ORIGINAL ARTICLE

A flower with several secretions: anatomy, secretion composition, and functional aspects of the floral secretory structures of *Chelonanthus viridiflorus* (Helieae—Gentianaceae)

Bianca El Ajouz[1] · Adriano Valentin-Silva[2] · Dayana Maria Teodoro Francino[3] · Valdnéa Casagrande Dalvi[1]

Abstract

Floral secretory structures have been reported for Gentianaceae; however, morphoanatomical studies of these glands are rare. We described the development and secretory activity of the colleters and nectaries throughout the floral development of *Chelonanthus viridiflorus*. We collected flower buds, flowers at anthesis, and fruits to be investigated using light and scanning electron microscopy. We performed histochemical tests on the secretion of colleters and used glycophyte to confirm the presence of glucose in nectar. Colleters are located on the ventral surface of sepals and nectaries occur in four regions: (i) the dorsal and (ii) ventral surfaces of sepals; (iii) apex of petals; and (iv) base of ovary. The colleters have a short peduncle and a secretory portion with homogeneous cells. They are active in flower buds and secrete polysaccharides and proteins. In flowers at anthesis, they begin to senescence presenting protoplast retraction, cell collapse, and lignification; these characteristics are intensified in fruit. The nectaries of sepals and petals have two to five cells surrounding a central cell through which the secretion is released. Nectaries are numerous, forming a nectariferous area on the dorsal surface of sepals, like that observed on petals, and can form isolated units on the ventral surface of sepals. They are active from flower buds to fruits. A region with secretory activity was identified at the base of the ovary. The secretion of colleters acts in the protection of developing organs, while nectaries are related to defenses against herbivores and the supply of nectar to potential robbers or pollinators.

Keywords Colleter · Gentianaceae · Histochemistry · Nectary · Secretion · Secretory structures

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✉ Valdnéa Casagrande Dalvi
  valdnea.dalvi@ifgoiano.edu.br

1. Laboratório de Anatomia Vegetal, Instituto Federal de Educação, Ciência e Tecnologia Goiano (IF Goiano, Campus Rio Verde), Rodovia Sul Goiana, Km 01, Zona Rural, Caixa Postal 66, Goiás CEP 75901-970 Rio Verde, Brazil

2. Departamento de Botânica, Universidade Federal de Minas Gerais (UFMG, Campus Pampulha), Avenida Antônio Carlos, 6627, Minas Gerais CEP 31270-901 Belo Horizonte, Brazil

3. Laboratório de Anatomia Vegetal, Departamento de Ciências Biológicas, Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM, Campus JK), Rodovia MGT-367, Km 583, 5000, Alto da Jacuba, Minas Gerais CEP 39100-000 Diamantina, Brazil

Introduction

Gentianaceae, the third largest family of the order Gentianales (Judd et al. 2009), has different floral secretory structures that are widely reported in the literature, like colleters and nectaries (Baillon 1889; Lindsey 1940; Nemomissa 1997; Vogel 1998; Struwe et al. 2002; Zanotti 2018; Dalvi et al. 2020). Colleters, one of the order's synapomorphies, are commonly reported in family taxonomic studies in both vegetative and reproductive organs (Struwe et al. 2002; Calió 2009; Judd et al. 2009). The secretion of colleters can be composed of mucilage or by a mixture of hydrophilic and lipophilic substances that lubricate and protect meristems and developing organs against desiccation and inhibit the growth of microorganisms (Fahn 1979; Thomas 1991; Silva et al. 2017). Thomas (1991) reviewed the terminology attributed to these structures over time, including "squamellae," "glandular hairs," "glandular shaggy hairs," or "glandular trichomes," which may have caused an underestimation of

the occurrence of colleters in some groups of plants, including Gentianaceae.

Calycinal secretory trichomes, which correspond to colleters, were first described for Gentianaceae by Baillon (1889) as common structures in the family. The occurrence of "calycine squamellae" in different genera of the family was later reported (Lindsey 1940). However, from a histochemical and/or anatomical point of view, these structures have only been described in the calyx and corolla of Gentianinae flowers (Renobales et al. 2001) and calyx of *Calolisianthus* (Zanotti 2018; Dalvi et al. 2020) and *Swertia* (Nemomissa 1997) species.

Another secretory structure common in the family is the nectary, specialized in secreting nectar, a sugary solution that mediates the plant's interaction with animals (Rudgers 2004; Nicolson et al. 2007). Among plant survival strategies, two evolutionary trends are related to this secretory structure: one for protection against herbivory and the other for pollination (Fahn 1979). Owing to their importance in mediating these biotic interactions, there are several ways to classify nectaries. Nuptial and extra-nuptial nectaries have been used when nectar is involved or not with pollination, respectively (Delpino 1873). According to the position, nectaries can be classified as floral (FNs) when present in floral organs such as sepals, petals, androecium, and gynoecium, and extrafloral nectaries (EFNs) when present in vegetative organs such as stem, leaf, and cotyledons (Caspary 1848). The FNs of Gentianaceae species are commonly related to pollination (i.e., nuptial nectary; Wolff 2006) and although they attract ants, these structures are associated with defense mechanisms against herbivores (i.e., extra-nuptial nectary; Norment 1988; Dejean et al. 2011). The EFNs are common in Neotropical Gentianaceae species and are generally related to the protection of the plant against herbivores and pathogens (Delgado et al. 2011a; Dalvi et al. 2013).

Nectaries can also be classified according to their anatomical structure. In some cases, nectar tissue does not differ from adjacent tissues and only nectar is detected (non-structured nectaries); when anatomically differentiated, nectaries are characterized by the presence of xylem and phloem conduction elements, nectariferous parenchymatous tissue, and epidermis (Zimmermann 1932). In Gentianaceae, the peculiar secretory structures of the nectar were defined by Vogel (1998) as nectarioles. In the traditional sense, nectarioles include anatomically heterogeneous secretory structures formed by one or more cells and are distributed individually or in clusters composing macroscopic nectaries (Vogel 1998); these have been widely observed (Dalvi et al. 2013, 2014a, 2017, 2020; Delgado et al. 2011a, b; Zanotti 2018).

The presence of a nectariferous disk at the base of the ovary is one of the taxonomic characteristics used for the recognition of Gentianaceae, although it is shared with other families of the order, as well as with Lamiales and Solanales

(Calió 2009). Nectaries on the dorsal surface of the calyx (called glandular areas) are characteristic of Helieae species (Struwe et al. 2002), and on corolla lobes are common in *Swertia* species (Nemomissa 1998). However, the anatomical structure of the sepal and petal nectaries in Gentianaceae has only been described for species of *Calolisianthus* and *Chelonanthus* (Vogel 1998; Zanotti 2018; Dalvi et al. 2020).

In this context, the diversity existing in Gentianaceae in relation to the type and location of secretory structures in flowers is evident. The presence of a persistent calyx in fruits of *Chelonanthus* species (Lepis 2009) makes it possible to investigate the functional aspects of colleters and nectaries throughout the development of reproductive organs. However, the scarcity of information on floral secretory structures in this genus and the observation of nectariferous areas in petals and sepals of *Chelonanthus viridiflorus* (Mart.) Gilg as well as ants patrolling the entire plant motivated the selection of this species for the study. Thus, we aimed to describe the development and secretory activity of colleters and nectaries at different stages of the floral development of *C. viridiflorus*. In addition, we established the functional relationships of these secretory structures in the species studied.

