory aspects of the extranuptial nectaries in *Pitcairnia burchellii* Mez, briefly reviewing the current knowledge of extranuptial nectaries in bromeliads, with prospective for future efforts.

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More than scales: evidence for the production and exudation of mucilage by the peltate trichomes of *Tillandsia cyanea* (Bromeliaceae: Tillandsioideae)

I

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Complex exudates in inflorescences of *Vriesea* species reveal evidence of lipophilic secretions by scales of bromeliads

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II

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ABSTRACT

Trichomes are an important feature of bromeliads. These structures are able to absorb water and minerals, and potentially provide protection against high irradiance, attraction of pollinators and dispersers, or mediate digestive processes. Recent studies have shown a glandular function associated with mucilage-secreting scales in inflorescences of bromeliads. During a prospective study of secretion in bromeliad inflorescences, we observed presence of an oily exudate in three species of *Vriesea* sect. *Xiphion.* To verify whether or not scales in bromeliads are able to produce lipophilic secretions, we investigated the secretory activity in these species to identify the source and chemical nature of the secretions covering the inflorescences. We used standard methods of histochemistry, light and electron microscopy applied to samples of floral bracts in two distinct phases of development. The secretions comprised both hydrophilic and lipophilic substances secreted by trichomes on the adaxial side of bracts. In *V.* aff. *bituminosa* and *V. fenestralis* the exudate was heterogeneous, with lipophilic phase composed of terpenoids and lipids. *Vriesea* aff*. bituminosa* also presented alkaloids. In *V. platynema* var. *rosea* the lipophilic content was lipidic. Secretory activity was restricted to early stages of trichome development and might be characterized by a temporal disjunction between secretion of hydrophilic and lipophilic contents. We suggest that the functions of the secretion include protection against herbivores, but might also relate to protection against pathogens, water loss, high-irradiance, and attraction of pollinators or other visitors. To our knowledge, this is the first report of bromeliad scales involved in the secretion of lipophilic substances.

Key-words: resin, Bromeliaceae, peltate-trichomes, plant-environment interactions, terpenoids, colleters, carnivory.

1.1 INTRODUCTION

Trichomes seem to be a key adaptive trait in bromeliads, accounting for important ecological and evolutionary roles in the history of this plant group (Givnish, 2014). Much of this status come from absorptive leaf scales - complex and specialized peltate trichomes that occur on leaves in many atmospheric and tank-forming bromeliads. These scales, which are capable of absorbing moisture and nutrients from the atmosphere or impounding-tanks (Benzing, 1976, 2000), are an important trait in understanding the remarkable independence from the soil achieved by many members of Bromeliaceae (Benzing, 1976, 2000; Givnish, 2014).

Scales of bromeliads have also been related to other ecological roles, besides their ability to absorb water and minerals. For instance, some bromeliad trichomes might not absorb water at all, instead being hydrophobic and acting in the maintenance of gas exchange that would otherwise be affected by collected moisture (Pierce et al., 2001). Other functions presented in the literature include protection against high irradiance, attraction of pollinators and dispersers, and even mediation of digestive processes in tanks of carnivorous bromeliads (Givnish et al., 1984; Benzing, 2000). Recently, a new unique secretory function was revealed in inflorescence trichomes of *Aechmea blanchetiana* (Baker) L.B.Sm. and *Tillandsia cyanea* Linden ex K.Koch, which produce a mucilaginous secretion that cover the young portion of the inflorescences acting as colleters, thus protecting the developing inflorescence from desiccation and/or herbivores (Ballego-Campos and Paiva, 2018; Ballego-Campos et al., 2020). Previous accounts on glandular trichomes in bromeliads were mostly speculative and devoid of details regarding the secretory activity. An intracellular deposition of polysaccharides was reported for the uppermost stalk cell in foliar scales of *Tillandsia usneoides* (L.) L. (Brighigna et al., 1988) and *Ananas comosus* (L.) Merr. (Sakai and Sanford, 1980), but despite the fact that this secretion might be related to improve water absorption (Brighigna et al., 1988), no further efforts have been made in this regard. Likewise, capitate trichomes observed first in *Ronnbergia petersii* L.B.Sm. (Gross, 1991) and later in *Navia glandulifera* B.Holst (Holst, 1996) were suggested to produce deterrent, toxic secretions for defense against biotic agents (Benzing, 2000), but their secretory activity and composition remain unknown.

During a prospective study of secretion in floral bracts of several bromeliad species (Ballego-Campos et al., *unpubl.*), we observed the presence of complex exudates covering the inflorescences in three species of *Vriesea* sect*. Xiphion*. This section is loosely characterized by certain floral traits such as campanulate corolla, distichous flowers, filaments enlarged at the apex, involute floral bracts (Moura, 2011; Moura and Costa, 2014), and many species are known to produce characteristic secretions covering the inflorescences, often with oily or bituminoid aspect (Moura, 2011; Moura and Costa, 2014; Gomes-da-Costa and Souza-Chies, 2018). Reports of secretion include the ability to trap herbivorous on the stick inflorescences (Monteiro and Macedo, 2014). Also, the presence of a "*sui generis"* scent is often reported for species of *Vriesea* sect. *Xiphion* (Moura, 2011, Costa et al., 2022). This group has historical circumscription problems, and has been shown to be non-natural by many authors (Moura, 2011; Machado et al., 2020 and references therein).

Despite these reports, there have been no studies regarding the source or composition of the exudate in oily/bituminoid species in bromeliads to date. Thus, in this study we investigated the secretory activity in *V.* aff. *bituminosa* Wawra, *V. fenestralis* Linden & André and *V. platynema* var*. rosea* (Antoine) Mez to identify the source and chemical nature of secretions covering their inflorescences. Following the findings of Ballego-Campos and Paiva (2018) and Ballego-Campos et al. (2020), we specifically looked for involvement of trichomes in the secretory activity to test whether bromeliad scales are also able to produce compounds other than mucilage that might be involved in distinct ecological roles.

2. MATERIALS AND METHODS

2.1 ***Plant Material***

Plant material was obtained from single individuals of *V. fenestralis*, *V. platynema* var. *rosea* and three specimens of *V.* aff. *bituminosa,* all of which growing in cultivation at the Coleção de Plantas Vivas do Jardim Botânico da Fundação de Parques Municipais e Zoobotânica (Belo Horizonte, Minas Gerais, Brazil). Due to contrasting/absent information regarding the specimens' collection site, as well as the young stage of the inflorescences, we were unable to safely determine the individuals related to *V. bituminosa*. These individuals were then labelled as *V.* aff. *bituminosa*, and will be referred as such hereafter. Difficulties in the identification and systematics of this species have been acknowledged before (Moura and Costa, 2014) and recently the monophyly of *V. bituminosa* was questioned (Machado et al., 2020). Individuals of *V.* aff. *bituminosa* and *V. fenestralis* flowered from late spring throughout the summer (nov/2019 – mar/2020), while *Vriesea platynema* var. *rosea* flowered in the late autumn (jun/2019). Access number for all specimens studied are as follow: CVJBFZB593; CVJBFZB713; CVJBFZB1111; CVJBFZB1151; CVJBFZB 1452; BHZB (*in prep.*); BHCB (*in prep.).*

2.2 *Light Microscopy and histochemistry*

For light microscopy and histochemistry, young inflorescences of all studied individuals were sampled. The sampling was carried out during the early hours of the morning. To maximize the chance of obtaining distinct phases of secretion, fragments of the bracts (middle third, aprox.1cm^2) were collected from both the apical and basal portions of the inflorescences. The studied species show acropetally maturation of the floral parts, and therefore apical and basal samples were considered young (≤50% of total expansion) and mature portions (≥50% of total expansion), respectively. We sampled both scape and floral bracts, and do not distinguish them hereafter since they did not differ in secretory activity. The collected samples were fixed under slightly vacuum in Karnovsky fixative (pH 7.2 in 0.1M phosphate buffer; modified from Karnovsky, 1965) for 24h, dehydrated in an increasing ethanol series and embedded in synthetic resin (2-hydroxyethyl methacrylate, Leica®, Heidelberg, German). Thin sections (6–8µm) were then obtained under a rotary microtome (Hyrax M40, Carl Zeiss Mikroskopie, Jena, Germany), stained with toluidine blue (pH 4.7 in acetate buffer, modified from O'Brien et al., 1964) and counterstained with ruthenium red (aqueous solution, 0.002%). The sections were mounted in glass slides using a synthetic mounting-medium (Entellan®, Sigma-Aldrich, St. Louis, USA) and observed under a CX41RF microscope (Olympus Scientific Solutions, Waltham, US) equipped with a digital camera and image capturing system (TV0.5XC-3, Olympus Scientific Solutions, Waltham, USA).

Following Ballego-Campos and Paiva (2018) and Ballego-Campos et al. (2020), the adaxial face of young and mature freshly-collected bracts were carefully scrapped with the aid of a razor blade and the material obtained submitted to the following histochemical tests: ruthenium red (Johansen, 1940) and alcian blue (Pearse, 1980) for mucilage; Sudan Red 7B for total lipids (modified from Brundrett et al., 1991), NADI reagent for terpenoids (David and Carde, 1964), Wagner reagent for alkaloids (Furr and Mahlberg, 1981), ferric chloride for phenolics (Johansen, 1940) and Xylidine Pounceau for proteins (Vidal, 1970). In addition, unstained sections of fixed and embedded material were submitted to the same tests. Control tests were performed accordingly.

2.3 *Electron Microscopy*

For transmission electron microscopy, fragments of young and mature floral bracts (aprox. 1mm^2) were obtained from inflorescences of *V.* aff. *bituminosa* and *V. platynema* var. *rosea.* These fragments were fixed in Karnovsky fixative (pH 7.2 in 0.1M phosphate buffer; modified from Karnovsky, 1965) under slight vaccum for 24h, postfixed in 1% osmium tetroxide for 2h, dehydrated in an increasing acetone series and embedded in epoxy resin (Spurr, 1969). Ultrathin sections were obtained with the aid of an ultramicrotome (UC6, Leica, Wetzlar, Germany) and contrasted with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Prepared sections were observed under a transmission electron microscope (Tecnai G2–Spirit, Philips/FEI).

3. RESULTS

3.1 *Structure and secretory aspects*

Secretion accumulates on the adaxial surface of bracts, covering the young floral buds, adjacent bracts and inflorescence axis of all species studied (Fig. 1A-E). This secretion was usually clear and viscous in unexposed young portions, but turn pale yellow to brown in mature parts, as it was exposed. The secretion was especially viscous and dense in unexposed portions of *V.* aff. *bituminosa*, rendering a grease-like aspect to the exudate (Fig. 1D). In this species, and also in *V. fenestralis*, the secretion remained as a shiny, sticky film that covered the entire reproductive axis during its development (Fig. 1C, E). In *V. platynema*, however, such a film was not seen, and the secretion appeared to be easily washed off from the surface of the inflorescences, remaining within the bracts and partially on the surface of the axis and young floral buds, sometimes forming dried strands (Fig. 1A-B). Secretions in all studied species were characteristically fragrant, although presenting distinct scents.

The adaxial surface of bracts presents many trichomes (Fig. 2A-B), comprising of two basal cells, a stalk and a flattened shield (Fig. 2C-L). The stalk is uniseriate and short, usually with 2-3 cells, but up to 4 in some scales of *V. platynema* (Fig. 2C-D) and as few as a single cell in *V.* aff. *bituminosa* (Fig. 2J). The shield is relatively well organized in *V. fenestralis* and *V. platynema*, comprising of a group of four central cells surrounded by one or two rings of isodiametric cells (the outermost usually incomplete), and a wing comprised of numerous peripheral elongated cells (Fig. 2C- H). In *V.* aff.

bituminosa, the shields are usually asymmetric and unorganized, with small compressed central cells, inflated ring cells forming numerous incomplete rows, and wing cells of various lengths showing transverse and oblique divisions (Fig. 2I-L). Trichomes in young bracts usually showed living cells with dense protoplast (Fig. 2C, F, H-J). In trichomes of mature bracts, however, cells of the shield, especially those comprising the wing, showed progressive decrease in density, in some cases presenting empty lumens with collapsed walls (Fig. 2D-E, G, K). In *V. fenestralis* and *V.* aff*. bituminosa*, these changes were also accompanied with alterations in the walls and protoplasts of shield and stalk cells, which often accumulated phenolics (Fig. 2 G, K).

The trichomes were always associated with conspicuous exudates, which histochemical tests revealed to comprise of both lipophilic and hydrophilic substances (Fig. 3). In *V.* aff. *bituminosa* and *V. fenestralis*, this exudate was heterogenous, composed of a mucilaginous hydrophilic phase and a lipophilic phase showing terpenoids (essential oils) and lipids (Fig. 3A-J); in *V.* aff. *bituminosa*, alkaloids were also seen in the lipophilic phase (Fig. 3K). The mucilaginous content was often seen inside the protoplast of young, secreting trichomes (Fig. 3B-C, E-F) or as a thin film that cover and spread around the trichomes (Fig. 3A-C, F-G, I). Lipophilic substances were often seen as small to large circular droplets disposed around the trichomes shield (Fig. 3A-F, H-J), or inside the protoplast of young secreting trichomes (Fig. 3H). In *V. platynema*, both mucilage and lipids were observed, but they did not present distinct phases. In fact, exudated material would rarely remain attached to the trichomes shields. In this species, mucilage was seen in the protoplast of very young, unexpanded trichomes (Fig. 3L) or as a thin film (Fig. 3M). Lipids, however, were observed only as small droplets inside the protoplast of somewhat mature trichomes or as small isolated droplets in the released secretion (Fig. 3N). Proteins were detected in the protoplast of young shield cells, but not in the associated secretion, and phenolics were not detected. Results of histochemical tests are summarized in Table 1.

In sections stained with toluidine blue and counterstained with ruthenium red, secretions associated with young trichomes were usually seen as purplish films above the shields (Fig. 2C, F). This secretion accompanied alterations described for cell walls and protoplast, acquiring a greenish tone (Fig. 2K).

3.2 *Trichome ultrastructure*

3.2.1 *Vriesea* aff. *bituminosa*

In *V.* aff. *bituminosa*, TEM analysis revealed that the trichomes of young bracts possess a shield comprising living cells with remarkably dense and organelle-rich cytoplasm (Fig. 4A-D). Shield cells are connected by plasmodesmata (Fig. 4C). Plastids, mitochondria and endoplasmic reticulum were the most abundant organelles, while dictyosomes were rarely seen. All organelles were seen randomly disposed throughout the cytoplasmic matrix, along with numerous polyribosomes (Fig. 4A-F). The plastids showed poorly developed inner membrane system and dense stroma with several inclusions of osmiophilic material, as well as electron-dense and electron-lucent lipophilic droplets (Fig. 4B-C). The endoplasmic reticulum was predominantly smooth and formed a prolific network within the cytoplasmic matrix (Fig. 4D-F). Several vesicles and small vacuoles filled with granular material were seen scattered along the protoplast and close to the plasma membrane, sometimes forming multivesicular structures or paramural bodies (Fig. 4D-G); some of these vesicles were seen to arise from the endoplasmic reticulum (Fig. 4E). Osmiophilic material and lipophilic droplets were seen freely in the cytoplasmic matrix (Fig. 4H-I), accumulating in the periplasmic space (Fig. 4D), on the surface of cell-walls and in the middle lamellae at points of connection between adjacent cells (Fig. 4G, J). Fibrillar material was also seen to accumulate on the surface of cell-walls, sometimes forming large compact bodies (Fig. 4G, K-L).

In mature bracts, the cells of the trichome shields showed empty lumens with few protoplast debris (Fig. 4M). Secretory products released in previous stages of the cell cycle were seen as osmiophilic material and lipophilic droplets accumulated on the surface of cell-walls (Fig. 4M, insert). Fibrillar material were also seen on these cells, usually as compact large bodies (Fig. 4M).

3.2.2 *Vriesea platynema* var. *rosea*

In *V. platynema* var. *rosea*, trichomes of young bracts also showed living shield cells with conspicuous nuclei and cytoplasm rich in organelles, prevailing mitochondria and smooth endoplasmic reticulum (Fig. 5A-C). The endoplasmic reticulum usually formed an extensive network or was seen around large vesicles filled with granular material (Fig. 5B-C). Segments of the rough endoplasmic reticulum were rarely observed (Fig. 5B). Small vesicles were present scattered along the cytoplasmic matrix, often next to the plasma membrane (Fig. 5B-C). Large lipophilic droplets were also observed along this matrix, often associated with osmiophilic material disposed around the droplets or as inclusions (Fig. 5A-C). Large bodies of compact fibrillar material were present on the surface of the cell-walls; distinct degrees of density were seen in these structures, including relatively lose strand-like zones (Fig. 5D). Distinct degrees of vacuolation and cell degradation were seen in trichomes of young bracts, ranging from the presence of peripheral vacuoles filled with cytoplasmic residues to loss of tonoplast and organelle integrity (Fig. 5A, E).

In samples of mature bracts, the cells comprising the trichomes shield were always seen with degenerating protoplasts, in which organelles and the plasma membrane had lost their integrity and were no longer recognizable (Fig. 5F-G). In this stage, large lipophilic droplets were seen to accumulate in the lumen, especially in the cells of the wing (Fig. 5G).

4. DISCUSSION

4. 1 *Structure and secretory activity*

The association between the studied trichomes and complex exudates, along with data from histochemical and microscopic analysis, allow us to conclude that these structures comprise the secretory apparatus responsible for the synthesis and release of exudates that cover the inflorescences of the studied species. In young bracts, the presence of scales with shields exhibiting living cells and dense protoplast is a good indicative of their secretory capacity. Plant tissues involved in secretory processes have been extensively demonstrated to display dense cytoplasm with numerous vesicles, mitochondria and other organelles (Fahn, 1979, 2000; Oliveira et al., 2017, Fernandes et al., 2017; Tresmondi et al., 2017; Teixeira et al., 2021). In these glands, the presence of mitochondria usually reflects high metabolic activity, while the frequency of other subcellular structures varies according to the composition of the secreted substance (Fahn, 2000; Fernandes et al., 2017).

In *V.* aff. *bituminosa,* the presence of well-developed endoplasmic reticulum and numerous plastids is coherent with the secretion of lipophilic substances, as pointed by several authors (Fahn, 1979; Tresmondi et al., 2017; Giuliani et al., 2017; Paiva et al., 2022). The occurrence of plastids in association with the ER, specifically, has been often associated with secretion of terpenes in resin-based plant secretions (Fahn, 1979; Machado et al., 2006, 2015; Giuliani et al., 2017). In fact, the absence of plastids in secretory trichomes of *V. platynema* var. *rosea* seems to be coherent with the lack of terpenoids in the exudate of this species, notably monoterpenes whose synthesis is usually associated with these organelles. In this species, the lipophilic content comprises mostly lipids whose origins are likely associated with the extensive endoplasmic reticulum network observed in the secretory cells.

The presence of lipophilic droplets and osmiophilic material in the protoplast of the glandular cells further corroborates the secretory capacity of trichomes in *V. platynema* var. *rosea* and *V.* aff. *bituminosa*. In the latter, secretory products showing distinct aspects and sources (i.e., osmiophilic material, lipophilic droplets, vesicles and small vacuoles with granular content) reinforces the secretory activity and corroborates the complex nature of the exudate. To our knowledge, there is no other evidence of scales producing lipophilic substances in Bromeliaceae. These findings represent a novelty for the secretion biology in the group and expand the intricate plant-environment interactions mediated by the typical indumentum of bromeliads.

