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Leaf and calycine colleters in *Odontadenia lutea* (Apocynaceae – Apocynoideae – Odontadenieae): their structure and histochemistry[1]

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ABSTRACT – (Leaf and calycine colleters in *Odontadenia lutea* (Apocynaceae – Apocynoideae – Odontadenieae): their structure and histochemistry). The structure and histochemistry of colleters found on the vegetative and floral apices of *Odontadenia lutea* are described. Colleters occur on vegetative apices starting at the fourth node, with 68 to 80 colleters being found at each node. Each leaf primordium has only one colleter of axillary origin, 3-5 intra-petiolar, and 12-16 inter-petiolar (intra-stipular). There are four types of colleters: standard, bipartite standard, sessile, and bipartite sessile. Colleters on the reproductive apices alternate with the sepals and are sessile, reduced sessile, tripartite laminar sessile, or asymmetrical. All of the colleters have a central nucleus of parenchymatous cells covered by a palisade uniseriate secretory epidermis and a thin cuticle. Secretory idioblasts were observed in the parenchymatous axis. Vascularization was observed only in standard axillary and laminar colleters. Crystals were observed in the parenchyma of the axillary colleter. Histochemical tests demonstrated that there was no rupturing or distension of the cuticle during the secretion process. Mucilage was identified using the PAS reaction as well as by Mayer's reagent and Ruthenium red staining. The calycine colleters had two distinct secretory phases, the first synthesizing mucilage and the second producing phenolic compounds.

Key words - mucilage, phenolic compounds, secretory structures

INTRODUCTION

Secretory structures vary widely in terms of their morphology, position, function, and types of secretion (Fahn 1979). Accordingly, studies of the chemical composition of the secreted materials and anatomical characterizations of colleters are needed to fully understand their roles and the functions of their exudates (Schnepf 1974). Additionally, histochemical studies are needed to detect the principal metabolites that compose the exudates, as the identification of secretory structures is largely based on the predominant substance produced (Fahn 1979).

The structural similarity of colleters with other secretory structures and the general lack of studies identifying their exudates have led many researchers to confuse colleters with nectaries or resin glands (Arekal & Ramakrisna 1980, Mohan & Inamdar 1986, Subramanian et al. 1989, Thomas 1991).

Colleters have an extremely important function in plant growth, being responsible for secretions that can protect and lubricate meristems (Fahn 1979). The material liberated by a colleter may be composed solely of mucilage (Fahn 1979, Thomas 1991) or a mixture of mucilage with lipophilic substances, including terpenoids (Fahn 1990); additional substances have been detected in colleters in the Apocynaceae such as resins (Subramanian et al. 1989), lipids (Appezzato-da-Glória & Estelita 2000), proteins (Thomas et al. 1989), mucopolysaccharides (Dave et al. 1987, Thomas et al. 1989), phenolic compounds (Rio et al. 2002, Demarco 2005, Rio 2006), and fatty acids (Demarco 2005). Only fairly reduced numbers of researchers have described the ontogeny and histochemistry of colleters, including Brazilian workers such as: Appezzato-da-Glória & Estelita (2000), who described the development, structure, and distribution of colleters in *Mandevilla illustris* (Vell.) Woodson and *M. pohliana* (Stadelm.) A.H. Gentry (= *M. velutina* (Mart. ex Stadelm.) Woodson; Rio et al. (2002), who characterized the anatomy of the leaf colleters of *Prestonia coalita* (Vell.) Woodson; Simões et al. (2006), who described the anatomy of the calycine colleters of seven species of Apocynaceae; and Martins et al. (2010), who described the vegetative and floral colleters of *Temnadenia violacea* (Vell.) Miers.

In order to contribute to our knowledge about the colleters of the Apocynaceae, the present work describes the structure of the vegetative and calycine colleters of *Odontadenia lutea* (Vell.) Markgr. and characterizes the chemical nature of their secretions.