Material and methods

Plant species and sampling

Chelonanthus viridiflorus is a terrestrial herb endemic to Brazil, commonly found along headwater streams in wet savannas and forest edges and on white-sand soils with low nutrient content (Lepis 2009). We collected samples of flower buds, flowers at pre-anthesis and anthesis, and fruits (in early development) from five individuals of *C. viridiflorus*, in the region of Diamantina, Minas Gerais, Brazil, in an area of *campos rupestres* (18°25′ S, 43°41′ W). We obtained photographic records with a Nikon D7000 (16.2 Mp; Tokyo, Japan). Vouchers were deposited in the Rio Verde herbarium (numbers 1760 and 1762), Instituto Federal Goiano, campus Rio Verde (IFRV).

Light microscopy (LM)

We used the samples collected to describe the development of secretory structures (colleters and nectaries). To do so, we fixed samples at different stages of development in FAA (formalin, acetic acid, 50% ethanol, 1:1:18 by volume) for 48 h and then stored in 70% ethanol (Johansen 1940). We dehydrated these samples in increasing ethanol series and embedded them in synthetic resin (2-hydroxyethyl methacrylate; Historesin, Leica Microsystems, Heidelberg, Germany). We sectioned the samples (paradermic, transversal,

and longitudinal sections) with a thickness of 7 μm in a rotating microtome (model 1508R, Logen Scientific, China) using low profile disposable steel razors. We stained with 0.05% toluidine blue (0.1 M sodium phosphate buffer, pH 4.7; O'Brien et al. 1964) and mounted the cuts under coverslips with synthetic resin (Permount, Fisher Scientific, NJ, USA).

We performed the diaphanization of petals and sepals in flowers at anthesis to observe the presence of nectaries. We used 10% sodium hydroxide and 10% sodium hypochlorite until complete clarification. Then we stained with 0.01% basic fuchsin in alcoholic solution and mounted under a coverslip with synthetic resin. We observed the anatomical cuts and diaphanized material with a light microscope (model BX61, Olympus Optical, Tokyo, Japan), equipped with an image capture system (DP-72 camera, Olympus Optical, Tokyo, Japan).

Scanning electron microscopy (SEM)

To study the micromorphology of secretory structures, we used samples of sepals and petals at the different stages of floral development analyzed, which were fixed in 2.5% glutaraldehyde (0.2 M sodium phosphate buffer, pH 7.2) for 48 h and stored in 70% ethanol (Gahan 1984). Subsequently, we dehydrated the samples in an acetone series to wash the secretion and subjected them to critical-point drying using CO_2 (Autosamdri 815—Series A, Tousimis, MD, USA). After fixing the samples onto stubs with double-sided tape, the material was sputter-coated with a gold protective film (25 nm thickness; Desk V, Denton Vacuum, NJ, USA). We observed and captured the images in a scanning electron microscope (Jeol JSM—6610, equipped with EDS, Thermo Scientific NSS Spectral Imaging, Tokyo, Japan).

Secretion composition

We used part of the material, which was embedded in synthetic resin, to perform histochemical tests and evaluate the secretion composition of colleters at different stages of floral development. The sections were treated with periodic acid–Schiff (PAS) to determine total polysaccharides (McManus 1948), 0.002% ruthenium red aqueous solution for pectins (Johansen 1940), 10% potassium dichromate aqueous solution for general phenolic compounds (Gabe 1968), 0.3% Sudan III in alcoholic solution for structural lipids (Pearse 1985), and 0.1% Xylidine Ponceau prepared in 3% acetic acid for proteins (O'Brien and McCully 1981). We also analyzed samples not subjected to histochemical tests. We observed the slides and captured the images as described.

We demonstrated the presence of sugars in the secretion produced by nectaries using a glucose enzyme test band, considering that glucose is the main sugar found in nectar (Baker and Baker 1983). We analyzed the secretion accumulated in bagged flowers from pre-anthesis to the third and last day of anthesis with the aid of blood glucose monitoring system equipment (Accu-Chek Active, Roche, São Paulo, Brazil).

Results

Structure and development of colleters

Calycinal colleters of *C. viridiflorus* were present from the beginning of the formation of the flower bud to the fruit (Fig. 1) because the calyx is persistent in the fruit. They were numerous and located at the base of sepals, on the ventral surface (Fig. 1a, b). Colleters in flower buds were observed at different stages, from cells in division forming small protuberances to fully formed colleters (Fig. 1c), evidencing asynchrony of development.

A large accumulation of secretion was observed covering the colleters (Fig. 1d) during pre-anthesis. At this stage, most colleters were still in secretory activity (Fig. 1e), characterized by the presence of a multicellular peduncle with hyaline content and secretory portion with similar cells in shape and size, intensely stained cytoplasm, large nuclei, and centralized nucleoli (Fig. 1e). They were avascularized and covered by a thin cuticle (Fig. 1f). However, asynchrony in development was still evident and colleters in the onset of senescence were also observed presenting protoplast retraction (Fig. 1g).

Although flowers still had active colleters during anthesis, most of them began to show signs of senescence (Fig. 1h–k). Cells with lignified walls in the basal region of the peduncle were common (Fig. 1h, i). Secretory cell collapse was also observed in most colleters (Fig. 1j, k), as well as hyaline cytoplasm, nucleus, and cell walls losing conformation (Fig. 1k).

Colleters were still persistent in fruits; however, the senescence signs reported for flowers during anthesis were intensified. Cell walls in the secretory portion became lignified (Fig. 1l), in addition to those at the base of the peduncle. The cells of colleters were completely collapsed in the fruit, and it was not possible to distinguish any cell contour or portion of this secretory structure (Fig. 1m, n). Although persistent in the fruit, *C. viridiflorus* colleters were inactive at this stage. The extravasated secretion observed was probably only a remnant of previous stages of floral development.