In turn, mucilage secretion by bromeliad scales has been demonstrated twice, in inflorescences of *A. blanchetiana* (Bromelioideae; Ballego-Campos and Paiva, 2018) and *T. cyanea* (Tillandsioideae; Ballego-Campos et al., 2020). In both cases, the trichomes show intense secretory activity at early stages of development, producing mucilage that covers the young portions of the inflorescence. In the present study, although mucilage was clearly observed in histochemical tests, ultrastructural data does not provide good evidence of non-cellulosic polysaccharide secretion. Specifically, the presence of poorly developed Golgi apparatus is noteworthy since dictyosomes seem to be closely associated with mucilage secreting cells (Fahn, 2000; Paiva and Machado, 2006; Ballego-Campos and Paiva, 2018; Ballego-Campos et al., 2020). We suggest that a certain temporal disjunction in the secretion of hydrophilic and lipophilic components might explain this incongruence. Based on this idea, mucilage is secreted first, in very early stages of trichome development that were not apprehended by our sampling. Secretion of lipids and terpenoids is carried out later, after the initial expansion of the trichomes, but still in young portions. This secretory process would also help explain why the secretion appear as a complete heterogeneous substance in *V.* aff. *bituminosa* an *V. fenestralis*, and why lipids were detected only in mature trichomes of *V. platynema* var. *rosea*. Despite the fact that heterogeneous mixtures are expected between hydrophilic and lipophilic substances, mixed secretions are not uncommon in plants (Tresmondi et al., 2015, 2017), and usually the separation of their component phases are not as conspicuous as observed here. The presence of large bodies of fibrillar material accumulated on the walls of secreting cells might constitute another piece of evidence. A similar material was seen to accumulate during the secretory phase in mucilage secreting trichomes of *T. cyanea*, appearing as compacted bodies in trichomes of mature bracts (Ballego-Campos et al., 2020). This material was interpreted to be exuded mucilage that changed its aspect due to dehydration caused by exposition to the environment (Ballego-Campos et al., 2020). In addition, when stained with toluidine blue, the distinct aspect of the secretion seen in young and mature trichomes of *V.* aff. *bituminosa* and *V. fenestralis* (i.e., from mucilage to phenolics) might indicate that distinct components are being added to the secretion as the secretory process continues. Alternatively, lipophilic and hydrophilic components could be secreted at the same time, but secretion of mucilage ceases first, rendering secretory phases in which the subcellular apparatus does not explain the entire chemical complexity of the secretions.

In *V.* aff. *bituminosa*, ultrastructural data suggests that the secretion is released to the periplasmic space both in membrane bound vesicles (granulocrine secretion), or via reverse pinocytosis of lipophilic droplets (see Fahn, 1979). The presence of osmiophilic and lipophilic content accumulated in the periplasmic space, in the middle lamella region and on the surface of cells indicate that the lipophilic content directly transposes the cell wall towards the exterior.

It is important to note that, despite the presence of numerous vesicles, it is unclear whether the lipophilic content in *V. platynema* var. *rosea* reaches the exterior of the cells or remains as an intracellular secretion. We did not observe any evidence of secretion transposition or accumulation outside the cell, although lipidic droplets were seen in samples of secretion treated with Sudan Red. Furthermore, the distinct aspect of this secretion compared with mucilaginous exudates of *A. blanchetiana* (Ballego-Campos and Paiva, 2018), *T. cyanea* (Ballego-Campos et al., 2020) and other *Vriesea* species (Ballego-Campos et al., *unpubl.*) suggest distinct composition compatible with resinous or mixed secretions in plants.

The negative results for terpenoids in *V. platynema* var. *rosea* also raise questions regarding the fragrant properties of the secretion. Fragrances in plants are often associated with volatile terpenes present either in essential oils or resins (Fahn, 1979). In this sense, the characteristic scent observed in the secretion of *V.* aff. *bituminosa* and *V. fenestralis* is probably associated with the terpenoid content in the lipophilic phase of the secretion. In *V. platynema* var. rosea, however, the absence of such compounds might indicate alternative sources for the fragrance. Alternatively, terpenoids might still be present in the secretion, but in quantities or quality that difficult their detection in histochemical tests. Recently, Paiva et al. (2019) demonstrated that NADI reagent was unable to detect terpenoids present in fragrant secretions of *Caryocar brasiliensis* Cambess., although chemical analysis and sensorial tests confirmed their presence. A possible explanation for this would be that low volatile terpenes could diffuse rapidly from the cells, not persisting trough the procedures required for histochemical tests.

In mature bracts, the presence of trichomes with empty or collapsed shield cells indicate that the secretion is ephemeral, culminating with cell death. This feature was observed in secretory trichomes of *A. blanchetiana* and *T. cyanea* (Ballego-Campos and Paiva, 2018; Ballego-Campos et al., 2020). In both species, signs of protoplast disintegration were observed, apparently evidencing processes of programmed cell death. In fact, with the exception of trichomes in some species of *Brocchinia*, shield cells in scales of bromeliad usually lose the protoplast at maturity (Tomlinson, 1969; Benzing, 1976). In *V. platynema* var. *rosea*, signs of cell degradation in samples of young bracts might indicate that the secretory phase is particularly short, starting at very early stages of the inflorescence development and ending before the total expansion of bracts.

4.2 *Functional aspects*

Secretions produced and released on young portions of the plant body are often associated with colleters. These glands produce hydrophilic, lipophilic or a mixture of secretions that are related to the protection against desiccation, herbivores and pathogens (Thomas, 1991; Paiva, 2012). The secretory scales found in inflorescences of *A. blanchetiana* and *T. cyanea* were suggested to act as colleters, protecting the reproductive axis against desiccation by providing a hygroscopic coverage to the young, vulnerable parts (Ballego-Campos and Paiva, 2018; Ballego-Campos et al., 2020). This protective role has been associated with glands of several angiosperms (Thomas, 1991; Paiva, 2009, 2012; Machado et al., 2015; Silva et al., 2017; Tresmondi et al., 2017), including glandular trichomes in many monocots (Mayer et al., 2011; Ferrari and Oriani, 2017; Cassola et al., 2019).

The sticky secretion of *V. bituminosa* was previously demonstrated to protect the inflorescences by trapping potentially damaging insects (Monteiro and Macedo, 2014). This secretion is remarkably distinct in aspect when compared with the exudates of *T. cyanea* and *A. blanchetiana*, which do not seem capable of immobilizing predators as in *V. bituminosa* (Ballego-Campos and Paiva, 2018). Since the secretion in the former species lack lipophilic contents, we suggest that these chemicals are responsible for the adhesive properties seen in *V. bituminosa*. Sticky secretions with potential to trap insects are relatively common in plants (LoPresti et al., 2015, 2018; Paiva et al., 2022), and they are usually associated with complex secretions rich in lipophilic substances (Voigt et al., 2020; Paiva et al., 2022). Adhesive secretion may also play a role in the indirect defense, by providing carrion that attract predators and protect the plants (LoPresti et al., 2018). Moreover, the potential of insect carcasses and debris in the nutritional input of *V. bituminosa* has been previously discussed (Romero et al., 2010; Monteiro and Macedo, 2014). Many bromeliads possess water impounding tanks that, together with foliar absorptive scales, might serve as a nutritional resource in saprophytic, diazotrophic-assisted nitrogen fixation and carnivorous strategies (Benzing, 2000). In *V. bituminosa*, dead termites used to simulate insects trapped in the bromeliad tank contributed to 49.6% of the total nitrogen, and frogs that shelter in the water impounding tank also contribute providing additional nutrients (Romero et al., 2010). In *V. gigantea*, Inselsbacher et al. (2007) showed that microorganisms actively transformed nitrogen compounds into ammonium in the water-impounding tank, thus providing this mineral in a form preferred by the bromeliad. For insects trapped in the inflorescences, at least some nutrients can be washed down to the phytotelma, either by dew or rain (Monteiro and Macedo, 2014).

In addition to the adhesive properties of many lipophilic substances, their presence might also contribute to the protection against abiotic environmental factors, such as dehydration, irradiation and insulation (Dell, 1977; Tresmondi et al., 2017). Similarly, lipophilic secretions are waterproof and may reduce cuticular transpiration and control temperature by increasing radiation reflectance (Dell, 1977; Machado et al., 2017). The shiny coverage seen in the inflorescences of *V.* aff. *bituminosa* and *V. fenestralis* might serve in this manner. In addition, the mucilaginous content in all species studied might also protect against abiotic environmental stresses. Due to its hygroscopic properties, mucilage is thought to improve water retention and reduce excessive water loss by transpiration (Paiva, 2012). Nonetheless, this putative protection against water loss and irradiation needs further verification, since the studied species usually grown in humid habitats in the Atlantic Forest (Moura, 2011; Costa et al., 2022).

The presence of distinct fragrances in the inflorescences might reflect additional functions for the exudate observed in the studied *Vriesea* species. Scents are common in flowers, serving as pollinator attractants; they are often associated with low volatile terpenes and are produced by secretory structures called osmophores (Vogel, 1990; Fahn, 1979). Such volatile compounds may be also deterrent, providing selective defense against antagonists (Farré-Armengol et al., 2013). *Vriesea bituminosa*, *V. fenestralis* and *V. platynema* var. *rosea* all display flowers with nocturnal anthesis and pale to yellow bell-shaped corollas, features shared with most members of section *Xiphion* and typically associated with chiropterophilous syndrome (Moura, 2011; Sazima et al., 1995; Aguilar-Rodríguez et al., 2019). In fact, bat pollination has been demonstrated in *V. bituminosa* and reported to *V. fenestralis* (Aguilar-Rodríguez et al., 2019). There is no data on the pollination biology of *V. platynema* var. *rosea*, although this variety differs from *Vriesea platynema* Gaudich. (bird pollinated) by the nocturnal anthesis and fragrant inflorescence secretion, among other features (Moura, 2011). In addition to acoustic cues, floral scents comprise an important sensory signal for the location of opened flowers by bats (Gonzalez-Terrazas, 2016). At present, reported floral scents associated with chiropterophily in bromeliads are the garlic-like fragrance of opened flowers (Aguilar-Rodríguez et al., 2019). To our knowledge, no connection has been drawn, experimentally or not, regarding bat pollination and the scent emanated by the inflorescence secretions in bromeliads. It is noteworthy, however, that many species with chiropterophilous flowers are also described to bear floral bracts filled with fragrant oily secretions (see Moura, 2011). Further research on the chemical composition of inflorescence secretions in species of *Vriesea* section *Xiphion*, along with detailed accounts on the pollination biology of this species might reveal additional function for the bromeliad indumentum.

5. CONCLUSIONS

The secretion observed in the inflorescences of *V.* aff. *bituminosa*, *V. fenestralis* and *V. platynema* var. *rosea* comprise both mucilage (hydrophilic) and lipophilic substances secreted by trichomes on the adaxial face of bracts. In *V.* aff. *bituminosa* and *V. fenestralis*, this exudate is remarkably heterogeneous, with a lipophilic phase composed of terpenoids and lipids. In *V. bituminosa*, alkaloids were also seen in the secretion. The lipophilic content in *V. platynema* var. *rosea* appear to comprise of lipids only. Our results, especially on the ultrastructure of trichomes, suggest that the secretion initiate in early stages of development, culminating in cell death. The secretory activity might be characterized by a temporal disjunction between secretion of hydrophilic and lipophilic contents. The presence of these exudates is related with protection against herbivores at least in *V. bituminosa*, but their function might extend to protection against pathogens, water loss and damage from irradiation, as well as attraction of pollinators or other visitors. To our knowledge, this is the first report of bromeliad scales involved in the secretion of lipophilic substances. Further efforts on the functional role of the secretion in *Vriesea* section *Xiphion* might shed light into new and exciting strategies mediated by the versatile lepidote indumentum of Bromeliaceae.

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FIGURE CAPTIONS

Figure 1. Inflorescences of *Vriesea* species and their secretions. **A–B**. *Vriesea platynema var. rosea*. Notice the secretion (arrows) on the surface of flower buds (fb) and main axis (ax). In B, the secretion (arrows) is seen to collect inside the bracts (br) and around young flower buds (fb), sometimes forming strands of dried material**. C–D**. *Vriesea aff. bituminosa*. Young inflorescence covered by a shiny film of secretion (arrows). In D, a bract was removed to exposed the axis (ax) and the accumulated secretion beneath (arrows). **E**. *Vriesea fenestralis*. Notice the shiny film covering the entire inflorescence. Two bracts were removed to expose the flower buds (fb) and axis (ax). ax=axis; br=bract; co=corolla; fb=flower bud; se=sepal.

Figure 2. Position and structure of the secretory trichomes in *Vriesea* species. **A–B.** *Vriesea aff bituminos*a. Transversal sections of the bract showing adaxial surface of the epidermis (ep) covered with trichomes (tr). In B, trichomes associated with heterogeneous secretion (arrows). **C–E.** *Vriesea platynema var. rosea*. **C.** Young trichome showing secretion film (arrow), and wing cells (wc) with dense protoplast. **D.**

Mature trichome showing wing cells (wc) with empty lumens and partially collapsed cell walls. **E.** Mature trichome showing general structure of the shield; stained with ruthenium red. **F–H**. *Vriesea fenestralis*. **F**. Young trichomes showing secretion film (arrow), and wing cells (wc) with dense protoplast. **G**. Mature trichome with alterations in wing and stalk cells, staining in green. **H.** Shield of a young trichome, stained in ruthenium red. **I–L.** *Vriesea aff. bituminosa*. **I–J**. Young trichomes with dense protoplasts. Notice large ring cells (rc), and secretion covering the shield in I (arrow). **K.** Mature trichome showing collapsed shield (sh) and accumulated secretion (arrow). Notice the alterations in color observed in the cell-walls and secretion. **L**. Young trichome displaying irregular shield, and associated secretion (arrows). bc=basal cell; cc=central cells; ep=epidermis; rc=ring cells; sh=shield; st=stalk; tr=trichomes; wc=wing cells.

Figure 3. Histochemical characterization of secretory trichomes in *Vriesea species*. **A–D.** *Vriesea fenestralis*. **A-C.** Trichomes (tr) showing associated secretion and protoplasts with positive results for mucilage in pink (ruthenium red). Notice the heterogeneous secretion with lipophilic contents (lp, arrows). **D.** Trichome (tr) showing secretion stained in red for lipids (Sudan Red7B). The insert shows heterogeneous aspect of the secretion. **E–K.** *Vriesea aff. bituminosa*, **E–G.** Positive reaction for mucilage in the protoplast and associated secretory films (mu); polysaccharides stain in pink (ruthenium red) and blue (alcian blue). Notice lipophilic components (lp, arrows) **H-J.** Positive reaction for lipophilic substances (lp, arrows); lipids stain in red (Sudan Red7B) and terpenoids in blue (NADI reagent). **K.** Trichome showing alkaloids in dark-brown Wagner reagent). **L–N.** *Vriesea platynema var. rosea*. **L.** Young trichome showing wing cells with positive reaction for mucilage in pink (ruthenium red). **M.** Detached secretory film showing positive reaction for mucilage in pink (ruthenium red). **N.** Positive reaction for lipids in the wing cells of a mature trichomes (in red). The insert show lipid droplets outside the trichome.

Figure 4. Ultrastructural aspects of secretory trichomes in *Vriesea aff. bituminosa*. A. Shield cell showing dense protoplast with abundant plastids, and endoplasmic reticulum (insert). **B–D.** Protoplast of secretory cells showing organelle-rich cytoplasm with numerous polyribosomes (circles). Notice the plasmodesmata in C (arrows) and osmiophilic material accumulated in the periplasmic space in D**. E–F**. Detail of endoplasmic reticulum network. Notice numerous vesicles, small vacuoles, and

multivesicular structures in **F.** The arrow shows a vesicle arising from the endoplasmic reticulum. **G.** Detail showing paramural bodies, osmiophilic material impregnating the cell-wall (arrowhead) and fibrillar material deposited on the surface (asterisk). **H–J**. Lipophilic substances freely in the cytosol or impregnated in the middle lamellae region (arrowheads). **K–L**. Fibrillar material deposited on the surface of cells walls (asterisks). Notice loose (K) and compact arrangements (L). **M**. Secretory cell of a mature trichome showing empty lumen with cytoplasm residues and cell wall impregnated with osmiophilic material (arrowhead) and covered with compact bodies of fibrillar material (asterisks). cw=cell wall; er=endoplasmic reticulum; ld=lipophilic droplet; mi=mitochondria; ms=multivesicular structures; pb=paramural bodies; pl=plastid; pt=protoplast va=vacuole; ve=vesicle.

Figure 5. Ultrastructural aspects of secretory trichomes in *Vriesea platynema var. rosea*. **A.** Shield cell showing large nucleus, vacuole and numerous lipophilic droplets with osmiophilic material (arrowheads). **B–C.** Details showing extravacuolar cytoplasm rich in mitochondria and endoplasmic reticulum. Notice lipophilic droplets associated with osmiophilic material (arrowhead), and large vesicles surrounded by endoplasmic reticulum in B. **D.** Detail of fibrillar material (asterisk) deposited on the surface of the cell wall. Notice compact and lose arrangements. **E.** Cell of a trichome of young bracts showing signs of protoplast degeneration. **F-G**. Trichomes of mature bracts showing protoplasts degenerated and large lipophilic droplets with osmiophilic inclusions (arrowheads) in the wing cells. cw=cell wall; er=endoplasmic reticulum; ld=lipophilic droplet; mi=mitochondria; nu=nucleus; pl=plastid; pt=protoplast va=vacuole; ve=vesicle.