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MATERIAL AND METHODS

The study material was collected in two areas of *Cerrado* (Brazilian savanna) vegetation in the Mogi-Guaçu Biological Reserve and the Bauru Botanical Garden in São Paulo State, Brazil. Reference material was deposited in the UEC Herbarium, São Paulo: Bauru, 10/II/2004, *F Martins* s.n. (UEC 147884); Mogi-Guaçu, 22/II/2004, *F Martins* s.n. (UEC 147886); 22/II/2004, *F Martins* s.n. (UEC 147885); 22/II/2004, *F Martins* s.n. (UEC 147883).

Branches with shoot apices, leaf buds, inflorescences with floral buds at different development states, and flowers in anthesis were collected and fixed in formaldehyde-acidic acetic- 50% ethyl alcohol, 1:1:18 v/v (FAA) for 24 hours (Johansen 1940); neutral-buffered formaldehyde solution (FNT) for 48 hours (Lillie 1948 *in* Clark 1973); formalin-ferrous sulfate (SFF) for 48 hours (Johansen 1940). All of the material was exposed to vacuum desiccation during the process of fixation and was subsequently transferred to 70% ethyl alcohol.

Samples were isolated and kept in 70% tertiary butyl alcohol for approximately 7 days, dehydrated in a butilic alcohol series, and subsequently embedded in histological paraffin (Histosec/Merck; Johansen 1940). Serial transversal and longitudinal sections (ca. 10 µm) were made in a rotary microtome. The sections were stained with 1.5% alcoholic Safranin O and 1% aqueous Astra Blue (Gerlarch 1969); permanent slides were mounted with synthetic resin (Permount/Fisher).

Histochemical tests of the calycine colleters were performed on serial sections of floral buds (15 and 30 mm long) that were fixed: in FAA and tested for polysaccharides, proteins, or water-soluble phenolic compounds; in FNT to identify total lipids and lipid soluble phenolic compounds; in SFF to test for all classes of phenolic compounds. The treatments utilized are described in table 1. The slides used in the histochemical tests were mounted with glycerinated gelatin.

The controls for the tests for lipophilic substances used an extraction solution composed of chloroform/methanol/water/HCl (66:33:4:1 v/v, High 1984). The samples were immersed in this solution for 48 hours at room temperature and subsequently fixed in FAA or FNT and submitted to the same treatments as the other samples.

To identify the presence of polysaccharides in vegetative colleters, sections of the vegetative apex were fixed in FAA and treated with Ruthenium red (Gregory & Baas 1989) or exposed to the PAS reaction (Periodic acid-Schiff; McManus 1948). The controls for these hydrophilic substances were carried out as described in the specific references.

Photomicrographs were taken using an Olympus BX51 microscope and Kodak Pro Image 100 film. The photomicrographs were digitalized using Adobe Photoshop 9.0 software. The figure scales were obtained by photographing/digitalizing a millimeter scale under the same optical conditions. Measurements of the lengths of the colleters were performed on three vegetative and floral apices from each individual using a camera lucida apparatus. The classification of the colleters followed Lersten (1974).