The secretion of the colleters reacted to the total polysaccharides (Fig. 1o), pectins (Fig. 1p), and proteins (Fig. 1q). These compounds were detected in all the floral development stages analyzed, being more abundant in the flower bud stage.
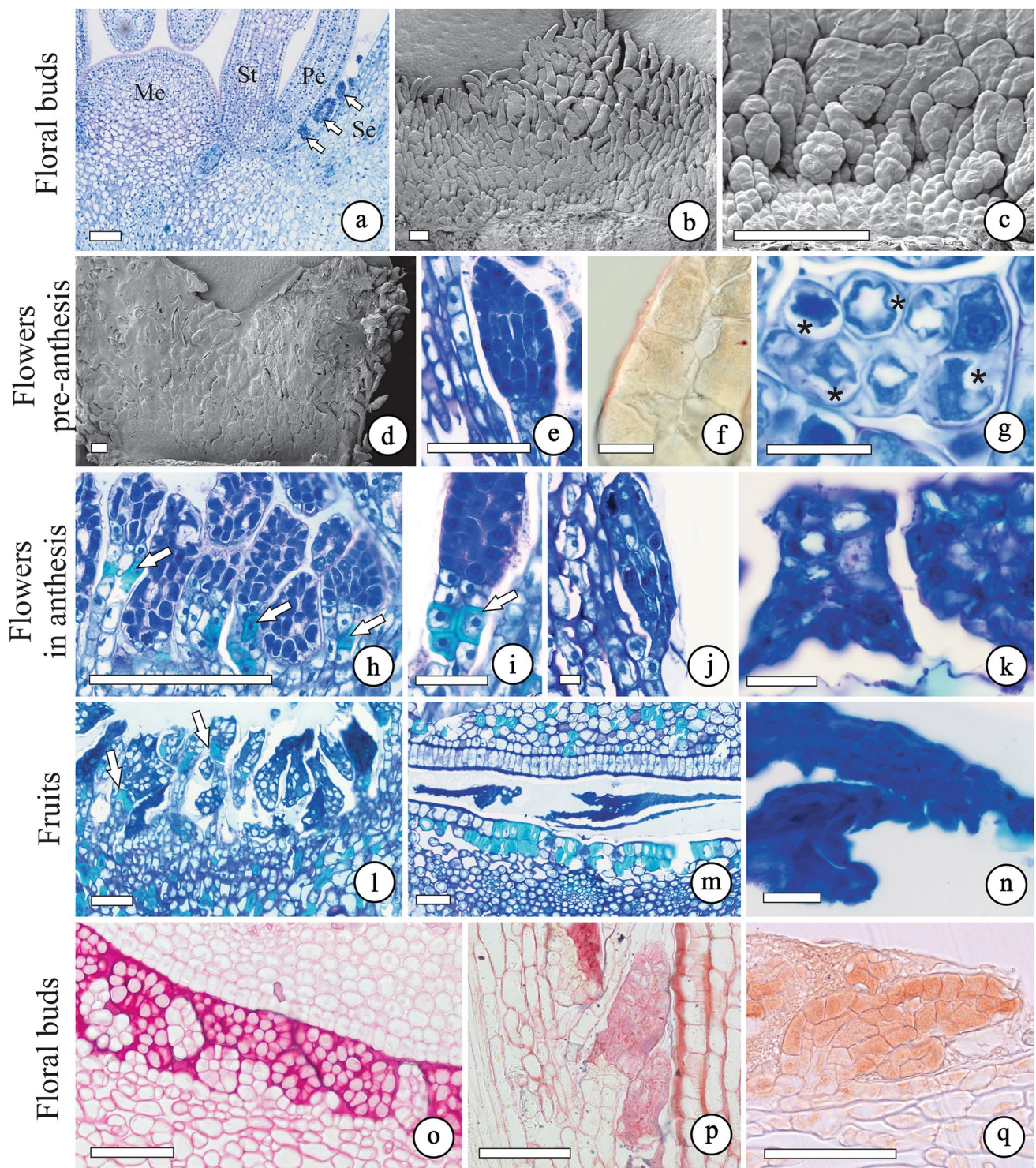
Fig. 1 Characterization of colleters at different stages of floral development of *Chelonanthus viridiflorus*. **a**, **e**, **g–n** Staining with toluidine blue; **b–d** scanning electron microscopy; and **f**, **o–q** histochemical tests. **a** Location of colleters (arrows) in longitudinal section. **b** Distribution of colleters on the ventral surface of sepals. **c** Asynchrony in the development of colleters. **d** Secretion accumulation over the colleters. **e** Differentiated colleters in a non-secretory peduncle and a multicellular secretory portion. **f** Thin cuticle evidenced by the reaction with Sudan III. **g** Cells of the secretory portion with protoplast retraction (*). **h–i** Lignified peduncle cells (arrows). **j** Active colleters with signs of senescence. **k** Cells with hyaline cytoplasm, nucleus, and walls with loss of conformation. **l** Persistent, degraded colleters with lignified cells of secretory portion (arrow). **m**, **n** Completely degraded colleters. **o–q** Presence of total polysaccharides, pectins, and proteins, respectively, in the secretion and/or in the cells of the secretory portion. Me, meristem; Pe, petal; Se, sepal; St, stamen. Bars **f–h**, **k**, **n** 20 μm, other images 100 μm

Structure and development of nectaries

Chelonanthus viridiflorus nectaries were observed on the dorsal and ventral surfaces of sepals and the ventral surface of petals (Figs. 2 and 3). A nectariferous region was also seen at the base of the ovary (Fig. 4).

Nectaries in the calyx

In each sepal, the nectary occurred in the median region of the dorsal surface, mild depressions, and was differentiated by the yellow-green appearance (Fig. 2a–c). Droplets of colorless and translucent sugary secretion (positive test for glucose) were observed in these nectaries from the beginning of the development of flower buds (Fig. 2a–d), in flowers at anthesis (Fig. 2e), and fruits in early development (Fig. 2f). These nectaries were often visited by ants (Fig. 2d). This glandular area (Fig. 3a) consisted of numerous small nectariferous units (Fig. 3b–d), which occurred interspersed with the stomata (Fig. 3b, c). The distinction of nectaries and stomata in frontal view, in SEM, was practically imperceptible,

evidenced only by the cuticle rupture when secretion was released (Fig. 3d). Scattered nectariferous units occurred in the ventral surface of sepals (Fig. 3e), where no secretion was observed.

In the frontal view, each nectariferous unit consisted of 2–5 secretory cells (Fig. 3c) with labyrinthine walls, arranged in a rosette (Fig. 3f). Secretory cells differed from other epidermal cells in shape and size and by the intense staining of the cytoplasm (Fig. 3g). Subepidermal cells with denser content, resembling a nectariferous parenchyma, and numerous vascular bundles were observed (Fig. 3b). On the ventral surface of sepals, the epidermis of the central portion had cells with lignified walls present in flower buds (Fig. 3h). This lignification, intensified throughout the floral development and in fruits, was seen in the epidermal cells and in 2–3 subepidermal layers (Fig. 3i).

Nectaries in corolla

At the apex of each petal, on the ventral surface, the nectariferous region can be identified by the slightly greenish color