FIGURES

Figure 1

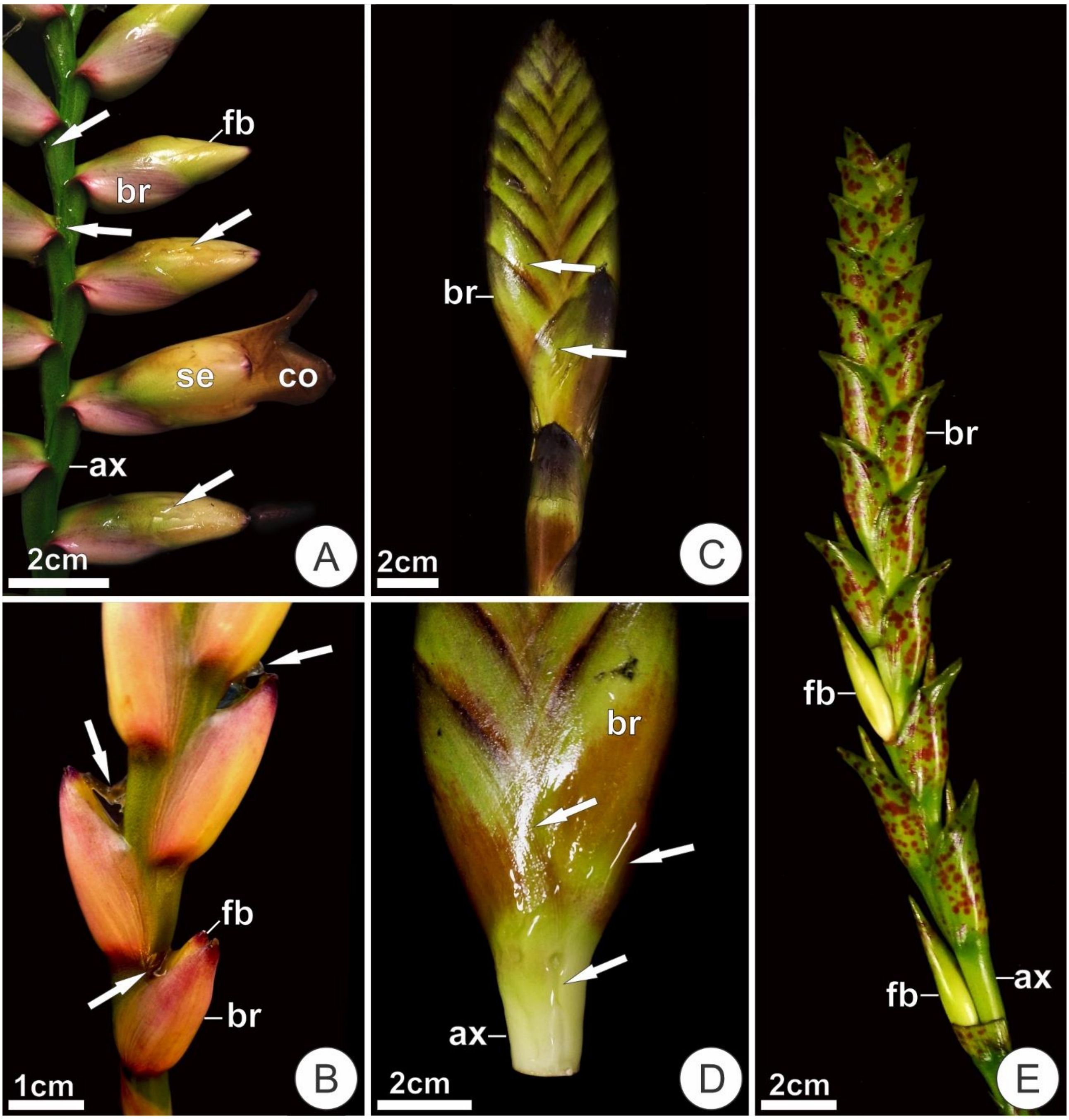

Figure 2

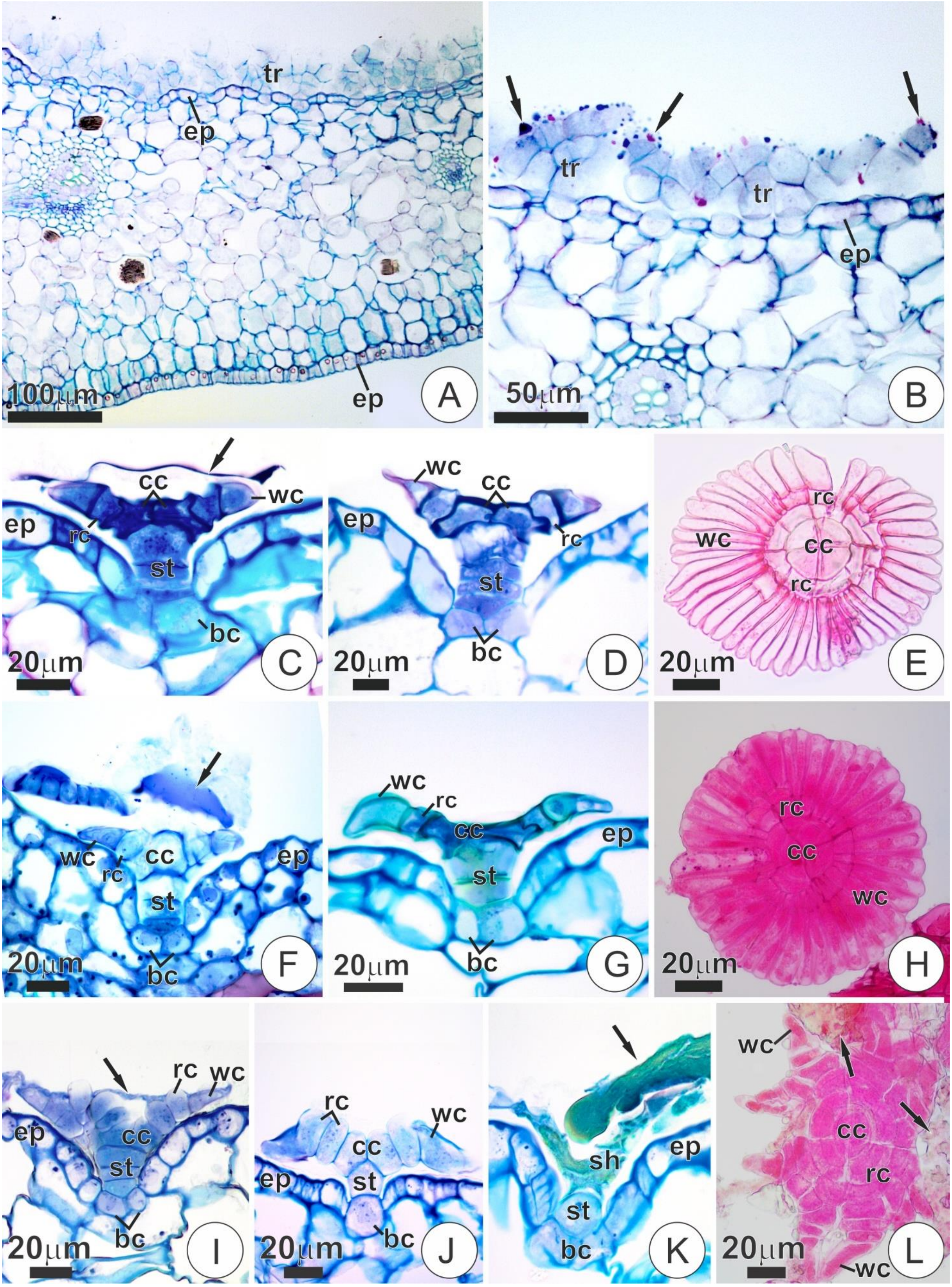
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Figure 3

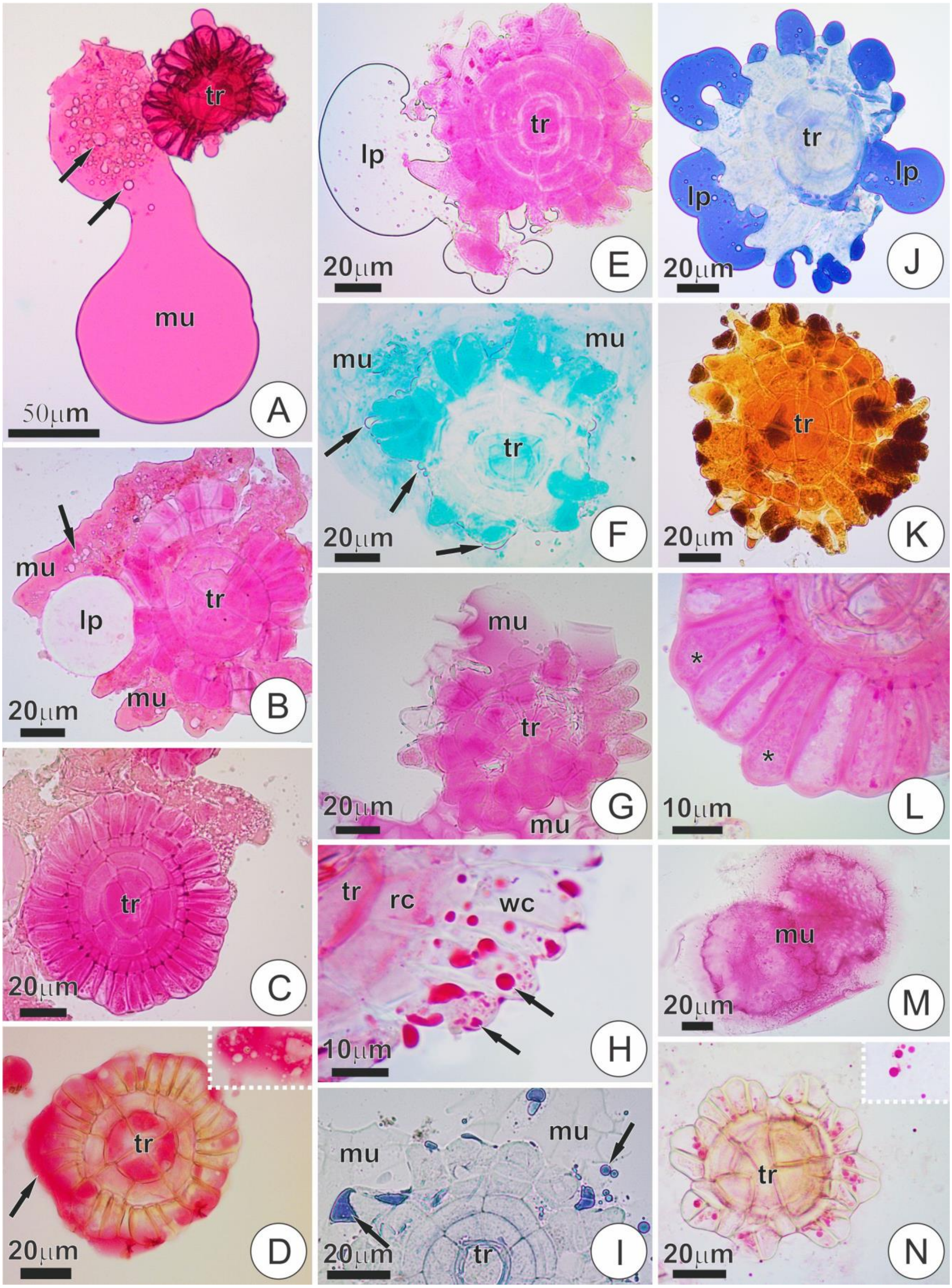
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Figure 4

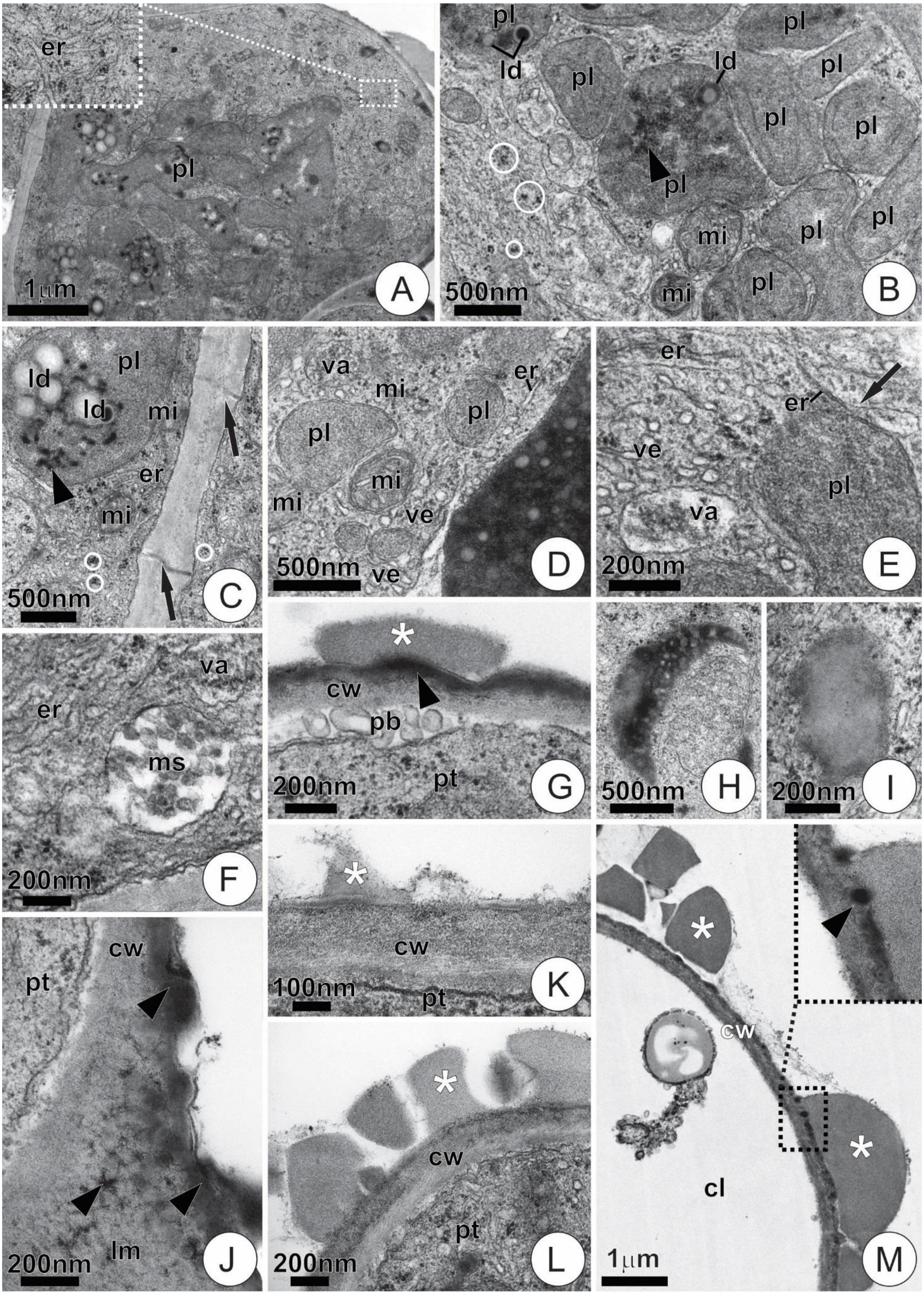
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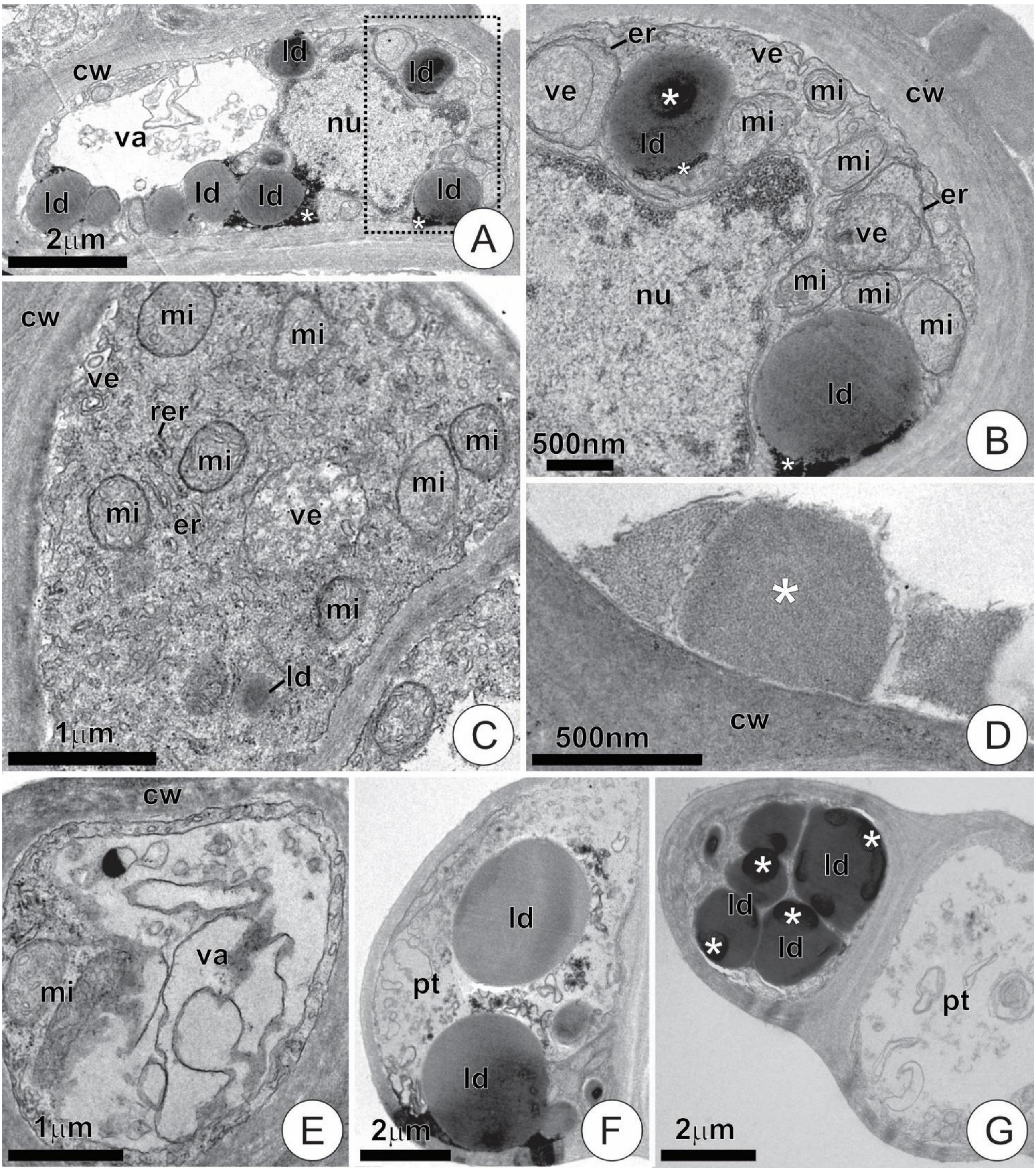
Figure 5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TABLES

Table 1. Histochemical profile of secretory trichomes in *Vriesea* species

Test	**Target substance**	***V.* aff. *bituminosa***		***V. fenestralis***		***V. platynema* var. *rosea***	
		Protoplast	Secretion	Protoplast	Secretion	Protoplast	Secretion
NADI reagent	Terpenoids	+	+	+	+	-	-
Ruthenium Red/ Alcian blue	Mucilage	+	+	+	+	+	+
Sudan Red B	Lipids	+	+	+	+	+	+
Xylidine Ponceau	Proteins	+	-	+	-	+	-
Ferric chloride	Phenolics	-	-	-	-	-	-
Wagner reagent	Alkaloids	+	+	-	-	-	-

An overview of secretion in floral bracts of Tillandsioideae (Bromeliaceae), with emphasis in the secretory scales

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III

Igor Ballego Campos – Rafaela C. Forzza – Élder A. S. Paiva

ABSTRACT

Bromeliaceae present many water-use strategies, from leaf impounding tanks, to CAM photosynthesis and absorbing trichomes. Recent studies show that trichomes in inflorescence of bromeliads can exude viscous secretions, providing protection against various factors, including excessive water loss. In light of this, and considering the knowledge gap regarding inflorescence trichomes, we conducted a prospection in 52 species of Tillandsioideae (Bromeliaceae) and one outgroup species. We used histochemical tests and standard methods of light microscopy to investigate presence, source and chemical nature of inflorescence secretions, and to describe the anatomy of floral bracts focusing on trichome structure and position. Secretion appears in all species of tribe Vrieseeae (except *Vriesea guttata*), in *Guzmania spp.*, *Wallisia cyanea, Tillandsia streptopylla* (Tillandsieae) and *Catopsis morreniana* (Catopsideae). It is absent in *V. guttata* (Vriesseae), *Racinaea crispa* and in various *Tillandsia* species (Tillandsieae). Secretion is produced by adaxial peltate trichomes of young bracts and comprise hydrophilic and lipophilic compounds. Bract anatomy revealed an internal mucilaginous tissue with wide distribution within subtribe Vrieseinae. Our results point to a widespread occurrence of secretion associated with bracteal scales in inflorescences of Tillandsioideae. Ecological significance is discussed based on secretion presence, composition and trichome structure. A few evolutive and taxonomical considerations are presented, as well as directions for future research.

Key-words**:** mucilage, resin, plant-environment interactions, colleters, desiccation, epiphytes.