Fig. 2 Field photographs of the different phases of floral development of *Chelonanthus viridiflorus*, showing the secretion droplets produced by nectaries and differentiated coloration of the nectariferous region. **a–c** Flower buds during early development; in **c** secretion droplets in sepals and at the apex of petals (arrow). **d** Ants on patrol activity on the floral bud. **e**, **f** Nectar production still evident in flowers at anthesis and fruit in early development, respectively. Bars 1 cm
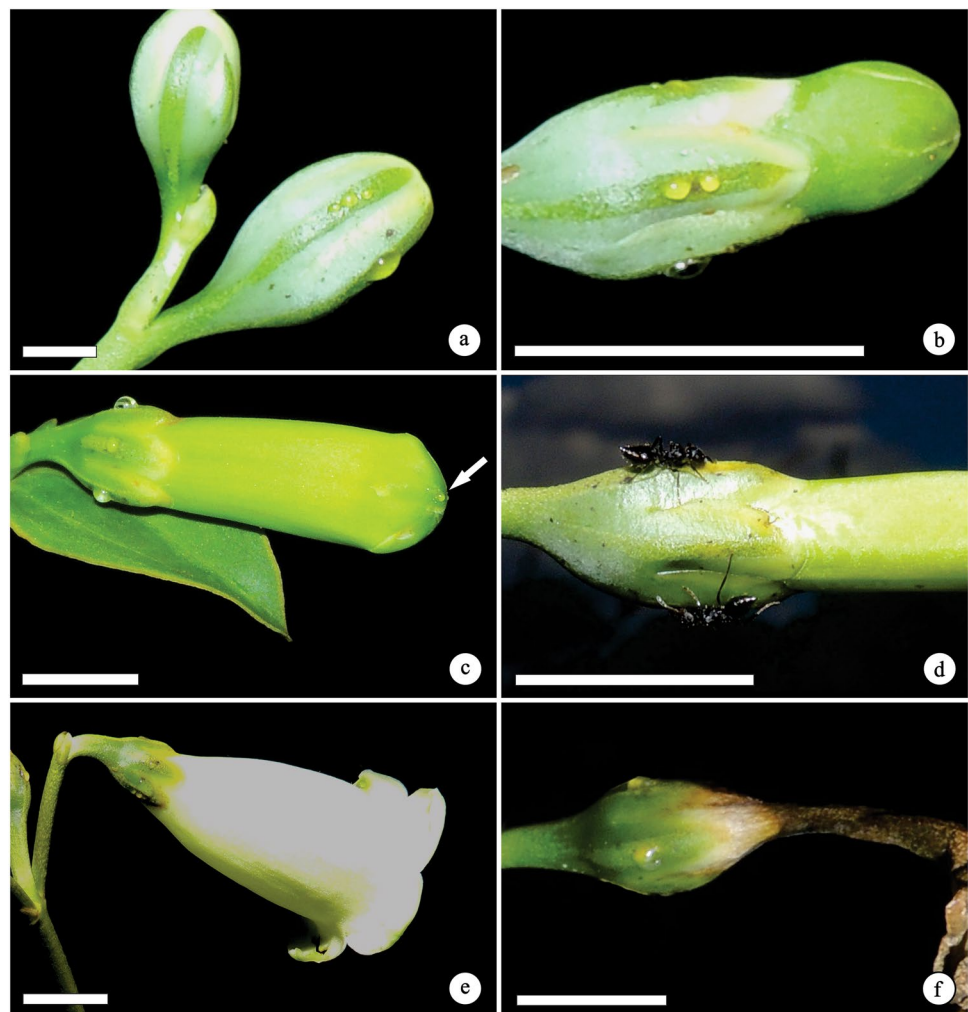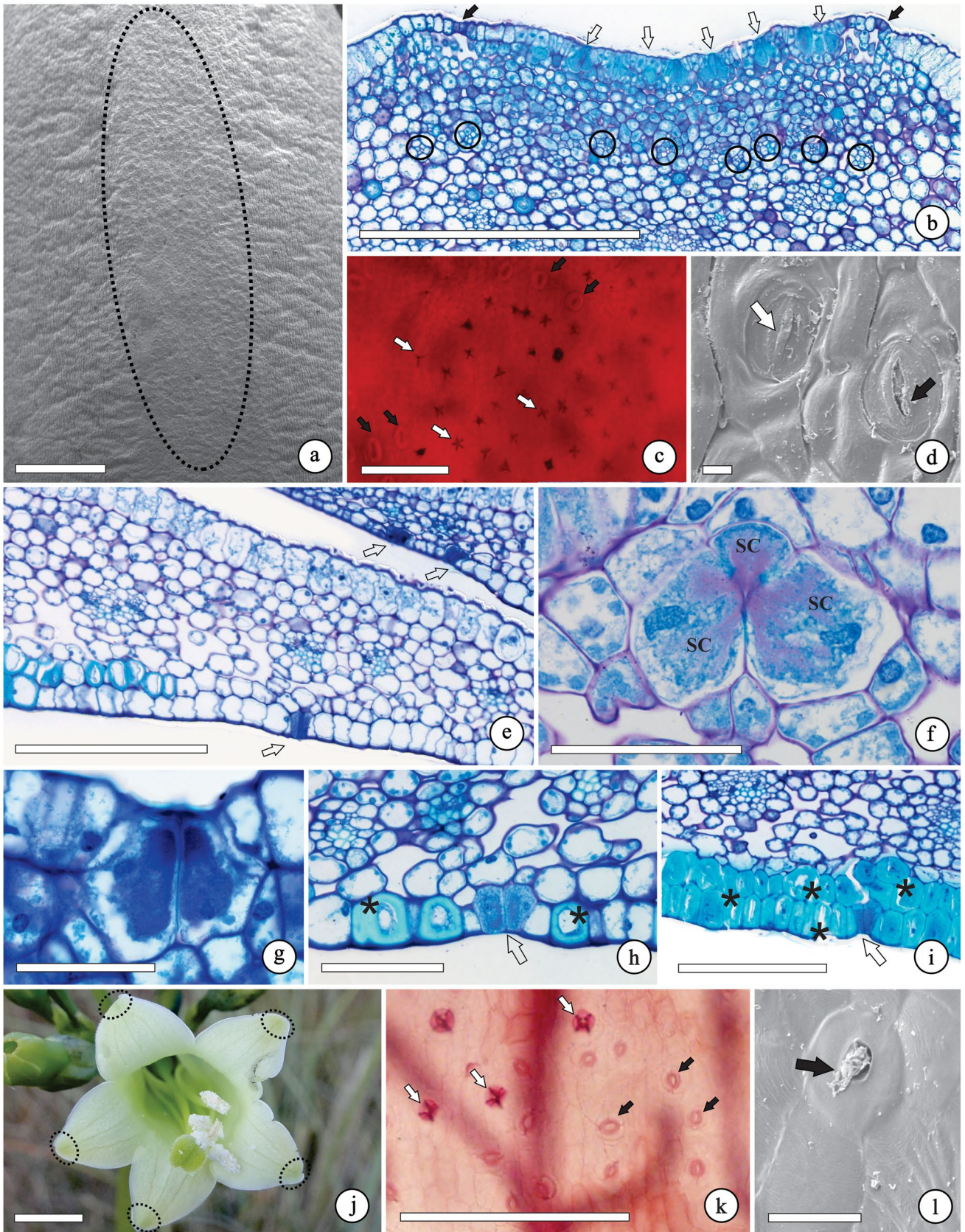
◀**Fig. 3** Characterization of nectaries in sepals (**a–i**) and petals (**j–l**) of *Chelonanthus viridiflorus*. **a** Nectariferous region on the dorsal surface of the sepal using scanning electron microscopy. **b** Nectariferous area with several nectariferous units (white arrows) interspersed with stomata (black arrows) and vascularization (circles). **c** Diaphanized sepals showing stomata (black arrows) and nectaries (white arrows). **d** Cuticle on the nectary (white arrow) and broken cuticle (black arrow). **e** Nectaries on the ventral surface of sepals (arrows). **f** Nectary in frontal view showing secretory cells (SC) and labyrinthine walls stained with toluidine blue. **g** Cross section of the nectary on the dorsal surface of the sepal. **h**, **i** Nectaries (arrows) on the ventral surface of sepals with different degrees of lignification (*) on the cell walls surrounding the nectariferous units. **j** Nectariferous region (dotted circles) at the apex of petals. **k** Diaphanized petals showing stomata (black arrows) and nectaries (white arrows). **l** Extravasated secretion (arrow). Bars **a**, **b**, **e**, **k** 500 µm. **c** 200 µm. **f**, **g**, **h**, **i** 50 µm. **d** 10 µm. **l** 100 µm. **j** 1 cm

(Fig. 3j), where secretion droplets were observed (Fig. 2c), as well as ants on patrol. The glucose enzyme test band confirmed the presence of this sugar in the secretion. Structurally, this region consisted of nectariferous units like those of sepals, but they were less numerous (Fig. 3k, l) and occurred interspersed with numerous stomata (Fig. 3k). Extravasated secretion could be observed (Fig. 3l).

Nectary in gynoecium

There was no formation of a nectariferous disk at the base of the ovary; however, it was possible to differentiate a region by color: while the largest extension of the ovary appeared green, the base was yellowish (Fig. 4a, b). This region was observed at the base of the ovary in flower buds, at pre-anthesis, and anthesis. Anatomically, this basal region, strongly stained with toluidine blue (Fig. 4c–f), had epidermis with palisade cells, thin cuticle, and dense cytoplasm (Fig. 4e, f); stomata were not observed. The subepidermal region had 3–5 layers with palisade cells and dense cytoplasm (Fig. 4d, e). This dense cellular content was not observed in the non-secretory region of the ovary, which had small, isodiametric thin-walled cells (Fig. 4g). Xylem and phloem vascular terminations converged on the subepidermal parenchyma (Fig. 4d). Glucose was present in the secretion accumulated in flowers on the third day of anthesis. This region did not present a glandular aspect in the fruit.

Discussion

The flowers of *C. viridiflorus* have two types of secretory structures, colleters and nectaries, which can be found in the calyx, corolla, and ovary. Here we highlight the first record of nectaries on the ventral surface of sepals for Gentianaceae.

Colleters

The presence of calycinal colleters, adhered to the internal surface of sepals, such as those described for *C. viridiflorus* by Lindsey (1940) and Struwe et al. (1994, 2002), is a common characteristic of Gentianaceae. In most species in this family, floral colleters are restricted to sepals (Lindsey 1940; Nemomissa 1997; Renobales et al. 2001; Zanotti 2018; Dalvi et al. 2020), as noted here. However, some taxa such as *Comastoma tenellum* (Rottb.) Toyok and *Swertia perennis* L. (Renobales et al. 2001) also have colleters in the corolla.

The colleters of *C. viridiflorus* differ from those described by Lersten (1974) but resemble the structures of Leguminosae reported by Barros et al. (2017) and Silva et al. (2017) and other Gentianaceae species (Zanotti 2018; Dalvi et al. 2020). These authors demonstrated the same morphology present in the colleters of *C. viridiflorus*, that is, the presence of a peduncle and a homogeneous multicellular secretory portion. In Gentianaceae, both floral (Renobales et al. 2001; Zanotti 2018; Dalvi et al. 2020) and foliar colleters (Delgado et al. 2011a; Dalvi et al. 2014a; Zanotti 2018) show this secretory portion morphology with homogeneous cells. A single exception has been registered for foliar colleters of *Macrocarpaea obtusifolia* (Griseb.) Gilg, which have a central axis of cells covered by a palisade secreting epidermis (Dalvi et al. 2014b). It is interesting to note that colleters with this morphology are not found in other species of Gentianales, which could support ontogenetic/phylogenetic studies in this order.

Silva et al. (2017) found that, although there is a predominance of a type of colleter in *Chamaecrista*, there are variations in shape and/or type within the same taxon according to the position that these structures occupy in the plant. Simões et al. (2006) highlighted variations in the type and position of calycinal colleters in Apocynaceae species. These records highlight the taxonomic potential of these secretory structures for Gentianaceae, as suggested by Nemomissa (1997) and Renobales et al. (2001), and should be investigated in a greater number of species of this family.

In the case of colleters in floral organs, such as those observed here, Mayer et al. (2013) found that their secretion covers and keeps the petals together until the beginning of anthesis in coffee flowers. In *Ipomoea cairica* (L.) Sweet, the highly hygroscopic secretion forms a film on the exposed surface of the corolla, protecting it from dehydration (Paiva and Martins 2011). Dalvi et al. (2020) found active colleters in *Calolisianthus pedunculatus* (Cham. & Schltdl.) Gilg. even during fruit development. These authors suggested that the secretion of colleters could seal the calyx and corolla (which are persistent) until environmental conditions are favorable for seed dispersal. However, in the present study, colleters were senescent in flowers at anthesis and during fruit development, and no