1. INTRODUCTION

Water is the major limiting resource for growth in land plants (Chaves, 1991; Xiong and Nadal, 2020). It is required in large quantities and is critical for most physiological processes, from transport of nutrients and metabolites to mechanical support in herbaceous plants (Lambers and Oliveira, 2019). Accordingly, strategies to cope with water-related stresses have been discussed profusely in terrestrial plants, both in physiological and structural grounds (Aroca, 2012). In Bromeliaceae, the characteristic presence of leaf-impounding tanks has been traditionally interpreted as a source of water and nutrients for the plant (Benzing, 2000). Moreover, the presence of impounding tanks was recently related to a protective mechanism in the development of inflorescences in *Nidularium innocenttii* Lem., effectively acting as a thermal regulator (Nogueira et al. 2019, 2021). In fact, the biology of adaptations related to water-use strategies in Bromeliaceae is extremely diverse, including not only leaf impounding tanks, but also the mesophyll structure, photosynthetic pathways, and trichomes (Benzing, 2000; Males, 2016). These adaptations, in turn, are related to occupation of diverse niches, including epiphytic and lytophytic life forms, through an extensive process of adaptive radiation (Benzing, 2000; Givnish, 2014; Males, 2016).

Among these adaptative features, the typical peltate trichomes of bromeliads are thought to be a key trait, exhibiting correlated evolution with epyphitism due to its capacity of efficient water absorption (Givnish, 2014). In fact, together with other features, these absorbing scales account from a variable independence from functional roots, leading to extreme atmospheric life forms that have expanded bromeliads adaptive zones even beyond that of other known epiphytes (Givnish, 2014). Not surprisingly, the absorptive capacities of these trichomes have received more attention in the literature, but other functions of the bromeliad indumentum include protection against herbivores/pathogens and reduction of water loss via transpiration and insulation (Benzing, 2000; Pierce et al., 2001) although further investigation is needed (Males, 2016). However, it is noteworthy that most of the knowledge on bromeliad trichomes come from studies with the vegetative axis, especially from leaves of atmospheric *Tillandsia* spp. and water-impounding taxa.

In the reproductive axis, trichomes of certain bromeliads were suggested to produce visual signs for pollinators and dispersers (Benzing, 2000), but beyond these putative functions, very little is known regarding their functional biology in inflorescences of bromeliads. Carvalho et al. (2017), showed extreme polymorphism in floral peltate trichomes of three *Dyckia* species, and found this character valuable for species delimitation. Recent findings in inflorescence trichomes of bromeliads showed that the mucilage found covering the young inflorescences of *A. blanchetiana* and *T. cyanea* is produced and exuded by the peltate trichomes of the reproductive axis, and is possible involved in mechanisms to cope with water-related stresses (Ballego-Campos and Paiva, 2018; Ballego-Campos et al. 2020). In bromeliads, the presence of secretion covering the inflorescence has been known from anecdotal observations, or from species characterization in herbarium material and taxonomic studies (Benzing, 2000; Moura, 2011; Moura and Costa, 2014; Gomes-da-Silva and Souza-Chies, 2018). The presence of an oily secretion is reported for many *Vriesea* spp., and it has been demonstrated that this secretion act as sticky trap for herbivores, effectively reducing damage in *Vriesea bituminosa* (Monteiro and Macedo, 2014). A study with three *Vriesea* species demonstrated that the scales in floral bracts are responsible to produce these secretions, which might play diverse ecological roles, including protection from abiotic and biotic environmental stresses (Ballego-Campos et al. *unpubl.*). These recent findings provide new insight in the functional biology of bromeliad trichomes, and call for a broader investigation of scales in the reproductive axis of Bromeliaceae.

Tillandsioideae is the large of the eight subfamilies currently accepted for Bromeliaceae (Barfuss et al. 2016). It comprises a morphological diverse group of plants, counting more than 1,500 species that range from epiphytes on humid forests to xerophytic lithophytes in rock outcrops (Smith and Downs, 1977; Givnish et al., 2011; Barfuss et al., 2016; Gouda and Butcher, *cont. updated*). The phylogenetic relationships within the subfamily have received great attention in the recent years (Barfuss et al. 2016; Gomes-da-Silva and Souza-Chies, 2018; Machado et al., 2020). Based on molecular and morphological data, species of Tillandsioideae were recovered with good support in four distinctive tribes: Tillandsieae, Vrieseeae, Catopsideae and Glomeropticairnieae (Barfuss, 2016; Gomes-da-Silva and Souza-Chies, 2018; Machado et al., 2020). Among these, Tillandsieae and Vrieseeae comprise the core group, with the vast majority of species. Vrieseeae, in turn, splits into two major lineages generally ascribed to eastern Brazil (subtribe Vrieseinae), and to the Andes, Central America and Caribbean (Cipuropsidineae; Barfuss, et al. 2016; but see Machado et al. 2020). While the phylogenetic relationships in Tillandsioideae have seen great advancement in the recent years (Barfuss et al., 2016; Gomes-da-Silva and Souza-Chies, 2018; Machado et al., 2020), generic and infrageneric systematics are still in need of further resolution, especially regarding species-rich lineages and underrepresented clades, with emphasis in genus *Tillandsia* (Tillandsieae) and the so-called *Cipuropsis-Mezobromelia* Complex (Vrieseeae; Barfuss et al. 2016; Machado et al., 2020).

In light of the lack of knowledge regarding inflorescences trichomes in bromeliad, as well as the recent discoveries regarding secretion of mucilage in inflorescences of Tillandsioideae species (Ballego-Campos et al. 2018, Ballego-Campos et al., *unpubl.*), in this study we investigated the presence of secretion in the floral bracts of 52 species of Tillandsioideae and one outgroup species, *Brocchinia reducta* Baker (Brocchinioideae). Our main goals were to investigate the presence and source of secretion in inflorescences of the studied species, including the overall nature of the exudate, as well as to describe the anatomy of floral bracts in the studied species, focusing on trichome structure and position. We specifically tested the following hypothesis:

a) secretion by inflorescence bracts is widespread within Tillandsioideae, potentially comprising a synapomorphy of the subfamily;

b) Secretion, when present, is produce by peltate trichomes;

c) Secretion is mucilaginous in nature, the presence of lipophilic secretions in *Vriesea* spp. (Ballego-Campos et al., *unpubl.*) being a derived trait of members within *Vriesea*. sect. *Xiphion*.

2. MATERIAL AND METHODS

2.1 *Sampling and plant material*

Plant material was obtained during multiple flowering episodes over the years 2017–2020, from material growing in the living collections of the Jardim Botânico do Rio de Janeiro (RBvb; Rio de Janeiro, Brazil) and Jardim Botânico de Belo Horizonte (CPVJBBH; Minas Gerais, Brazil). Additionally, specimens of *Tillandsia loliaceae* Mart. ex Schult. f. and *T. recurvata* (L.) L. were collected from natural population growing in the campus of the Universidade Federal de Minas Gerais (Belo Horizonte, Brazil). We sampled a total of 52 species of Tillandsioideae (12 genera) and one outgroup species, *Brocchinia reducta* (Brocchinioideae; Table 1). Nomenclature and phylogenetical inferences followed Barfuss et al. (2016) and Machado et al. (2020). The taxonomic revision proposed by Barfuss et al. (2016) has been receiving some criticism (Gomes-da-Silva and Souza-Chies, 2018), but recent advancements have greatly supported this revision (Machado et al., 2020). The adoption of this new approach includes, among other changes, the replacement of *Tillandsia cyanea* Linden ex K. Koch in a new combination within *Wallisia* (Tillandsieae: Tillandsioideae)*,* as *Wallisia cyanea* Barfuss & W. Till (Table 1).

Samples were collected from inflorescences at early stage of flowering and divided in two categories, following Ballego-Campos et al. (2020): (a) young portions (with less than 50% of expansion) and (b) mature portions (with 50% or more of total expansion). Based on the acropetally maturation of the floral parts, samples were often obtained from the same inflorescence, in which case young portions comprised the apical third of the inflorescence and mature portions comprised the middle to basal inflorescence (Ballego-Campos et al., 2020). In ramified inflorescences, this categorization applied to each ramification individually.

2.2 *Fresh-material prospecting*

Inflorescences at early stage of flowering were screened for the presence of secretion both in the field, and in the laboratory with the aid of a stereomicroscope (M205 C, Leica Microsystems Inc.). We searched for the presence of secretion outside the overall inflorescence and in the interior (adaxial face) of floral bracts, recording the aspect (viscosity, color, presence/absence of scent) and overall abundancy (abundant, scarce, absent/indetectable). Additionally, drops of secretion were deposited onto glass slides and left to dry; we observed whether this secretion changed viscosity over time and if there was residual material left on the slide, such as solid films and gums. The dried product was also stained with ruthenium red (0.002%, aqueous solution) and Alcian Blue (Pearse, 1980) to detect the presence of mucilage, and Sudan Red 7B (modified from Brundrett et al., 1991) for lipids.

To further corroborate the secretion and to investigate the role of trichomes in the secretory process, the adaxial surface of young and mature floral bracts were carefully scrapped (Ballego-Campos et al. 2020) and the material obtained (i.e. trichomes and secretion) was placed onto glass slides and tested using Ruthenium red, Alcian blue and Sudan red 7B (see above). Control tests were performed accordingly.

2.3 *Light microscopy*

Fresh samples of young/mature floral bracts (middle third portion) were obtained, fixed under slight vacuum in Karnovsky solution (pH 7.2 in 0.1 M phosphate buffer; modified from Karnovsky, 1965) for 24h, dehydrated in an increasing ethanol series and embedded in synthetic resin (2-hydroxyethyl methacrylate, Leica®, Heidelberg, German). Transversal sections (6-8μm) were obtained using a rotary microtome (Hyrax M40, Carl Zeiss Mikroskopie, Jena, Germany), placed onto glass slides and stained with toluidine blue (pH 4.7 in acetate buffer, modified from O'Brien et al. 1964) and ruthenium red (aqueous solution, 0.002%). Slides were then mounted with synthetic resin (Entellan®, Sigma-Aldrich, St. Louis, USA) and observed under a light microscope (Olympus Scientific Solutions, Waltham, US) equipped with camera and an image capture software (TV0.5XC-3, Olympus Scientific Solutions, Waltham, USA). Additionally, unstained sections were subjected to histochemical tests using ruthenium red alone, and Sudan Red 7B (modified from Brundrett et al., 1991). Control tests were performed accordingly.

3. RESULTS

3.1 *Secretion presence and aspect*

Within tribe Vrieseeae, all studied species displayed a secretion that accumulates on the adaxial surface of floral bracts, with the exception of *V. guttata*, in which no exudate could be observed (Table 1). Secretory abundance and aspect varied between and within genera. In *Alcantarea*, secretion was seen in all studied species (Table 1) as a clear, gelatinous and remarkably abundant exudate, especially in *A. burle-marxii*, *A. extensa, A. farneyi* and *A. pataxoana* (Fig. 1A, B). In the latter, secretion not only accumulated in the bracts, but sometimes dripped off the inflorescence (Fig. 1A). Likewise, in most *Vriesea* spp., secretion was conspicuous and could be seen accumulating in the interior of bracts, but it was usually retained within the spaces of accumulation (Fig. 1C-E), sometimes appearing only after manipulation of the inflorescence (Fig. 1F, G). The secretion in *Vriesea* spp. was usually a clear, gelatinous exudate, but in *V.* aff. *bituminosa, V. botafoguensis, V. fenestralis, V. friburguensis, V. minarum, V. platynema*, *V. procera, V. ruschii* and *V. stricta* the exudate was particularly viscous, oily and with a sticky aspect (Fig. 1H). In *V. aff. bituminosa*, *V. fenestralis, V. ruschii*, and *V. stricta*, specifically, this exudate assumed a yellowish tone. In *Stigmatodon goniorachis*, secretion was abundant, viscous and dense, with an oily aspect in young, unexposed parts, but assuming a more gelatinous appearance in mature, exposed bracts with increased volumes of secretion (Fig. 1I). *Goudea* spp. and *Werauhia viridiflora* also exhibited exudation of a clear, viscous secretion in the adaxial side of the bracts, whereas the two studied species of the *Cipuropsis-Mezobromelia* Complex showed little secretion, with very fluid aspect, only perceptible in young bracts.

In species of tribe Tillandsieae, clear presence of secretion was observed on the adaxial surface of bracts only in *Wa. cyanea* (Fig. 1J) and in all studied *Guzmania* species (Table 1). In the former, the exudate was similar to the secretion of many *Vriesea* spp., while in *Guzmania* spp., secretion was scarce and often difficult to distinguish from accumulated water due to its less viscous aspect. *Racinaea crispa* and most of the *Tillandsia* spp. did not show signs of accumulated secretion on the adaxial surface of floral bracts (Table 1). The same was observed for inflorescences of *B. reducta* and *Catopsis morreniana* (Table 1), in which the floral bracts always appeared dry, with no apparent secretion (Fig. 1K).

3.2 ***Trichome presence and position***

Peltate trichomes were often seen in the bracts of the studied species, with position and structure varying between and within groups (Table 1). In members of tribe Vrieseeae, the trichomes were usually present in the adaxial surface of the floral bracts (Fig. 2A; Table 1). Abaxial trichomes were only seen in some species, usually sparsely distributed. *V. poenulata* and *W. viridiflora*, however, displayed trichomes in both surfaces (Fig. 2B, C; Table 1).

Within species of the tribe Tillandsieae, trichomes were absent in the floral bracts of *T. araujei*, *T. stricta* and *T. tenuifolia* (Fig. 2D; Table 1). *T. loliaceae* and *T. recurvata* showed trichomes exclusively on the abaxial side (Fig. 2E; Table 1) while *T. streptophylla* and *R. crispa* had trichomes in both surfaces of the bracts, but predominantly in the abaxial one (Fig. 2F; Table 1). *Guzmania* spp. and *Wa. cyanea* comprised exceptions, their trichomes appearing predominantly in the adaxial side of the bracts (Fig. 2G, H; Table 1).

The presence and position of trichomes in *C. morreniana* was similar to the majority of Vrieseeae species, with the scales predominantly covering the adaxial surface of floral bracts (Table 1). In *B. reducta*, trichomes were rarely seen either in the adaxial or abaxial surface of bracts (Fig. 2I; Table 1).

3.3 *Trichome structure and secretory activity*

The trichomes in all studied species were generally formed by two basal cells, a short uniseriate stalk (usually 3-4 cells) often exhibiting a larger, dome-shaped uppermost cell (i.e., dome cell), and a distal flattened shield (Fig. 3A-T). The shield comprises a group of few central cells surrounded by one or more rings of concentric cells and a peripheral wing formed by long, radially-arranged cells (Fig. 4). In all studied species, a conspicuous cuticle was seen in the anticlinal walls of the stalk, usually forming projections towards the periclinal plane (Fig. 3G). In *B. reducta*, the trichomes did not present a recognizable flat shield. Instead, the head of the trichomes in this species comprised one or more long, free multicellular appendages (Fig. 3U).

Variation in the structure of the trichomes was observed especially in regards to the shield organization. Within tribe Vrieseeae, most *Vriesea* spp*.,* as well as *S. goniorachis,* showed trichomes with relatively well-organized, symmetrical shields in which wing cells were juxtaposed and roughly the same size, ending in a round tip that rendered a circular outline to the trichomes (Fig. 4A, B). *Vriesea* aff. *bituminosa*, *V.* aff. *rubra* and *V. stricta* comprised exceptional cases, showing remarkably irregular shields formed by many oblique division and numerous incomplete rings and wing (Fig. 4C, D). *Vriesea brusquensis*, *V. erythrodactylon*, *V. flammea*, *V. poenulata* and *V. psittacina* also showed distinct shield configuration, with numerous, narrow wing cells with variable sizes and acute free tips (Fig. 4E-G). In these species, thickening of the outer periclinal wall of central cells was usually observed (Figs. 3E, F, 4E, F). In *Alcantarea* spp., trichomes were numerous, and often seen juxtaposed in groups of two or more (Fig. 3N). The shield of the trichomes were also relatively regular, symmetrical and with wing cells juxtaposed forming a circular outline (Fig. 4H, I). In these species, wing cells were remarkably short (Figs. 3M, N, 4H, I), with exception of *A. farneyi*, in which the often-asymmetrical wing comprised long, numerous cells with variable sizes (Fig. 4J). In the studied species of *Goudea*, *Go. ospinae* showed trichomes with relatively well-organized, symmetrical shields (Fig. 4K), whereas *Go. chrysostachys* displayed shields with irregular outline in which the wing was often incomplete and wing cells were not juxtaposed thoroughly (Fig. 4L).

Scales in *Guzmania* and *Racinaea* were usually asymmetrical with an irregular outline, but strong variation was observed (Fig. 4M-P). Trichomes in the studied *Tillandsia* spp. displayed shields with broad wings comprised of numerous, long and narrow cells with free, acute tips (Figs. 3R, 4Q). In these species, distinctive cell-wall thickening was observed in the central cells, ring and wing portions (Fig. 3R-T). Similar wall features were observed in mature scales of *R. crispa* and *C. morreniana* (Fig. 3P). *Wa. cyaneaWa. cyanea*, in turn, showed shields with a similar configuration from that of most *Vriesea* spp., although a conspicuous thickening was observed in the shield of mature trichomes and the wing was also formed by numerous, narrow wing cells (Fig. 4R).

In species with scales in both surfaces of the bract, we did not detect difference between the adaxial and abaxial indumentum, with exception of *V. poenulata* and *T. streptophylla*. In these species, trichomes of the abaxial surface often showed broader shields with stronger cell wall thickening when compared with trichomes of the adaxial side, often including irregular thickening in cells of the ring (Fig. 3E, F, S, T).

Based on the variable structure of the shield, trichomes within the studied Tillandsioideae species can be roughly divided into three types: type A , with rounded-tip wings cells thoroughly juxtaposed and forming a circular outline (Fig. 5A); type B, with wing-cells not thoroughly juxtaposed, often with incomplete wings, oblique divisions and irregular outline (Fig. 5B); and type C, presenting broad, many celled-wing formed by narrow cells with free acute tips, frequently with characteristic thickening of the cells at maturity, especially those of the central portion (Fig. 5C). Table 2 list the main features of each trichome type.

Trichomes were associated with secretion in several species (Table 1), either as a film around and over the shields or accumulated inside the protoplast in trichomes of young bracts (Fig. 6A-K). In *V.* aff. *bituminosa* and *V. fenestralis*, the secretion appeared as a strongly heterogeneous material (not shown). Secretions tested positive for mucilage in all cases (Fig. 6A-H; Table 1), whereas lipids were observed exclusively in *A. burle-marxii, A. extensa*, *A. pataxoana*, *S. goniorachis, V.* aff. *bituminosa, V. botafogensis, V. fenestralis*, *V. minarum*, *V. platynema* var. *rosea* and *V. stricta* (Fig. 6I-K, Table 1). In *C. morreniana* and *T. streptophylla*, the shield of the adaxial trichomes were often seen in association with a film of mucilaginous secretion (Fig. 6G, H), even though no secretion was observed to accumulate in the surface of the bracts. In the latter, as well as in *V. poenulata*, where trichomes were distinct between abaxial and adaxial side, secretion was only observed in trichomes of the adaxial portion.