Fig. 4 Nectary at the base of the *Chelonanthus viridiflorus* ovary. **a**, **b** Base of the ovary with a light-yellow tone, different from the predominant green color throughout the structure. **c** Region near the epidermis (arrows) of the ovary densely stained with toluidine blue. **d** Subepidermal parenchyma and vascularization close to this region (circles). **e**, **f** Detail of the epidermis and subepidermal parenchyma with palisade cells and dense cytoplasm. **g** Non-secretory portion of the ovary. Ep, epidermis. Bars **a**, **b** 1 cm. **c**, **d** 500 μm. **e**, **f**, **g** 100 μm
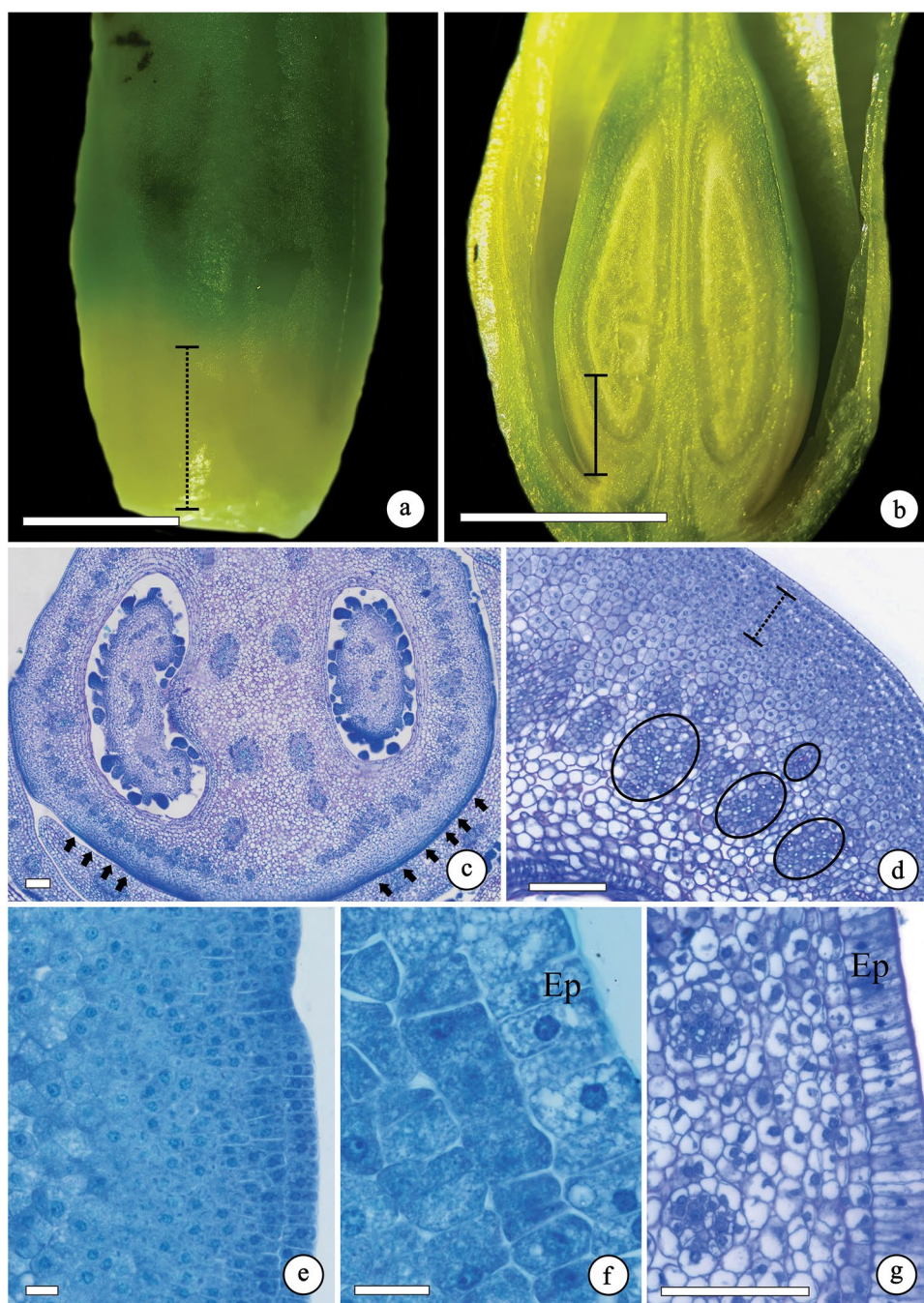
secretion leaked out of the calyx. Because *C. viridiflorus* commonly occurs in more open vegetation, such as wet savannas, forest edges (Lepis 2009), and in *campos rupestres* (present study), and flowering can occur throughout the year (Lepis 2009), individuals are exposed to variations in air humidity and solar radiation. In addition, the asynchronous development of these colleters, already observed in calycinal colleters from Gentianaceae (Zanotti 2018; Dalvi et al. 2020), and their greater activity in flower buds is related to the function of protecting the meristem and developing organs against desiccation (Thomas

1991). The intense mucilage production by the colleters of *C. viridiflorus* from flower buds to anthesis reinforces this hypothesis, as they could act in the maintenance of a humid environment due to the hygroscopic properties of the mucilage (Nobel et al. 1992).

We also found proteins in the secretion of *C. viridiflorus* colleters. The proteins in *Bathysa nicholsonii* K. Schum. colleters, for example, have an antifungal role (Miguel et al. 2006). The presence of proteins is common in species of Apocynaceae and Rubiaceae (Gentianales; Klein et al. 2004; Miguel et al. 2006; Barreiro and Machado 2007; Demarco

2017; Ribeiro et al. 2017). In Gentianaceae, the presence of proteins has been reported for foliar colleters of *Macrocarpaea obtusifolia* (Dalvi et al. 2014b), calycinal colleters of *Calolisianthus pedunculatus* (Dalvi et al. 2020), and calycinal and foliar colleters of *Calolisianthus speciosus* (Cham. & Schltdl.) Gilg (Zanotti 2018). These three species belong to the Helieae tribe and occur in Neotropical environments (Struwe et al. 2002). In contrast, Gentianaceae species, common in temperate regions, exclusively secrete polysaccharides (Renobales et al. 2001). These differences in the composition of the secretion of colleters may be related to different selective pressures of these environments, whether related to vegetation type (Tresmondi et al. 2015, 2017) or climatic seasonality (Costa et al. 2020).

Nectaries

The nectaries present in sepals and petals of *C. viridiflorus* are classified as floral nectaries (FNs; sensu Caspary 1848) and have already been recorded in other Gentianaceae species (Vogel 1998; Zanotti 2018; Dalvi et al. 2020). These secretory structures are similar to the nectarioles described by Vogel (1998), also found in other species of this family (Delgado et al. 2011a, b; Dalvi et al. 2014a). The same structures, referred to as nectaries, have been described in different species of Gentianaceae (Dalvi et al. 2013, 2017, 2020; Zanotti 2018). We chose to use "nectary" and not "nectariole" in order not to perpetuate its use and avoid confusion, since the term nectariole proposed by Vogel (1998) also refers to other secretory structures such as hydropotes in *Cabomba*.

The FNs on the sepals and petals of *C. viridiflorus* can be considered unusual (Vogel 1998). This type of nectary is more difficult to distinguish when the secretion is not observed, and is considered rare in Angiosperms (Bernadello 2007). Gentianaceae nectaries have a format like stomata, which makes it difficult to differentiate the structure, especially using scanning electron microscopy. In our study, one of the distinguishing criteria may be the cuticle covering the entire FN before the release of secretion, as highlighted by Zanotti (2018).

The FNs located on the dorsal surface of sepals and the apex of petals of *C. viridiflorus* are probably related to the defense against florivory (i.e., the consumption of flowers prior to seed coat formation; MacCall and Irwin 2006), owing to the presence of ants in the field patrolling the flowers. Forty-four species of ants were recorded around *Chelonanthus alatus* (Aubl.) Pulle individuals, patrolling their flowers mainly during the day, from flower buds to fruit formation, to prevent attacks by cockroaches and curculionid and chrysomelid beetles (Dejean et al. 2011). The period of secretory activity of these nectaries was similar to that observed here. This association between ants and FNs has been recorded from other Gentianaceae species, in the genera *Chelonanthus* (Lepis 2009) and *Calolisianthus* (Zanotti 2018). Besides this, the calyx with glandular areas on its dorsal surface is persistent in fruit in these genera (Calió 2009; Lepis 2009). Based on this, we can infer a common facultative mutualism between Gentianaceae species with FNs in the perianth and the workers of several species of ants. According to the optimal defense theory, this association may have evolved as an indirect defense mechanism of organs with high adaptive value, such as flowers (Zangerl and Bazzaz 1992; Marquis 2012).

On the other hand, this is the first record of nectaries on the ventral surface of sepals in flowers of Gentianaceae (see Bernadello 2007). Although descriptions of FNs are scarce for this family, reports of nectaries in leaves and stems (Delgado et al. 2011a, b; Dalvi et al. 2013, 2014a, 2017) have shown the taxonomic potential of this structure owing to variations in presence, structure, and location. FNs may also have taxonomic importance, as recorded in *Swertia* species, where there is variation in the position and number of nectaries in the corolla lobes (Nemomissa 1998). Furthermore, the distribution of these nectaries interspersed with stomata and their vascularization deserve further attention. Thus, the characteristics of calycinal nectaries can also be used as taxonomic tools for Gentianaceae species.