Differences in the structure of trichomes from young and mature bracts were evidenced by the presence of living or dead shield cells, cell-wall structure, and phenolic deposition. In scales of young trichomes, cells of the shield showed living protoplasts and thin pecto-cellulosic walls; cells of the wing often showed distinctive pectic walls and dense protoplast with conspicuous nuclei (Fig. 6A-H, see also Fig. 3). In turn, the shield of trichomes in mature bracts usually undergone increased vacuolation and protoplast loss, showing empty lumens (Fig. 6 L-O, see also Fig. 3). In this stage, collapse of shield cells was common. This collapse was either exclusively of the wing portion (Fig. 3B) or of the entire shield (Fig. 3C). Deposition of phenolics was also observed in mature trichomes (Fig. 3K). Trichomes in species of *Tillandsia* and in *V. poenulata* did not show differences between young and mature portion. In these species, trichomes appeared totally differentiated even in the samples of young portions.

3.4 *Bract anatomy*

The floral bracts of the studied species comprised a uniseriate epidermis, a heterogenous mesophyll and vascular system consisting of collateral vascular bundles (Fig. 7A-K). The epidermis had small cells, usually with thick anticlinal and periclinal walls in the abaxial portion forming a sclerotic layer (Fig. 7B). Sclerification of the entire adaxial epidermis was uncommon, occurring only in *C. morreniana*, *Go. chrysostachys, T. streptophylla, V.* aff*. rubra* and *Wa. cyaneaWa. cyanea* (Fig. 7E). Localized sclerification of the adaxial surface occurred around the basal cells of adaxial scales in *Go. ospinae* and *G. sprucei* (Fig. 7F, G). With the exception of *R. crispa*, stomata were presented exclusively in the abaxial surface of the epidermis (Fig. 7C-D). In *R. crispa*, however, stomata were observed in both surfaces of the bract, being predominant in the adaxial side (Fig. 7H).

The mesophyll in all species was heterogeneous and usually comprised a central chlorenchymatous tissue loosely limited by an adaxial and abaxial multilayered hypodermis (Fig. 7A, C, D). The adaxial hypodermis is usually a water-storage parenchyma comprised of large cells with thin walls perforated by numerous primary pit fields (Fig. 7A, J). The thickness of this tissue was usually higher towards the midrib region and varied considered between species, being especially prominent in *A. burle-marxii, A. extensa, A. pataxoana,* and *S. goniorachis* (Fig. 7J, K). This tissue was usually comprised of one to two layers of cells in *T. loliacea, T. mallemontii*, *T. recurvata* and *T. tenuifolia* (e.g., Fig. 2D, I).

The abaxial hypodermal cells were either isodiametric or anticlinally elongated, and their delimitation from the inner tissue was not always clear (Fig. 7B, C). Distinct degrees of sclerification were seen in the abaxial hypodermis, sometimes only with discrete thickening of the outer layers (Fig. 7A, B, D). Alternatively, sclerified tissue was observed in up to four layers subtending the epidermis at the abaxial side (Fig. 7C, I). In *A. compacta, C. morreniana*, *R. crispa, T. streptophylla* and *V. poenulata*, the abaxial hypodermis had large, thin-walled cells and appeared also as a water-storage tissue (Fig. 2C, Fig. 4D, E). The same situation was noted in *B. reducta,* although in this species a water-storage tissue was not present towards the adaxial side, being limited to the thin-walled abaxial hypodermis (Fig 2I). The chlorenchyma was usually restricted to a central lacunose portion, often not well delimited (Fig. 7A-E, H, I). Intercostal air lacunae developed within the chlorenchyma in most species, being absent only in *C. morreniana, B. reducta, R. crispa*, *T. loliacea*, *T. mallemontii* and *T. recurvata (*Fig. 2F, I, 7H). In the remaining species, air channels were always observed, ranging from poorly developed, as in *G. wittimackii, S. goniorachis, T. araujei* and *T. tenuifolia* (Fig. 2D*,* 7K) to rather broad and conspicuous, as in mature bracts of *Goudea* spp., *G. patula*, *G. roezlii*, *G. sprucei, M. pleiosticha, V.* aff. *rubra* and *W. viridiflora* (e. g., Fig. 7I)*.* In *V.* aff. *bituminosa*, the entire chlorenchyma was formed by a lacunose parenchyma in which air-channels proper were not observed (Figs. 7A). An assimilating tissue was clearly distinct only in *A. farneyi*, *C. morreniana*, *M. pleiosticha, T. streptophylla, V.* aff. *rubra, V. flammea, V. lubersii, V. poenulata, V. scalaris* and *W. viridiflora,* in which conspicuous chloroplasts could be seen (e. g. Fig. 7E).

Within Vrieseeae, a distinct tissue characterized by the presence of dense cells with pectic walls, intumescent middle lamella and large intercellular spaces filled with mucilaginous content was present in the midrib region of the bracts (Table 1, Fig. 8A-H). This tissue was usually conspicuous, developing between the adaxial hypodermis and the chlorenchyma (Fig. 8A-H). In some cases, this tissue appeared as well-defined channels (e. g., *M. pleiosticha*, *V. poenulata*; Fig. 8E, F), but in several species there were no clear delimitation of a lumen (Fig 8A-C, G, H). The position of this tissue in relation to the vascular system varied in two ways: appearing either above the vascular bundles (Fig. 8A, B, D), or intercalating with the veins in an intrusive manner (Fig. 8C, F, G). In the latter, the large intercellular spaces of this tissue merges with the air-lacunae, making it difficult to distinct from the subtending chlorenchyma (Fig. 8C, F, G). In fact, in several species, mucilage was observed filling the air-lacunae (Fig. 8C, F, G), sometimes extending over to the substomatal chambers (Fig. 8C). In *S. goniorachis*, this tissue appeared along the vascular bundles, but did not seem to touch the poorly-developed air-lacunar (Fig. 8H).

Moreover, in some section of the studied bracts, this tissue appeared to substitute the adaxial hypodermis completely, directly contacting the epidermis towards the adaxial surface (e. g., *V. erythrodactylon*, *V. ruschii*; Fig. 8D). Conversely, in *M. pleiosticha*, this tissue seemed to substitute the air-lacunae, forming well-defined channels delimited by cells with distinct pectic walls (Fig. 8E).

The collateral vascular bundles were usually disposed in the median portion, with varying calibers, intercalating with the air lacunae. In most species, these bundles were usually surrounded by a sclerified sheath (Fig. 7C-E, I-J).

4. DISCUSSION

4.1 *Secretory activity*

Our results points to a widespread secretory activity associated with floral bracts in the sampled Tillandsioideae species (present in 44 out of 52 species). Particularly, the presence of dense cells and conspicuous nuclei in young trichomes of the adaxial bract surface is an indicative of secretory function (Fahn, 1979, 2000). The observations of associated secretion (either as films or inside the protoplast) and results from histochemical tests for mucilage and lipids, further corroborate the secretory activity of these scales.

The remarkable distinctions observed between trichomes of young and mature bracts (i. e. shield cells with dense protoplast and conspicuous nuclei vs. empty lumens and collapsed cell walls at maturity) indicate that the secretory activity is limited to young trichomes in bracts that did not fully complete their expansion (see Ballego-Campos and Paiva, 2018; Ballego-Campos et al., 2020). Although we did not measure the time for trichome development and bract expansion, asynchronous development and variation in the duration of the secretory phase are likely to occur, as we did occasionally observe young trichomes in mature bracts, and vice-versa. In the studied species of *Tillandsia*, and in *V. poenulata*, the fact that young, developing trichomes were not observed might be explained by a particularly early and rapid development.

The distinct aspect of the wing cells in several of the studied species, namely the relatively denser protoplast and distinctive pectic walls, suggest that this region constitute a separate functional portion of the trichome, which is especially involved in secretion production and release, as suggested previously in *Wa. cyanea* and *Aechmea blanchetiana* (Ballego-Campos and Paiva, 2018; Ballego-Campos et al., 2020). However, secretory activity in the central portion of the trichomes cannot be excluded without an in-dept analysis since protoplast density and cell wall structure might be difficult to distinguish in some cases. For instance, in very young trichomes, both the wing and the central portion might present active protoplasts related to cell expansion and differentiation. Likewise, dense cytoplasm in the axial portion of bromeliad scales might be associated with water-absorption control (Brighigna, 1988; Ballego-Campos et al., 2020). Furthermore, due to the often-irregular development of trichomes in some species, including the presence of incomplete rows of cells and oblique divisions, wing and ring cells cannot be determined accurately in terms of their developmental origin.

4.2 *Trichome structure and position*

Trichomes of type A seem to be characteristic of Vrieseinae species, although occurring also in *Go. ospinae* and *Wa. cyanea* (Table 1). We believe that this might be a synapormorphic trait of subtribe Vrieseinae, arising independently in *Wa. cyanea* and *Go. ospinae* via phenotypic convergence of the reproductive axis. Inflorescences of *Wallisia* species are remarkably similar with the flat, lanceolate and distichous inflorescences of many *Vriesea* spp. Likewise, *Go. ospinae* also show flat, strongly complanate distichous spikes (see Smith and Downs, 1977; Luther, 1983; Barfuss et al. 2016). Furthermore, the presence of thick cell walls in the central portion of *Wa. cyanea*, as well as numerous wing cells, indicate that these scales do not fully correspond structurally with Type A trichomes (i. e. are analogous). *V.* aff. *bituminosa*, *V. fenestralis* and *V. stricta* presented scales more closely related to type B trichomes, possibly representing a transformation in this character (Table 1). It is unclear whether the difference in the trichome structure in these species is related to the distinct lipophilic secretions observed to be produced by them, although secretion of lipophilic substances is not restricted to irregular trichomes (e. g. *Alcantarea* spp., *S. goniorachis*, *V. minarum*; see also Ballego-Campos et al. *unpubl.*). Traditionally, *V.* aff. *bituminosa*, *V. fenestralis* and *V. stricta* were grouped within *Vriesea* section *Xiphion*, but evolutive inferences are difficult since this section is not a natural group, and the monophylly of *V. stricta* and *V. bituminosa* have been recently questioned (Machado et al., 2020).

Type B trichomes seem to be particularly associated with members of tribe Cipuropsidinae (Table 1), but the significance of this feature, either in an evolutive or ecological perspective, needs further investigation. The consideration of type B trichomes as an informative trait is particularly challenging due to the lack of representants of some genera in our analysis (*Jagrantia, Josemania*, *Lutheria*, *Zizkaea*) and the unresolved phylogeny of the *Cipuropsis-Mezobromelia* Complex (Barfuss, 2016, Machado et al. 2020). The presence of a strongly irregular head in the inflorescence trichomes of the outgroup species *B. reducta*, might suggest that an irregular distal portion is plesiomorphic in the family. From an ecological point of view, the significance of irregular shields in Bromeliaceae is largely unexplored.

Type C trichomes are similar with foliar scales of most *Tillandsia* species, particularly those of extreme "atmospheric" lifeforms, which also show broad, many-celled wings and distinct cell wall thickening (Tomlinson, 1969; Benzing, 1976, Table 1). In these trichomes, such features are often interpreted as adaptations that ensure rapid and efficient water absorption via a sophisticated pump-like mechanism (Benzing, 1976). In fact, the scales in floral bracts of the extreme epiphytes *T. loliacea* and *T. recurvata* are very similar with the complex and sophisticated structure of leaf absorbing trichomes in atmospheric tillandsias (Tomlinson, 1969; Benzing, 1976, 2000). Nonetheless, trichomes with type C features also appear in other genera (Table 1), such as *Alcantarea* (*A. farneyi)*, *Catopsis* (*C. morreniana)*, *Racinaea* (*R. crispa)* and *Vriesea* (*V. brusquensis, V. erythrodactylon*, *V. flammea*, *V. poenulata* and *V. psittacina).*

Outside *Tillandsia*, the occurrence of type C features seem to be related with smaller, xeric and tankless lifeforms at least in three cases: in *V. poenulata*, in *A. farneyi* and in *R. crispa*. *Vriesea poenulata* is a small species, whose narrow linear-triangular leaf blades form a utriculiform rosette, rather than an infundibuliform one (Gomes-da-Silva and Costa, 2011). Furthermore, an analysis of morphological and anatomical data (not including inflorescence trichomes) showed that this species shares similarities with *Tillandsia* species, including extreme "atmospheric" taxa (e. g., *T. stricta, T. tenuifolia;* Gomes-da Costa et al., 2012). Similarly, the distinctly broad wings in trichomes of *A. farneyi* might also reflect adaptations to a reduced, tankless lifeform. Unlike all other *Alcantarea* species studied, this species has a graminoid habit, with narrow leaf blades forming a tankless sub-bulbous rosette which stores little or no water (Versieux, 2009). *Racinaea crispa* is also a small epiphyte with narrowly triangular blades (Smith and Downs, 1977), unlikely to rely in stored water in the rosette. Thus, as generally considered for the vegetative shoot, we believe that trichomes in the floral bracts are also specialized in xeric species that cannot rely on roots or a well-developed phytotelmata for water and nutrient supply (see Benzing, 1976, 2000; Givnish, 2014).

It is important to note, however, that these assumptions are based on the premise that inflorescence trichomes, just like foliar scales, are able to absorb water. To our knowledge, there is no empirical data of absorption by inflorescence trichomes of bromeliads, and efforts in this direction are strongly encouraged. Nonetheless, the absorptive capacities of inflorescence trichomes seem to be supported at least by their structure, i. e. the similar arrangement of cells in comparison with foliar scales, the metabolic active cells in the axial portion and, perhaps more importantly, the presence of an identical cuticular pattern which, in turn, is considered fundamental to the one-way valve-like mechanism of the absorptive foliar scales (Tomlinson, 1969; Brighigna, 1988; Benzing, 1976, 2000; Raux et al. 2020).

Regarding the position of the trichomes, our results show that, in general, trichomes at the adaxial surface are strongly related with secretory function, whereas the indumentum in the underside of bracts is either scarce (as in most *Vriesea* and *Alcantarea* spp.) or present type C features (e. g. *T. loliacea, T. streptophylla*, *V. poenulata*). This suggests that distinctive selective pressures have acted on the adaxial and abaxial surface of floral bracts in Tillandsioideae species. At least in tribe Vrieseeae, and in part of the Tillandsieae (i. e. *Guzmania* and *Wallisia*) these pressures seem to have favored a glandular indumentum in the adaxial surface of floral bracts, perhaps associated with a more mesic, tank-forming habit. Whitin the remaining Tillandsieae, our sampling does not allow for any meaningful insight regarding possible trends or patterns. The presence of secretion in *T. streptophylla* and its absence in the other atmospheric tillandsias sampled is unclear. It is possible that the lack of glandular trichomes in the underside of most atmospheric species is related to a reduction in the size of the inflorescence (and of the overall plant body) allied to additional means of water acquisition and conservation (e. g. specialized leaf absorbing trichomes, CAM; see Benzing, 2000; Givnish et al., 2011, 2014). *T. streptophylla* possess considerable larger and branched inflorescences in which the flowering period extends to a much broader spam of time. Nonetheless, future efforts with a broader sampling of *Tillandsia* are needed to further clarify these observations.

The presence of trichomes in both surfaces of *W. viridiflora* is unique in the sense that it is the only species in which a conspicuous indumentum in the underside of the bract was not associated with type C trichome features. As previously stated, within Vrieseeae, abaxial trichomes were conspicuous only in *V. poenulata*, which differs dramatically in habit. It is unclear whether this feature might be informative, either as a diagnostic character or in an evolutive or ecological perspective. Perhaps significantly, we noted that the floral bracts in *W. viridiflora* tend to become paleaceous shortly after their expansion, possible as part of a senescence process.

4.3 *Aspects of the secretions and their ecological function*

Histochemical tests revealed that the exudate produced by floral bracts in the studied species might be exclusively mucilaginous or a mixed secretion comprised of both mucilage and lipids. Mucilaginous and mixed secretions are common products in secretory systems associated with young reproductive and vegetative axis of several plants, and are usually involved in protective roles (Fahn, 1979, Thomas, 1991; Tresmondi et al, 2015). In this sense, the secretory trichomes in the adaxial surface of floral bracts likely act as colleters, producing an exudate that covers the young portions and avoid desiccation, herbivores or pathogens (Thomas, 1991; Mayer et al., 2013; Tresmondi et al., 2015; Paiva et al., 2021).

The highly heterogeneous secretions of *V.* aff. *bituminosa* and *V. fenestralis* differ from the mixed secretions observed in the remaining studied species of Tillandsioideae, and due to the strong presence of terpenes, might be considered true resins (Langenheim, 2003). In *V.* aff. *bituminosa*, the secretion covering the inflorescences were demonstrated to protect the reproductive axis from herbivores, which are entrapped in the sticky exudate (Monteiro and Macedo, 2014). Since many bromeliads seem to obtain nutrients from distinctive forms of nutrition, including carnivory (Romero et al. 2010; Inselsbacher et al., 2007; Givnish et al. 1984; Benzing, 2000), the involvement of secretion in the entrapment of insect carcasses represents a compelling line of investigation concerning the adaptative roles of these secretory systems (see Monteiro and Macedo, 2014).

The presence of lipid secretion in floral bracts of *Alcantarea* spp. and *S. goniorachis* does not support the proposition that lipophilic exudates are exclusive of certain members of *Vriesea* sect. *Xiphion* (Ballego-Campos et al., *unpubl.*). However, the apparent association of lipid secretion with species that often grow in rocky, exposed habitats is remarkable and might suggest a phenotypic convergence related to the heliophytic habit. Lipophilic secretions can protect young and developing organs from irradiance, excessive transpiration and heat by increasing reflectance and the resistance to water loss (Dell, 1977; Machado et al., 2012). In fact, Tresmondi et al. (2015) showed that stipular colleters in Rubiaceae had prevalence of lipids in savanna areas with higher luminosity when compared with colleters in forest areas. Nonetheless, if interpreted as an adaptation to exposed, high-radiance habitats, the lipophilic secretion in *V.* aff. *bituminosa*, *V. fenestralis* and *V. platynema* var. *rosea* must have arisen independently from distinct selective pressures, as these species are often umbrofilous in tropical forests (Moura, 2011). Perhaps, this could also explain the unique resinous secretions observed in *V.* aff. *bituminosa* and *V. fenestralis*. The scented, terpenoid-rich secretions of these species are likely to perform distinct functions yet to be determined (Ballego-Campos et al., *unpubl.*).

The distinct secretory activity observed in *C. morreniana* and *T. streptophylla* (i. e. localized exudation of mucilage, limited to the trichome shield) cannot be interpreted functionally in the same manner as the secretion in the remaining species. An inconspicuous coverage of secretion is likely not effective in the protective roles discussed above. Instead, the mucilage secretion observed in *C. morreniana* and *T. streptophylla* might be exclusively related with the putative absorptive capacity of the trichomes in these species, providing a highly hygroscopic surface associated with the scales.

Regarding the aspect and abundance of the secretion in the studied species, exudates with sticky, viscous and oily aspect are usually correlated with the presence of considerable amounts of lipophilic compounds, such as lipids or even terpenes (e. g. *V. fenestralis, V. stricta*; see also Ballego-Campos et al., *unpubl.*). Such exudates behave more like resins or gums, assuming a solid, vitreous aspect when exposed, whereas polysaccharide-rich secretions appear as gelatinous exudates that decrease dramatically in volume upon exposition, often reducing to thin films of colorless material. While swelling, gelification and hardening are among general characteristics of plant resins and mucilages (Langenheim, 2003; Western, 2012; Ballego-Campos and Paiva, 2018), secretions in inflorescences of the studied species are extremely variable and do not allow for characterizations based on aspect. For instance, despite the presence of lipids in *Alcantarea* species, secretion appear to be mucilaginous in aspect. The wide variety of forms in which the secretion can be perceived might be explained based on two factors: the secretion composition and its exposition to the environmental conditions, such as high/low humidity and precipitation. In the first case, mucilage is aways present as a component of the secretion in species in which secretion was observed, even when a lipophilic content was also present. In some cases, these two distinct classes of components are present in a heterogenous mixture, which might be secreted in distinct moments of the trichome secretory phase (Ballego-Campos et al., *unpubl.*). Thus, the aspect of the secretion might depend on which class of component is present, the ratio in which they are present and, potentially, the stage of development of a given bract/trichome. Likewise, exposition to the environment might alter the aspect of plant secretions (Cunningham et al., 1977; Langenheim, 2003; Paiva, 2009; Ballego-Campos and Paiva, 2018). Changes of this nature might be more dramatic in relation to the mucilaginous content due to its hygroscopic properties. This might explain, for instance, the differences in aspect observed in young and mature portions of *S. goniorachis*. In this species, the secretion is abundant and assumes a more gelatinous aspect in mature portions, precisely when the bracts are more exposed to water. In mature bracts, mucilage could also be potentially washed off, or even be reduced in volume during periods of drought. These observations call for a careful use of secretion as a diagnostic trait. A more accurate description of the secretion, preferably based on some form of chemical scrutiny (e. g. histochemical, solubility tests) should be employed if we are to produce a comprehensive understanding of the biology of secretion in inflorescences of bromeliads.

4.4 *Bract anatomy*

Floral bracts of Tillandsioideae species seem to share several features with the overall structure of bromeliads leaves, mostly notably xeric traits such as sclerotic epidermis and hypodermis, the latter often differentiated in an water-storage tissue (Tomlinson, 1969, Santos-Silva et al., 2013, Faria et al., 2021). In leaves, sclerification of the adaxial epidermal surface is common (Tomlinson, 1969, Santos-Silva et al., 2013, Faria et al., 2021), a feature mostly absent in the studied floral bracts. The presence of this trait in *Go. chrysostachys* comprise another distinction of this species with *Go. ospinae*, which might corroborate the position of *G. chrysostachys* as more closely related to the *Cipuropsis-Mezobromelia* Complex (Machado et al., 2020). In fact, a sclerotic epidermis toward the adaxial surface was also observed in *V.* aff. *rubra*, another member of the *Cipuropsis-Mezobromelia* Complex. Future investigations should verify whether or not species of *Vriesea* (*lato sensu*) included in this complex share the same feature. Nonetheless, the evolution and cladistic significance of this trait needs further scrutiny, since it also appears in non-related species such as *C. morreniana*, *Wa. cyanea* and *T. streptophylla*. Ecological significance of the presence/absence of this trait could be associated with the secretory function of the adaxial indumentum. In this sense, a coverage of secretion would minimize the water loss, effectively substituting a hydrophobic sclerotic layer. However, the presence of sclerification in *Wa. cyanea* and *Go. chrysostachys*, in which secretion covers the adaxial surface of bracts, as well as the absence of secretion and sclerotic epidermis in most *Tillandsia* spp., challenge this view.

In *R. crispa*, the presence of stomata predominantly in the adaxial surface of the bracts is a unique feature. Further investigations of the bract anatomy in species of *Racinaea* might indicate if this is an autapomorphic trait or a shared feature and, therefore, a synapomorphy of the genus. The presence of stomata in the adaxial surface might be an adaptive trait related to reduction of water loss via excessive transpiration. Unlike most of the studied species, *R. crispa* has inflate floral bracts (Smith and Downs, 1977), not adpressed to the developing flower. This arrangement, while allowing for gas exchange, might increase the boundary layer in the adaxial face, thus increasing the resistance to water vapor diffusion and minimizing water loss via excessive transpiration.

Our results suggest that the presence of a sclerotic hypodermis might be more associated with a structural function (mechanical support or protection against herbivores) than a xeric trait (see Santos-Silva et al., 2013; Faria et al., 2021). Many species that inhabit xeric environments or are usually considered highly xeromorphic, did not show a mechanical hypodermis (e. g. *A. compacta*, *A. farneyi*, *T. loliacea*, *T. stricta*). Conversely, mesic species often present such characteristic (*Guzmania* spp., *M. pleiosticha*, *V. warmingii*).

The poor distinction of the clorenchyma in most of the studied species indicates that floral bracts do not contribute significantly to carbon assimilation. In leaves, occurrence of air-lacunae within the chlorenchyma is usually interpreted to comprise an internal aerating system, especially for the submerged tissues in tank-forming species (Tomlinson, 1969). Their presence in bracts challenges this view. Alternatively, air-lacunae might be a plesiomorphic trait of the Poales, in bromeliads possibly related to vapour-phase transport (Males, 2016).

The presence of water-storage tissues is a common feature of bromeliad leaves (Tomlinson, 1969, Santos-Silva et al., 2013, Faria et al., 2021), and has been shown to provide water to the chlorenchyma in conditions of drought (Stiles and Martin, 1996; Nowak and Martin, 1997). Water stored in the bracts, however, might have a distinct fate since the assimilating tissue appears to be inconspicuous in most species.

In the midrib portion of bracts, the presence of a mucilaginous tissue is a unique feature, apparently exclusive to tribe Vrieseeae, although absent in all of the studied species of subtribe Cipuropsidinae but *M. pleiosticha*. Records of this tissue in the literature are virtually absent. Recently, Silva et al. (2020) showed the presence of secretory canals in the peduncle bracts in species of the *V. oligantha* Complex, which correspond to the mucilaginous tissue observed in the present study. Nevertheless, we preferred to use the term “mucilaginous tissue” in describing these structures, since in many of the observed cases, there is no delimitation of a secretory epithelium and, especially, a lumen. We believe these are important features in the delimitation of channels/cavities as traditionally recognized (Fahn, 1979). Well-delimited channels seem to be common in sepals of *Guzmania* species (*pers. observation*, but see also Sajo et al., 2004), and might occur in other species of Tillandsioideae. Further studies on the development, structure and position of internal mucilage-secreting tissues in bromeliad reproductive organs are strongly encouraged, as they could provide insights in the taxonomy, evolution and ecology of the group.

The contents of the mucilaginous tissue do not appear to exude to the outside of the bract, as we did not note any signs of release pathways, except possibly the stomatal pore in *V. erythrodactylon*. Nevertheless, mucilage exudation trough the stomata would not explain any significative contribution of these structures to the secretion observed in the adaxial side. Mucilage retention in the interior of the plant body, either inside the cells or in internal spaces, is not uncommon among plants (Fahn, 1979; Gregory and Bass, 1989). Internal mucilage is usually associated with water storage, protection from high-irradiance, protection against herbivory (upon mechanical damage) and carbohydrate storage (Fahn, 1979; Gregory and Bass, 1989; Costa et al., 2021). Due to the hygroscopic capacities of mucilage and the intumescent lamellae, the mucilaginous tissue could function in a similar way to the water-storage tissue, retaining water and avoiding desiccation.

4.5 *Evolutionary and taxonomic notes*

As previously stated, the presence of secretion in species of all major groups of Tillandsioideae points to a widespread occurrence of secretory scales in inflorescences of this subfamily. We believe that this feature comprises an ancestral trait of tillandsioid bromeliads, which then suffered transformations in *V. guttata*, *R. crispa*, and at least in some *Tillandsia* species (*T. araujei, T. loliacea, T. malemontti, T. recurvata, T. stricta, T. tenuifolia*). Particularly, the presence of non-secretory trichomes in the outgroup species *B. reducta* and the evidence of secretion by bract trichomes in the early-diverging genus *Catopsis*, corroborates this view. Nevertheless, the prospection of secretion in inflorescences of this subfamily is a challenging enterprise and a broader sampling, including additional species of *Brochinnia*, *Racinea* and *Tillandsia*, as well as members of the monotypic tribe *Glomeropitcairnieae*, is necessary to further corroborate this proposition. In *V. guttata*, the absence of secretion suggests an autapomorphic trait, at least within *Vriesea*. In *R. crispa*, besides the possible effects of reduction in size previously discussed, the presence of stomata on the adaxial surface of leaves might indicate that distinctive selective pressures acted on the bracts in this species, perhaps accounting for the lack of secretion.

The secretion observed in inflorescences of *Aechmea blanchetiana* (Ballego-Campos and Paiva, 2018) is interpreted as an independent acquisition via phenotypic convergence. *Aechmea*, as well as several other Bromelioideae taxa, appear to share various convergent traits with members of Tillandsioideae, including the tank-epiphyte habit (Givnish et al., 2014).

Our results might corroborate the finding of Machado et al. (2020) regarding the status of *Goudea*, which was recovered by these authors as a non-natural group, *Go. chrysostachys* being more closely related to the *Cipuropsis-Mezobromelia* Complex than *Go. ospinae*. These two species differ from each other by the configuration of the trichome shield, which present Type A features in *Go. ospinae*, and the sclerification of the adaxial surface of the epidermis (absent in *G.ospinae*). Moreover, *G. chrysostachys* share type B trichomes with *M. pleiosticha* and *V.* aff. *rubra* (*Cipuropsis-Mezobromelia* Complex) and the presence of an adaxial sclerotic layer with *V.* aff *rubra*.

We also found evidence that further corroborates the separation of *Wa. cyanea* from *Tillandsia* as proposed by Barfuss et al. (2016). This species differs from the remaining studied *Tillandsia* species by the presence of abundant secretion in the inflorescences and trichomes with type A features predominantly in the adaxial surface of the bracts.

4. 6 *Directions for future research*

Our results show that while the vegetative axis of bromeliads is relatively well-known, our knowledge on the anatomy, ecophysiology and evolution of the reproductive axis needs further scrutiny. Some points for future research in this area include:

(a) *broader analysis of the secretory capacity of scales in bromeliad inflorescences*. This includes broader sampling within Tillandsioideae, but also in the remaining subfamilies. The presence of secretory scales in *Ae. blanchetiana* (Ballego-Campos and Paiva, 2018) is likely an isolate instance of a much pervasive feature.

(b) *detailed accounts on the anatomy and development of the "mucilaginous tissue".* A better understanding of this unusual secretory tissue is strongly encouraged. Efforts in this direction should focus in both bracts and sepals.

(c) *experimental evidence on the modes of water acquisition by the reproductive axis.* While an extensive amount of research has been done on strategies to obtain and use water by the vegetative shoot of bromeliads, virtually nothing is known about the modes of acquisition of water and nutrients in the reproductive axis of bromeliads that lack functional roots.

(d) relationship between mucilaginous secretions and water balance. Due to the hygroscopic characteristics of mucilage, the secretion produced by the trichomes and the "mucilaginous tissue" could be associated with mechanisms of water acquisition and allocation in the inflorescences of some bromeliads.

(e) *relationships between lipophilic secretions, protection against desiccation, high-radiance, herbivory, and indirect nutrient supply.* The ability of lipophilic secretions to avoid excessive transpiration, provide insulation and protect against herbivores needs further evaluation. Likewise, the involvement of these secretions in an indirectly input of nutrients (via insect carcasses trapped in the secretion; Monteiro and Macedo, 2014; Ballego-Campos and Paiva, *unpu*bl.) is an interesting perspective that should not be neglected.

5. CONCLUSION

Secretion in inflorescences of Tillandsioideae species is a widespread phenomenon. The secretory activity is carried out by peltate trichomes in the adaxial surface of floral bracts during their expansion, and can result in either hydrophilic or mixed (i. e. with both hydrophilic and lipophilic contents) exudates. At least in some members of *Vriesea* sect. *Xiphion* (*V.* aff. *bituminosa* and *V. fenestralis*), the secretion is resinous. These distinct exudates are likely to engage in colleter-like roles, such as protection against desiccation, high-radiation and herbivores. An indirect association with nutrient supply via trapped insect carcasses, as well as with pollination syndrome is also suggested.

Bract anatomy revealed the presence of a distinct “mucilaginous tissue” in the midrib portion. This feature is largely unknown in bromeliads, and we present evidences of a widespread occurrence within subtribe Vrieseinae, possibly comprising a synapomorphy.

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FIGURE CAPTIONS

Figure 1. Secretion in inflorescences of some Tillandsioideae species. **A-B**. *Alcantarea extensa*. **A.** Lateral ramification of an inflorescence with a viscous, clear secretion dripping off the inflorescence (arrows). **B.** Young portion displaying secretion (arrows) associated with floral bracts. **C.** *Vriesea simplex* with secretion accumulated inside the bracts (arrows). **D-E.** *Vriesa pinottii*. **D.** Lateral ramification of an inflorescence in early stage of expansion. **E.** Detail of secretion associated with a floral bract. **F-G.** *Vriesea poenulata*. Young inflorescence with secretion (in G, arrow) appearing after manipulation. **H.** *Vriesa stricta*. Portion of and young inflorescence. Note the strands of sticky secretion (arrows). **I.** *Stigmatodon goniorrachis*. Portion of an inflorescence with secretion accumulated inside the bracts and around an exposed flower bud (fb; associated bract removed). **J.** Inflorescence of *Wallisia cyanea* with secretion appearing after manipulation (arrow). **K.** Young inflorescence of *Catopsis morrenniana* (lateral ramifications removed), in which secretion is not observable. br=flower bract; fb=floral bud.

Figure 2. Trichome position and structural aspects of bracts in cross-section. **A.** Young bract of *Vriesea aff. bituminosa* with trichomes on the adaxial surface of the epidermis. **B-C.** Young bracts of *Werauhia viridiflora* and *Vriesea poenulata*, respectively, with trichome coverage in both sides of the bracts. In C, note the trichomes predominant on the abaxial surface. **D.** Mature bract of *Tillandisa tenuifolia* with glabrous epidermis. **E.** Mature bract of *Tillandsia streptophylla* with scales predominantly in the abaxial surface. **F.** Mature bract of *Tillandsia loliaceae* with trichomes exclusively on the abaxial surface. **G-H**. Mature bracts of *Guzmania sprucei* and *Wallisia cyanea*, respectively, with trichomes predominantly in the adaxial surface. **I**. Mature bract of *Brocchinia reducta*. Notice the absence of trichomes, rarely seen in the species. ah=adaxial hypodermis, ab=abaxial hypodermis, cl=clorenchyma, ep=epidermis, st=stomata, tr=trichome, vb=vascular bundle.

Figure 3. Longitudinal sections of trichome in bracts of the studied species. **A-B.** Trichome of the adaxial surface in a young and mature bract of *Vriesea friburguensis*, respectively. Note wing cells with dense protoplast and pectic cell walls in A (in pink, as evidenced by ruthenium red), and the same cells collapsed and with empty protoplast in B. **C.** *Vriesea erytrodactylon*. Trichome of a mature bract with collapsed shield (sh). **D.** *Vriesea aff. rubra*. Trichome of a young bract displaying cells with dense

protoplast. **E-G**. *Vriesea poenulata*. Trichomes of the abaxial (E) and adaxial (F) surface of a young bract. Notice the cell wall thickenings in the central and wing cells, especially prominent in E. The insert shows a detail of the dotted area, stained with ruthenium red. In G, detail of the stalk, evidencing the cuticle (in red, Sudan Red 7B). Notice the protrusions of cuticle towards the periclinal walls of the stalk. **H.** Mature trichome in *Mezobromelia pleiosticha.* Note empty, collapsed wing cells with pectic walls. **I.** Adaxial trichome in a young bract of *Goudea crysostachys* showing shield cells with dense protoplast with conspicuous nuclei. **J-K**. *Stigmatodon goniorachis*. Trichome in a young and mature bract, respectively. In K, notice the presence of phenolics in the stalk (in green, toluidine blue). **L**. *Werauhia viridiflora*. Young trichome on the adaxial surface with dense wing cells. **M-N.** *Alcantarea extensa*. Trichomes of a young bract exhibiting short wing cells with dense protoplast. In N, trichomes appearing juxtaposed to one another. **O-P.** *Catopsis morreniana*. Trichomes of young and mature bracts, respectively. Note dense shield cells in O, and conspicuous thickening of the cell walls in P. **Q.** Young trichome in *Guzmania roezlii*. **R.** Abaxial trichome in a mature bract of *Tillandsia loliaceae*. Notice the thickening of cell walls in the shield portion. **S-T.** *Tillandsia streptophylla*. Trichomes of the abaxial (S) and adaxial (T) surface of a young bract. Notice the broad wings and the cell wall thickenings in S. **U.** Trichome in the abaxial surface of a mature bract in *Brocchinia reducta*. The head of the trichome (hc) does not differentiate in a distinct flattened shield. bc=basal cells, cc=central cells, dc=dome cell, hc=head cells, rc=ring cell, sh=shield, st=stalk, wc=wing cells.

Figure 4. Structure of the shield in some species of Tillandsioideae. **A-B**. Trichome shield in young bracts of *Vriesea carinata* and *Stigmatodon goniorachis*, respectively. Notice the regular, circular outline of the shield. **C-D.** Trichomes of *Vriesea stricta* and *Vriesea* aff. *rubra*, respectively, with shields of irregular outline, incomplete rings and wing. **E-G.** Trichomes of *Vriesea poenulata*, *Vriesea psitaccina* and *Vriesea erytrodactylon* presenting shield with many long and narrow wing cells with free acute tips. Note the thickening of the walls in the central portion (strongly in pink). **H-I.** Trichomes in *Alcantarea extensa* and *Alcantarea pataxoana*, respectively, with short wings of regular, circular outline. **J.** Trichome shield in *Alcantarea farneyi* with distinct shield comprising long, narrow wing cells. **K-L.** Trichome shields in *Goudea ospinae* and *Goudea crysostachys*, respectively. **M-N.** Intraspecific variation in the shield

structure of trichomes in *Racinaea crispa* . Note the central portion with thick cell walls (strongly in pink). **O-P.** Trichomes in *Guzmania pathula*, also with intraspecific variation in shield structure. **Q.** Adaxial trichome of *Tillandsia streptophylla*. Note the shield with thickening of central cells walls and wing comprising narrow cells with free acute tips. **R.** Shield of trichome in W*allisia cyanea* with regular, circular outline. The insert shows central cells with thick outer cell-walls. cc=central cells, rc=ring cells, wc=wing cells.

Figure 5. Secretions associated with trichomes in some Tillandsioideae species. **A.** *Vriesea carinata*. Film of secretion stained positive for mucilage (in pink); ruthenium red. **B.** *Vriesea friburguensis*. Portion of a trichome shield showing wing cells with dense protoplast filled whit mucilage (arrows); ruthenium red. **C.** Trichome shield of *Vriesea paraibica* associated with mucilage (arrows); alcian blue. **D-E.** Portions of the shield in *Goudea chrysostachys* and *Guzmania ronhofiana*, respectively. Note wing cells with dense protoplast filled with mucilage (in pink); ruthenium red. **F.** *Werauhia viridiflora*. The protoplast of wing cells is filled with mucilaginous content (in blue); alcian blue. **G-H**. Shield of trichomes in *Catopsis morrenniana* and *Tillandsia streptophylla*, respectively, associated with a film of mucilage (arrows); ruthenium red. **I-K**. Trichomes in *Alcantarea pataxoana*, *Vriesea stricta* and *Stigmatodon goniorachis*, respectively, associated with lipid secretion (in red). Secretion is seen as lipid droplets inside the shield cells (I, J) and as a film of secretion around the shield (I, K); Sudan Red 7B. **L-O**. Trichome shield from mature bracts of *Racinaea crispa*, *Vriesea carinata*, *Werauhia viridiflora* and *Guzmania pathula*, respectively. Notice the empty cell lumens in the shield, especially in the wing cells; ruthenium red. se=secretion, tr=trichome, wc=wing cells.