Although no secretion was observed, the anatomical data indicate that the FNs on the ventral surface of sepals are active. The nectar produced inside the calyx could be a food source for potential antagonists, such as the hummingbird *Phaethornis ruber* robbing the nectar accumulated at the base of the corolla of *C. alatus* flowers at pre-anthesis (Machado et al. 1998). Thus, this nectar produced on the ventral surface of the calyx could decrease the depletion of nectar available to pollinators inside the corolla tube. In this context, calycinal nectaries, although structurally similar on both sides of the sepals, would have different functions depending on their location.

The nectariferous region present at the base of ovaries differs from that reported for other species of the genus, as well as for the family, which have a nectariferous disk (Judd et al. 2009; Lepis 2009) that was not observed in the evaluated individuals of *C. viridiflorus*. Zanotti (2018) observed a more dilated region at the base of the ovary in *C. speciosus*, which, anatomically, consists of palisade epidermis, nectariferous subepidermal parenchyma vascularized mainly by the phloem. Despite being inconspicuous, the structure similar to the nectariferous disk of *C. speciosus* (Zanotti 2018), the presence of glucose in the secretion, and vascular tissue indicate that this nectariferous region of *C. viridiflorus* is also active, as observed in reduced nectaries of two *Zeyheria* species (Machado et al. 2017). The reduction of the nectariferous disk in the analyzed population of *C. viridiflorus* may be related to the absence or scarcity of

pollinators at the site. This species has characteristics compatible with chiropterophily, as well as other Gentianaceae species (Dobat and Peikert-Holle 1985). In fact, *C. alatus* is pollinated by the nectar-feeding bat *Glossophaga soricina* (Machado et al. 1998). Thus, studies of pollination biology may clarify whether *C. viridiflorus* depends on biotic vectors for reproduction to occur or whether there may have been a shift to autogamy as a reproductive assurance mechanism (see Kalisz and Vogler 2003; Kalisz et al. 2004) and consequent reduction of the nectariferous disk.

Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

References

Baillon HE (1889) Histoire des plantes. Libraire Hachette & Co, Paris

Baker HG, Baker I (1983) Floral nectar sugar constituents in relation to pollinator type. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold Company Inc., New York, pp 117–141

Barreiro DP, Machado SR (2007) Coléteres dendróides em *Alibertia sessilis* (Vell.) K. Schum., uma espécie não-nodulada de Rubiaceae. Braz J Bot 30:389–399. https://doi.org/10.1590/S0100-84042007000300005

Barros TC, Marinho CR, Pedersoli GD, Paulino JV, Teixeira SP (2017) Beyond pollination: diversity of secretory structures during flower development in diferente legume lineages. Acta Bot Bras 31:358–373. https://doi.org/10.1590/0102-33062016abb0291

Bernadello G (2007) A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E (eds) Nectaries and nectar, 1st edn. Springer, Dordrecht, pp 19–128

Calió MFA (2009) Sistemática de Helieae Gilg (Gentianaceae). Dissertation, Universidade de São Paulo

Caspary R (1848) De nectariis. PhD Thesis. Bonn: Elverfeldae

Costa ISC, Lucena EMP, Bonilla OH, Guesdon IR, Coutinho IAC (2020) Seasonal variation in colleter exudates in *Myrcia splendens* (Myrtaceae). Aust J Bot 68:403–412. https://doi.org/10.1071/BT20020

Dalvi VC, Meira RMSA, Azevedo AA (2013) Extrafloral nectaries in neotropical Gentianaceae: occurrence, distribution patterns, and anatomical characterization. Am J Bot 100:1779–1789. https://doi.org/10.3732/ajb.1300130

Dalvi VC, Meira RMA, Francino DMT, Silva LC, Azevedo AA (2014a) Anatomical characteristics as taxonomic tools for the species of *Curtia* and *Hockinia* (Saccifolieae-Gentianaceae Juss.). Plant Syst Evol 300:99–112. https://doi.org/10.1007/s00606-013-0863-1

Dalvi VC, Cardinelli LS, Meira RMSA, Azevedo AA (2014b) Foliar colleters in *Macrocarpaea obtusifolia* (Gentianaceae): anatomy, ontogeny, and secretion. Botany 92:59–67. https://doi.org/10.1139/cjb-2013-0203

Dalvi VC, Meira RMSA, Azevedo AA (2017) Are stem nectaries common in Gentianaceae Juss.? Acta Bot Bras 31:403–410. https://doi.org/10.1590/0102-33062016abb0404

Dalvi VC, de Faria GS, Azevedo AA (2020) Calycinal secretory structures in *Calolisianthus pedunculatus* (Cham. & Schltdl) Gilg (Gentianaceae): anatomy, histochemistry and functional aspects. Protoplasma 257:275–284. https://doi.org/10.1007/s00709-019-01436-5

Dejean A, Corbara B, Leroy C, Delabie JHC, Rossi V, Céréghino R (2011) Inherited biotic protection in a neotropical pioneer plant. PLoS One 6:1–11. https://doi.org/10.1371/journal.pone.0018071

Delgado MN, Silva LC, Báo SN, Morais HC, Azevedo AA (2011a) Distribution, structural and ecological aspects of the unusual leaf nectaries of *Calolisianthus* species (Gentianaceae). Flora 206:676–683. https://doi.org/10.1016/j.flora.2010.11.016

Delgado MN, Azevedo AA, Valente GE, Kasuya MCM (2011b) Comparative anatomy of *Calolisianthus* species (Gentianaceae-Helieae) from Brazil: taxonomic aspects. Edinb J Bot 68:139–155. https://doi.org/10.1017/S0960428610000284

Delpino F (1873) Ulteriori osservazioni e considerazioni sulla Dicogamia nel regno vegetale. Atti Soc Ital Sci Nat 16:151–349

Demarco D (2017) Floral glands in Asclepiads: structure, diversity and evolution. Acta Bot Bras 31:477–502. https://doi.org/10.1590/0102-33062016abb0432

Dobat K, Peikert-Holle T (1985) Blüten und Fledermäuse (Chiropterophilie). Kramer, Frankfurt am Main

Fahn A (1979) Secretory tissues in plants. Academic Press, London

Gabe M (1968) Techniques histologiques. Masson and Cie, Paris

Gahan PB (1984) Plant histochemistry and cytochemistry. Academic Press, Florida, An introduction

Johansen DA (1940) Plant microtechnique. McGraw-Hill Book Company, New York

Judd WS, Campbell CS, Kellog EA, Stevens PF (2009) Plant systematics: a phylogenetic approach. Massachusetts: Sinauer Associates, Sunderland

Kalisz S, Vogler DW (2003) Benefits of autonomous selfing under unpredictable pollinator environments. Ecology 84:2928–2942. https://doi.org/10.1890/02-0519

Kalisz S, Vogler DW, Hanley KM (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430:884–887. https://doi.org/10.1038/nature02776

Klein DE, Gomes VM, Silva-Neto SJ, Cunha M (2004) The structure of colleters in several species of *Simira* (Rubiaceae). Ann Bot 94:733–740. https://doi.org/10.1093/aob/mch198

Lepis K (2009) Evolution and systematics of *Chelonanthus* (Gentianaceae). Ph.D. dissertation, Rutgers University