Figure 6. Transversal sections showing bract anatomy in the studied species. **A-B.** *Vriesea carinata*. Overall structure of mature bracts. In B, detail of the abaxial portion. Note the sclerified epidermis and abaxial hypodermis with isodiametric cells and little distinction from the adjacent chlorenchyma. **C -D**. Mature bracts of *Vriesea ruschii* and *Alcantarea compacta*. Notice stomata (st) in the abaxial surface with substomatal chamber contacting the air-lacunae (asterisks). **E.** Mature bract of *Catopsis morrenniana* with sclerification in the adaxial surface of the epidermis (arrows), and abaxial hypodermis with large cells forming a water-storage tissue. **F-G**. Mature trichomes of *Goudea ospinae* and *Guzmania sprucei* with localized sclerification of the adaxial epidermis around the trichome stalk (arrows). **H.** Mature bract of *Racineae*

crispa. Note abaxial trichomes, and stomata in the adaxial surface. I. Mature bract of *Goudea crysostachys.* Note the abaxial surface with heavily sclerified epidermis and hypodermis, as well as pronounced air-lacunae (asterisks). **J-K**. Bracts of *Alcantarea extensa* and *Stigmatodon goniorachis* with conspicuous water-storage hypodermis towards the adaxial side (ah). The insert in J shows detail of cells of the water-storage parenchyma with thin walls with numerous primary pit fields (arrow). ah=adaxial hypodermis, bh=abaxial hypodermis, cl=clorenchyma, ep=epidermis, tr=trichome, vb=vascular bundle.

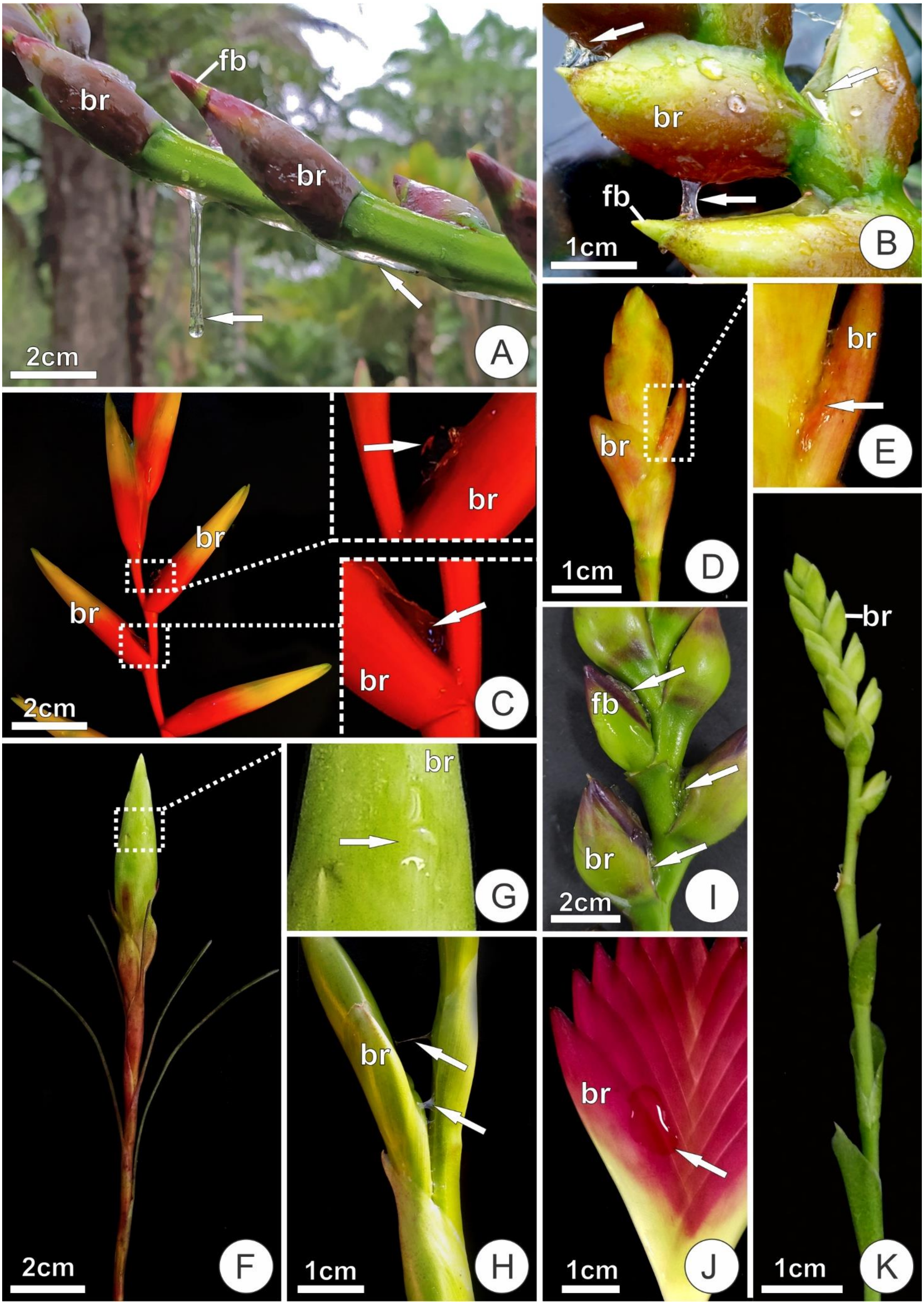
Figure 7. Cross-sections of the midrib portion in bracts of some Tillandsioideae species. **A.** *Vriesea aff bituminosa*. Notice the mucilaginous tissue (mt) between the adaxial hypodermis (ah) and the inner portion of the clorenchyma (cl). The insert shows a detail of the mucilaginous tissue evidencing cells with dense protoplast, large intercellular spaces and intumescent middle lamella (arrow). **B.** *Vriesea botafoguensis*. **C.** *Vriesea brusquensis*. Notice the air-lacunae filled with mucilage (arrows) extending over to and substomatal chamber (arrowhead). **D.** *Vriesea erytrodactylon*. The mucilaginous tissue takes over the entire water parenchyma, making direct contact with the adaxial surface of the epidermis. Notice the large intercellular spaces filled with mucilage. **E.** *Mezombromelia pleiosticha*. The mucilaginous tissue exhibits well defined lumens in between the vascular bundles. **F.** *Vriesea poenulata*. Notice the poorly developed mucilaginous tissue (mt) appearing as two small circular lumens in the central portion. **G.** *Alcantarea burle-marxii*. Region of transition between the margin (left) and midrib portion (right) of a mature bract. Notice the mucilaginous tissue differentiated within the adaxial hypodermis and the subtending air channel (asterisks) filled with mucilage. **H**. *Stigmatodon goniorachis.* Notice the mucilaginous tissue (mt) along the vascular bundles (vb), but not touching the small air-lacunae below (asterisks). ah=adaxial hypodemris, ab=abaxial hypodermis, cl=clorenchyma, ep=epidermis, mt=mucilaginous tissue, vb=vascular bundle, , asterisks=air-lacunae.

Figure 8. Types of trichomes in the studied species based on shield morphology. **A.** Type A, rounded-tip wings cells thoroughly juxtaposed and forming a circular outline. **B.** Type B, wing-cells not thoroughly juxtaposed, often with incomplete wings, oblique divisions and strongly irregular outlines. **C**. Type C, many-celled wing formed by narrow cells with free acute tips, often with thickening of the walls, especially those of the central portion. Black area=wing, White area=central portion, the dotted line indicates

the central cells position. The grey area represents thickening of the outer periclinal walls.

FIGURES

Figure 1



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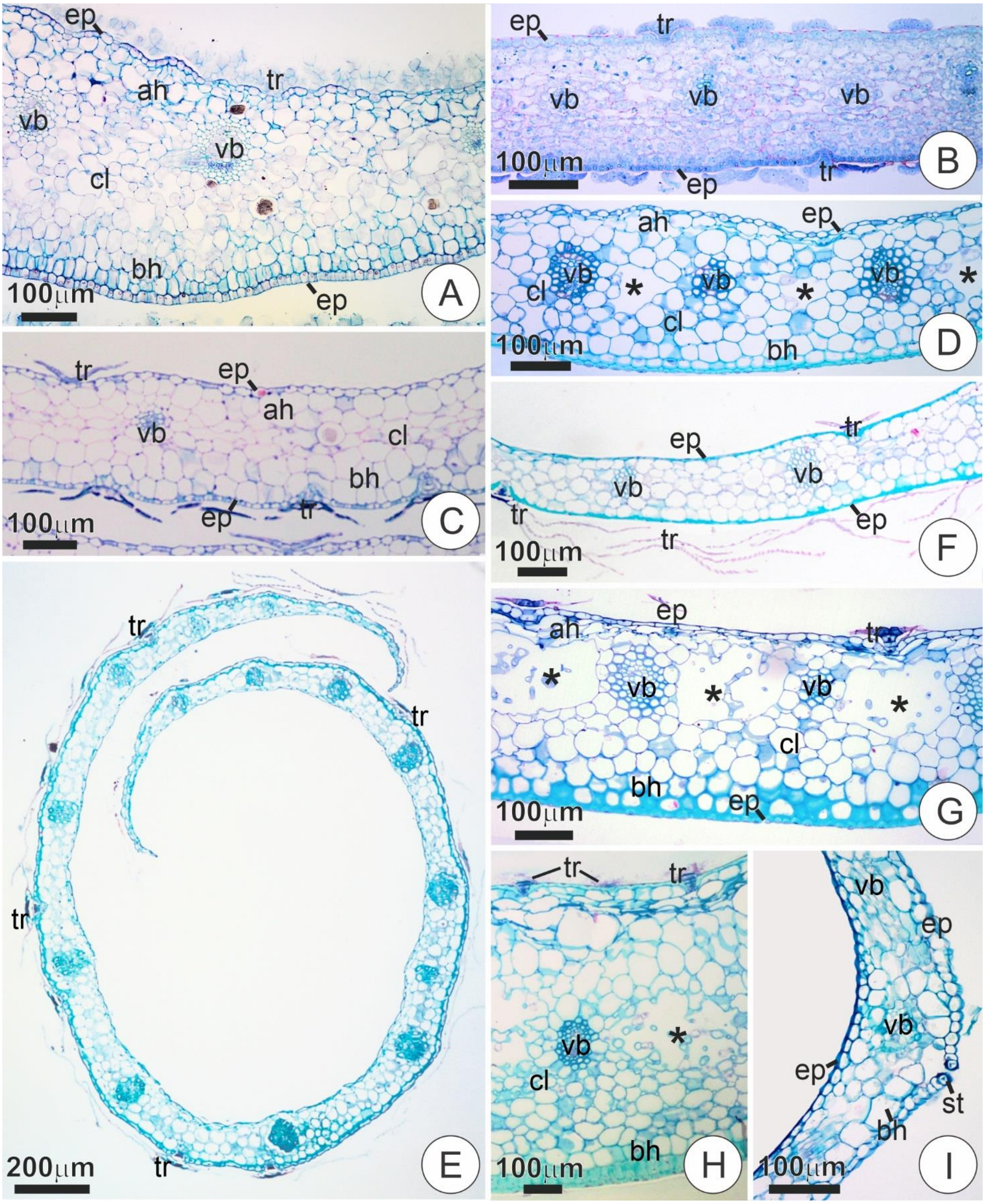
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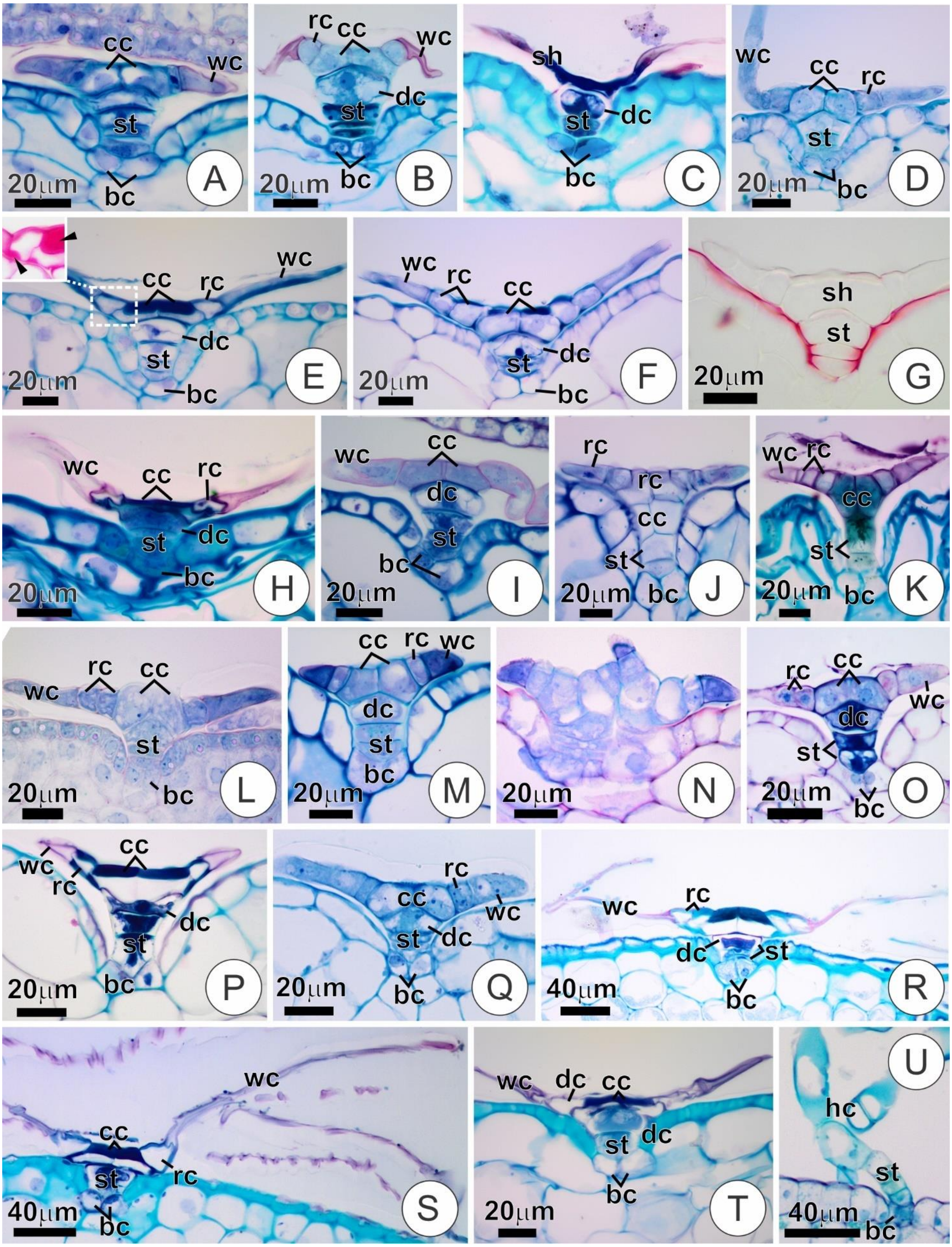
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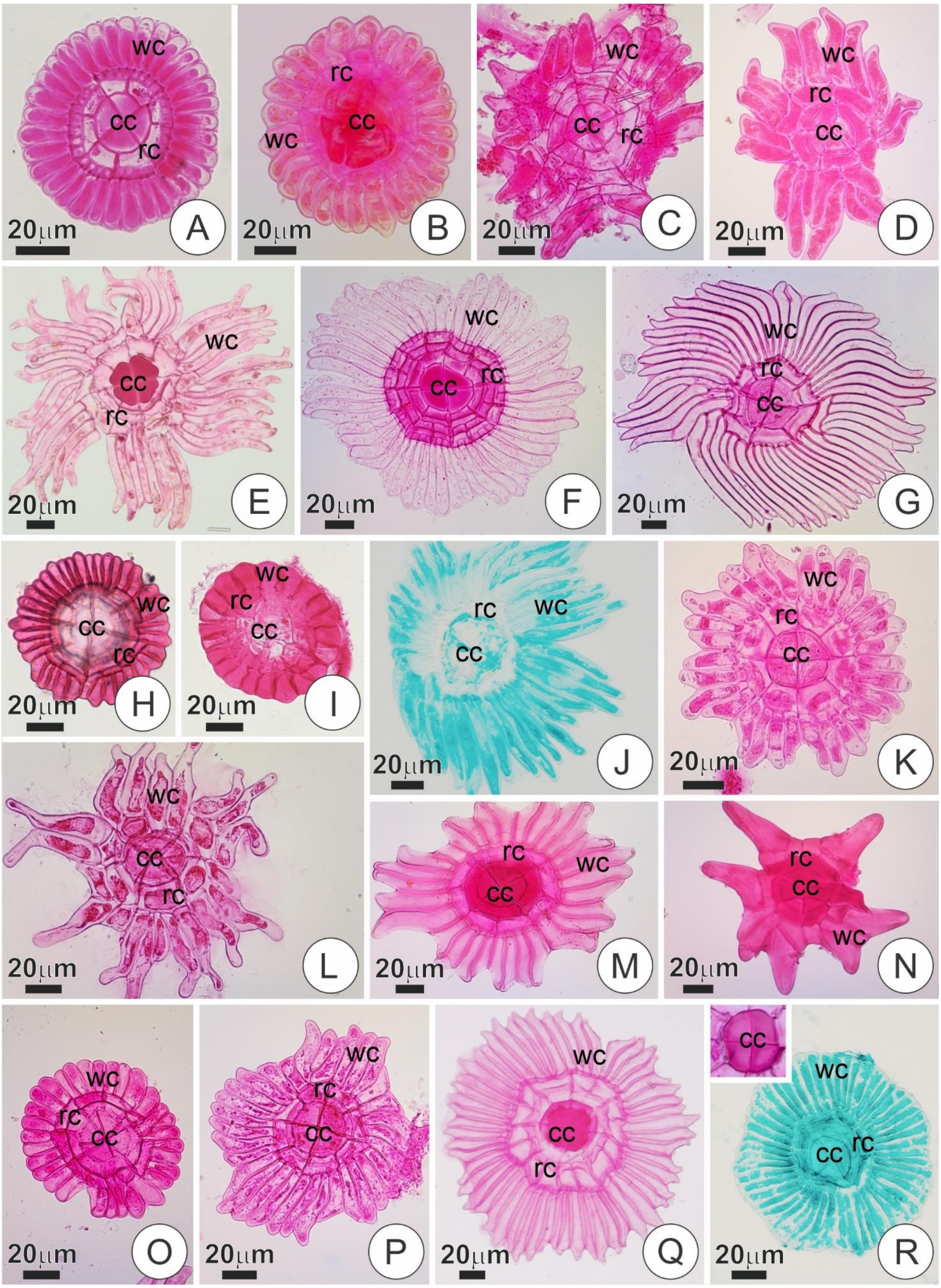
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Figure 5

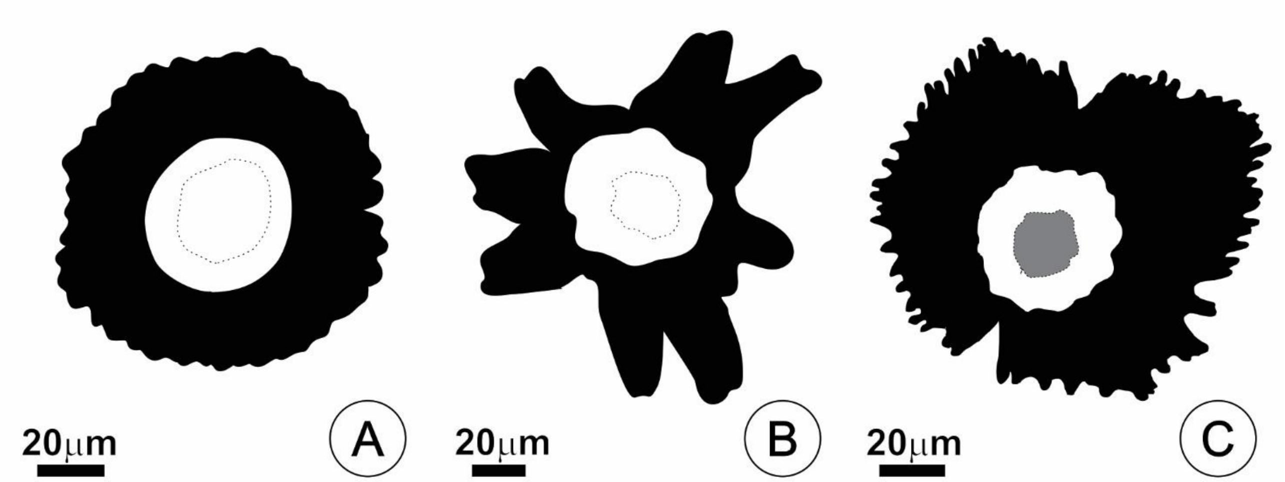

Figure 6


tr
se
tr
50µm
A
tr
wc
20µm
B
20µm
C
wc
20µm
D
tr
wc
20µm
E
wc
20µm
F
wc
tr
20µm
G
tr
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20µm
H
tr
tr
se
tr
tr
20µm
I
tr
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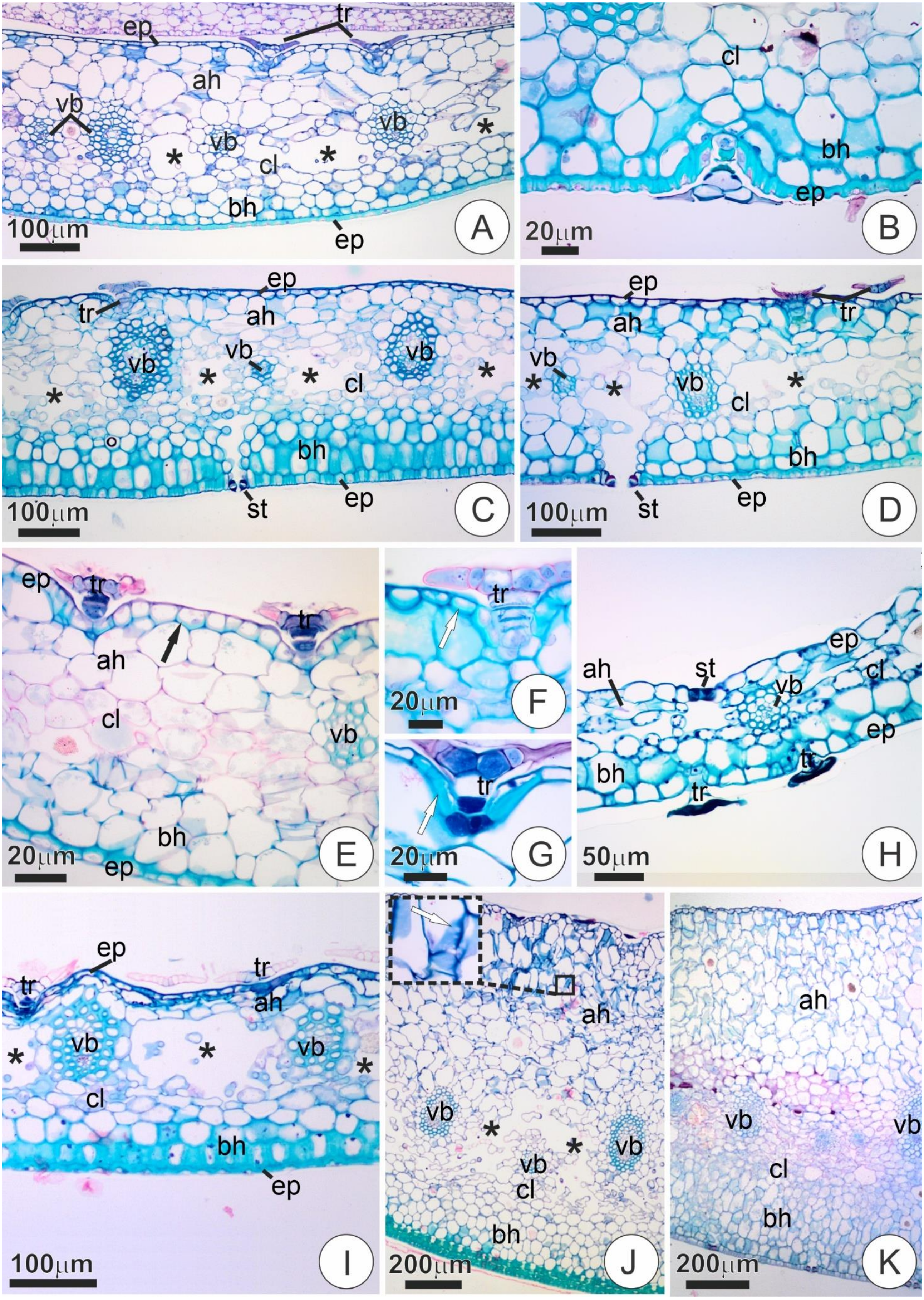
J
tr
20µm
K
wc
tr
20µm
L
wc
tr
20µm
M
wc
tr
20µm
N
wc
tr
20µm
O

Figure 7


ep
tr
ah
vb
vb
vb
*
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cl
bh
ep
100µm
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20µm
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100µm
I
ah
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*
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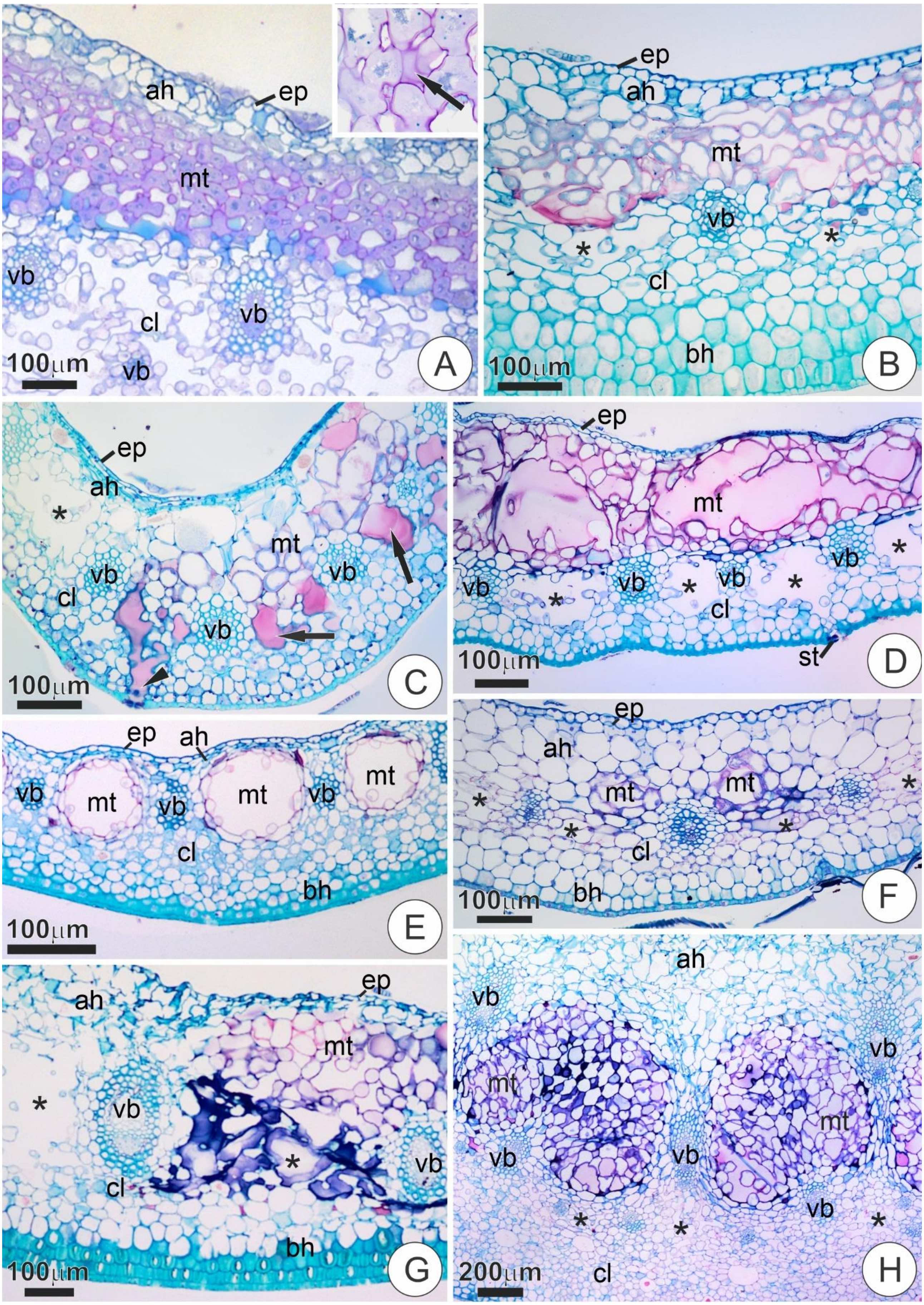
vb
vb
cl
bh
200µm
J
ah
vb
vb
cl
bh
200µm
K

Figure 8


ah
ep
mt
vb
cl
vb
vb
100µm
A
ep
ah
mt
vb
*
*
cl
bh
100µm
B
ep
ah
*
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vb
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100µm
C
ep
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100µm
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vb
cl
bh
100µm
G
ah
vb
vb
mt
mt
vb
vb
vb
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200µm
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H

TABLES

Table 1. Presence of trichomes, secretion and mucilaginous tissue in the floral bracts of the studied species

Taxa	Trichomes		Secretion		Mucilaginous tissue	Voucher specimens
	Presence	Type	Mucilage	Lipids		
Tillandsioideae subfamily						
Catopsideae tribe						
Catopsis						
C. morreniana Mez	**AD**/AB	B*	+	–	–	CVJBFZB 387; BHZB**
Tillandsieae tribe						
Guzmania						
G. aff*. strobilifera* Mez & Wercklé	**AD**/AB	various	+	–	–	CVJBFZB 283; BHZB**
G. patula Mez & Wercklé	**AD**/AB	various	+	–	–	CVJBFZB 3470; BHZB**
G. rhonhofiana Harms	**AD**/AB	various	+	–	–	CVJBFZB 2857; BHZB**
G. roezlii (E. Morren) Mez	**AD**/AB	various	+	–	–	CVJBFZB 589; BHZB 1147
G. sprucei (André) L.B. Sm.	**AD**/AB	various	+	–	–	CVJBFZB 388; BHZB11081
G. wittmackii (André) André ex Mez	**AD**/AB	various	+	–	–	CVJBFZB 237, 324, 2880; BHZB 11169
Racinaea						
R. crispa (Baker) M.A. Spencer & L.B. Sm.	AD/**AB**	various *	–	–	–	BHZB**
Tillandsia						
T. araujei Mez	–	C	–	–	–	RBvb2310
T. loliacea Mart. ex Schult. f.	AB	C	–	–	–	BHCB**
T. mallemontii Glaz. ex Mez	AB	C	–	–	–	CVJBFZB 3370

Table 1. *continued*

Taxa	Trichomes		Secretion		Mucilaginous tissue	Voucher specimens
	Presence	Type	Mucilage	Lipids		
T. recurvata (L.) L.	AB	C	–	–	–	BHCB**
T. streptophylla Scheidw. ex C. Morren	AD/**AB**	C	+	–	–	RB 802224
T. stricta Sol.	–	C	–	–	–	RB 802223
T. tenuifolia L.	–	C	–	–	–	CVJBFZB1194, CVJBFZB1249, BHZB**
Wallisia						
Wa. cyanea Barfuss & W. Till	**AD**/AB	A*	+	–	–	BHZB 4713; BHZB 13215; BHZB 13216
Vrieseeae tribe						
Cipuropsidinae subtribe						
Cipuropsis – Mezobromelia complex						
M. pleiosticha (Griseb.) Utley & H. Luther	**AD**/AB	B	+	**–**	+	CVJBFZB 435, BHZB**
V. aff*. rubra* (Ruiz & Pav.) Beer	**AD**/AB	B	+	**–**	–	CVJBFZB 557, BHZB**
Goudea						
Go. chrysostachys (E. Morren) W. Till & Barfuss	AD	B	+	–	–	CVJBFZB 262, 542; BHZB**
Go. ospinae (H. Luther) W. Till & Barfuss	**AD**/AB	A	+	–	–	CVJBFZB 2858; BHZB**
Werauhia						
W. viridiflora E. Morren	AD/**AB**	B	+	–	–	CVJBFZB 130; BHZB**
Vrieseinae subtribe						
Alcantarea						
A. burle-marxii (Leme) J.R. Grant	AD	A	+	+	+	RB596781, RBvb 2

Table 1. *continued*

Taxa	Trichomes		Secretion		Mucilaginous tissue	Voucher specimens
	Presence	Type	Mucilage	Lipids		
A. compacta Leme & O.B.C. Ribeiro	AD	A	+	–	–	CVJBFZB 3085, BHZB**
A. extensa (L.B. Sm.) J.R. Grant	AD	A	+	+	+	CVJBFZB1160, BHZB**
A. farneyi (Martinelli & A.F. Costa) J.R. Grant	AD	A*	+	–	–	CVJBFZB710, BHZB**
A. pataxoana Versieux	AD	A	+	+	+	RB342724, 596680; RBvb5
Stigmatodon						
S. goniorachis (Baker) Mez	AD	A	+	+	+	RBvb**, RB**
Vriesea						
V. aff. *bituminosa* Wawra	AD	B	+	+	+	CVJBFZB1111, 1151, 1452; BHZB*
V. botafogensis Mez	AD	A	+	+	+	RBvb2168
V. brusquensis Reitz	**AD**/AB	A*	+	–	+	CVJBFZB 180
V. carinata Wawra	**AD**/AB	A	+	–	+	RBvb266, 257, 2450; RB342436, 790354, 329084
V. erythrodactylon (E. Morren) E. Morren ex Mez	**AD**/AB	A*	+	–	+	CVJBFZB220, 3457, BHZB**
V. fenestralis Linden & André	AD	A	+	+	+	CVJBFZB713, BHZB**
V. flammea L.B. Sm.	**AD**/AB	A*	+	–	+	CVJBFZB 3, BHZB**
V. flava A.F. Costa, H. Luther & Wand.	AD	A	+	–	+	CVJBFZB 496, BHZB**
V. friburgensis Mez	AD	A	+	–	+	CVJBFZB 496, BHZB**
V. guttata Linden & André	AD	A	–	–	+	CVJBFZB 536

Table 1. *continued*

Taxa	Trichomes		Secretion		Mucilaginous tissue	Voucher specimens
	Presence	Type	Mucilage	Lipids		
V. incurvata Gaudich.	**AD**/AB	A	+	–	+	CVJBFZB197, 2910; RBvb 560; BHZB**, RB342547
V. lubbersii (Baker) E. Morren ex Mez	AD	A	+	–	+	CVJBFZB 2858; BHZB 7891
V. minarum L.B. Sm.	AD	A	+	+	+	CVJBFZB 1162, 1215; BHZB6487
V. paraibica Wawra	AD	A	+	–	+	RBvb 68; RB531299
V. pinottii Reitz	**AD**/AB	A	+	–	+	CVJBFZB11; BHZB**
V. platynema Gaudich.	AD	A	+	–	+	CVJBFZB 148, 535; BHZB**
V. platynema var*. rosea* (hort. ex Antoine) Mez	AD	A	+	+	+	CVJBFZB 593, BHZB**
V. poenulata (Baker) E. Morren ex Mez	AD/**AB**	C	+	–	+	RBvb2473
V. procera (Mart. ex Schult. f.) Wittm.	AD	A	+	–	+	RBvb 1036, RB 462661
V. psittacina (Hook.) Lindl.	**AD**/AB	A*	+	–	+	CVJBFZB252; BHZB**
V. ruschii L.B. Sm.	AD	A	+	–	+	CVJBFZB321; BHZB**
V. scalaris E. Morren	**AD**/AB	A	+	–	+	CVJBFZB6357; RBvb 637, 395, 396; RB 531298
V. simplex (Vell.) Beer	AD	A	+	–	+	RBvb 72, RB 439163
V. stricta L.B. Sm.	AD	B	+	+	+	CVJBFZB3085; BHZB**
V. warmingii E. Morren	AD	A	+	–	+	CVJBFZB1097, BHZB**

Table 1. *continued*

Taxa	Trichomes		Secretion		Mucilaginous tissue	Voucher specimens
	Presence	Type	Mucilage	Lipids		
Brocchinioideae subfamily(outgroup)						
Brocchinia						
B. reducta Baker	rarely seen	N/A	–	–	–	RBvb 899, RB 568168

AB, abaxial; AD, adaxial surface; bold indicate predominant presence; +, present: –, absent; * distinct with one or more type C features, ** in preparation.

Table 2. Characterization of trichome types based on shield arrangement

Trichome type	Features
A	• Shields with circular outline • Wing-cells with rounded-tips • Wing-cells thoroughly juxtaposed
B	• Shields with irregular outline • Rings and wings often incomplete • Oblique cellular divisions • Wing cells not thoroughly juxtaposed
C	• Wing relatively broad, many-celled • Wing cells narrow, with free acute tips • Cell walls of shield irregularly thickened

Extranuptial nectaries in bromeliads: a new record for *Pitcairnia burchellii* and perspectives for Bromeliaceae

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IV

Igor Ballego Campos – Rafaela C. Forzza – Élder A. S. Paiva

Ballego-Campos I, Forzza RC, Paiva EAS. 2022. Extranuptial nectaries in bromeliads: a new record for *Pitcairnia burchellii* and perspectives for Bromeliaceae. *The Science of Nature* 109: 1-15. https://doi.org/10.1007/s00114-022-01799-5.

FINAL REMARKS

It is clear that secretory processes in bromeliads are widespread. The little knowledge that we have on this regard is probably due to under evaluation rather than lack of diversity. In part, this might be a product of the strong research focus directed to the anatomy, physiology and ecology of the vegetative axis. Another issue could be the difficulty in obtaining fresh material from plants that, in many cases, are difficult to access in the field, especially when factors like developmental phases and sample fixation can alter the results significantly. To this point, it is important to reinforce the role and value of the scientific collections of living material, which are, in my judgement, very much underused.

Nonetheless, I am convinced that a better understanding of the secretory processes in bromeliads might comprise an important step in our understanding of the whole family. The many challenges that taxonomists and other researchers face when dealing with the diversity of bromeliads might be ameliorated by the discovery of new secretory structures and other anatomical novelties in the family. Of course, due to the great number of homoplastic features and other peculiarities associated with the rapid and robust diversification of the group, valuable insights will only be achieved with the accumulation of comprehensive data gathered from careful examination. As demonstrated, many records of secretory structures in bromeliads are incomplete or indirectly inferred.

Finally, the variety of secretions observed in bromeliads might prove useful for pharmacological or other applied processes, but the development of these approaches also depends on further documentation and investigation. As more and more researches turn their attention to secretory structures of bromeliads, new and exciting discoveries will certainly ensue.