Lersten NR (1974) Morphology and distribution of colleters and crystals in relation to the taxonomy and bacterial leaf nodule symbiosis of *Psychotria* (Rubiaceae). Am J Bot 61:973–981. https://doi.org/10.2307/2441988

Lindsey AA (1940) Floral anatomy in the Gentianaceae. Am J Bot 27:640–652

MacCall AC, Irwin RE (2006) Florivory: the intersection of pollination and herbivory. Ecol Lett 9:1351–1365. https://doi.org/10.1111/j.1461-0248.2006.00975.x

Machado ICS, Sazima I, Sazima M (1998) Bat pollination of the terrestrial herb *Irlbachia alata* (Gentianaceae) in northeastern Brazil. Pl Syst Evol 290:231–237. https://doi.org/10.1007/BF00985230

Machado SR, Souza CV, Guimarães E (2017) A reduced, yet functional, nectary disk integrates a complex system of floral nectar secretion in the genus *Zeyheria* (Bignoniaceae). Acta Bot Bras 31:344–357. https://doi.org/10.1590/0102-33062016abb0279

Marquis RJ (2012) Uma abordagem geral das defesas das plantas contra a ação de herbívoros. In: Del-Claro K, Torezan-Silingardi HM (eds) Ecologia das interações plantas-animais: uma abordagem ecológico-evolutiva. Technical Books, Rio de Janeiro, pp 55–66

Mayer JLS, Carmello-Guerreiro SM, Mazzafera P (2013) A functional role for the colleters of coffee flowers. AoB Plants 5:plt 029. https://doi.org/10.1093/aobpla/plt029

McManus JFA (1948) Histological and histochemical uses of periodic acid. Stain Technol 23:99–108. https://doi.org/10.3109/10520294809106232

Miguel EC, Gomes VM, Oliveira MA, Cunha MD (2006) Colleters in *Bathysa nicholsonii* K. Schum. (Rubiaceae): ultrastructure, secretion protein composition and antifungal activity. Plant Biol 200:715–722. https://doi.org/10.1055/s-2006-924174

Nemomissa S (1997) Floral character states of the Northeast and Tropical East African *Swertia* species (Gentianaceae). Nord J Bot 17:145–156. https://doi.org/10.1111/j.1756-1051.1997.tb00301.x

Nemomissa S (1998) A synopsis of *Swertia* (Gentianaceae) in East and Northeast Tropical Africa. Kew Bull 27:548–572. https://doi.org/10.2307/4114507

Nicolson SW, Nepi M, Pacini E (2007) Nectaries and nectar. Springer, Netherlands, Dordrecht

Nobel PS, Cavelier J, Andrade JL (1992) Mucilage in cacti: its apoplastic capacitance, associated solutes, and influence on tissue water relations. J Exp Bot 43:641–648. https://doi.org/10.1093/jxb/43.5.641

Norment CJ (1988) The effect of nectar-thieving ants on the reproductive success of *Frasera speciosa* (Gentianaceae). Am Midl Nat 120:331–336. https://doi.org/10.2307/2426005

O'Brien TP, McCully ME (1981) The study of plant structure principles and selected methods. Termarcaphi Ptey. Ltd., Melbourne

O'Brien TP, Feder N, McCully ME (1964) Polychromatic staining of plant cells walls by toluidine blue O. Protoplasma 59:368–373. https://doi.org/10.1007/BF01248568

Paiva EAS, Martins LC (2011) Calycinal trichomes in *Ipomoea cairica* (Convolvulaceae): ontogenesis, structure and functional aspects. Aust J Bot 59:91–98. https://doi.org/10.1071/BT10194

Pearse AGE (1985) Histochemistry theoretical and applied: preparative and optical technology. Churchill Livingston, Edinburgh

Renobales G, Diego E, Urcelay B, López-Quintana A (2001) Secretory hairs in *Gentiana* and allied genera (Gentianaceae, subtribe Gentianinae) from the Iberian Peninsula. Bot J Linn Soc 136:119–129. https://doi.org/10.1111/j.1095-8339.2001.tb00560.x

Ribeiro JC, Ferreira MJP, DeMarco D (2017) Colleters in Asclepiadoideae (Apocynaceae): protection of meristems against desiccation and new functions assigned. Int J Plant Sci 178:465–477. https://doi.org/10.1086/692295

Rudgers JA (2004) Enemies of herbivores can shape plants traits: selection in a facultative ant-plant mutualism. Ecology 85:192–205. https://doi.org/10.1890/02-0625

Silva MS, Coutinho IAC, Araújo MN, Meira RMSA (2017) Colleters in *Chamaecrista* (L.) Moench sect. *Chamaecrista* and sect. *Caliciopsis* (Leguminosae-Caesalpinioidae): anatomy and taxonomic implications. Acta Bot Bras 31:382–391. https://doi.org/10.1590/0102-33062016abb0339

Simões AO, Castro MM, Kinoshita LS (2006) Calycine colleters of seven species of Apocynaceae (Apocynoideae) from Brazil. Bot J Linn Soc 152:387–398. https://doi.org/10.1111/j.1095-8339.2006.00572.x

Struwe L, Albert VA, Bremer B (1994) Cladistics of the family level classification of the Gentianales. Cladistics 10:175–206. https://doi.org/10.1006/clad.1994.1011

Struwe L, Kadereit JW, Klackenberg J, Nilsson S, Thiv M, Von-Hagen KB, Albert VA (2002) Systematics, character evolution, and biogeography of Gentianaceae, including a new tribal and subtribal classification. In: Struwe L, Albert VA (eds) Gentianaceae: systematics and natural history. Cambridge University Press, Cambridge

Thomas V (1991) Structural, functional and phylogenetic aspects of the colleter. Ann Bot 68:287–305. https://doi.org/10.1093/oxfordjournals.aob.a088256

Tresmondi F, Nogueira A, Guimarães E, Machado SR (2015) Morphology, secretion composition, and ecological aspects of stipular colleters in Rubiaceae species from tropical forest and savanna. Sci Nat 102:73. https://doi.org/10.1007/s00114-015-1320-5

Tresmondi F, Canaveze Y, Guimarães E, Machado SR (2017) Colleters in Rubiaceae from forest and savanna: the link between secretion and environment. Sci Nat 104:17. https://doi.org/10.1007/s00114-017-1444-x

Vogel S (1998) Remarkable nectaries: structure, ecology, organophyletic perspectives II Nectarioles. Flora 193:1–29. https://doi.org/10.1016/S0367-2530(17)30812-5

Wolff D (2006) Nectar sugar composition and volumes of 47 species of Gentianales from a Southern Ecuadorian montane forest. Ann Bot 97:767–777. https://doi.org/10.1093/aob/mcl033

Zangerl AR, Bazzaz FA (1992) Theory and pattern in plant defense allocation. In: Fritz R Simms EL (eds) Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. Chicago University Press, Chicago, pp 363-391

Zanotti A (2018) Estruturas secretoras em *Calolisianthus specious* (Cham. & Schltdl.) Gilg. (Gentianaceae): ontogenia e biologia da secreção. Dissertation, Universidade Federal de Viçosa

Zimmermann JG (1932) Über die extrafloralen nektarien der angiospermen. Beih Bot Zentralb 49:99–196