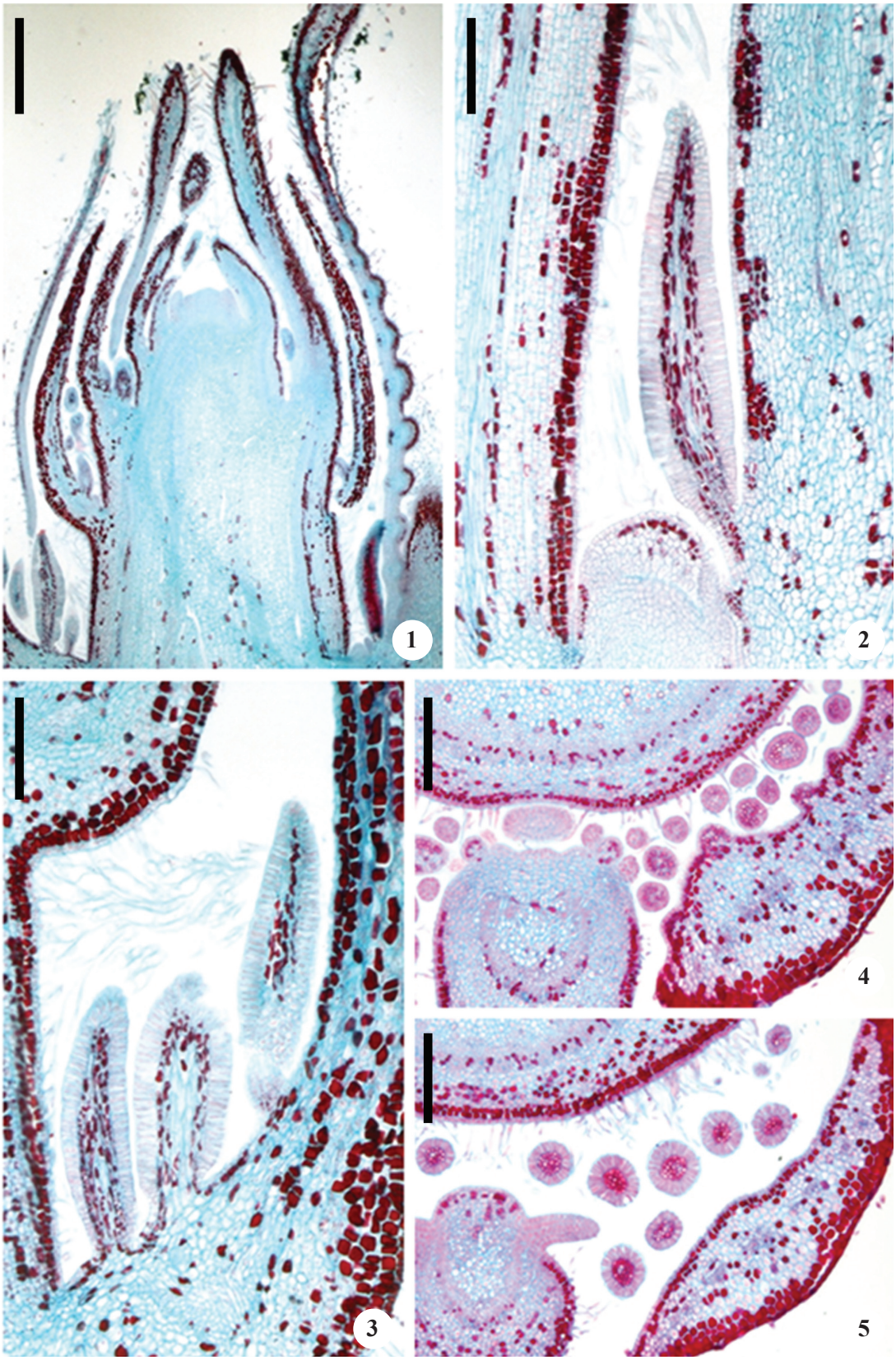
RESULTS

Leaf colleters (figures 1-7)

Vegetative apices approximately 5 mm long and having approximately seven nodes were used to describe the colleters. The colleters were found above the fourth

Table 1. List of the histochemical tests used to identify the principal metabolic compounds secreted by the calycine colleters in *Odontadenia lutea* (Apocynaceae – Apocynoideae – Odontadenieae).

Metabolic group		Reagent
Lipids	Total lipids	Sudan black B (Pearse 1985)
		Sudan IV (Pearse 1985)
	Acidic and neutral lipids	Nile blue sulfate (Cain 1947)
	Fatty acids	Copper acetate/rubeanic acid (Ganter & Jollés 1969)
Terpenes	Essential oils and resin oils	Nadi reagent (David & Carde 1964)
Phenolic compounds	General phenolic compounds	Potassium dichromate (Gabe 1968)
		Ferric chloride (Johansen 1940)
Alkaloids		Wagner reagent (Furr & Mahlberg 1981)
Polysaccharides	Total polysaccharides	PAS reaction (McManus 1948)
	Starch	Lugol reagent (Johansen 1940)
	Acidic mucopolysaccharides	Alcian blue (Pearse 1985)
	Acidic mucilage	Ruthenium red (Gregory & Baas 1989)
	Mucilage	Mayer's reagent (Pizzolato 1977)
Proteins	Total proteins	Coomassie blue (Fisher 1968)
		Bromophenol blue (Mazia et al. 1953)
		Aniline blue-black (Fisher 1968)



Figures 1-5. Longitudinal and transversal sections of the apical and vegetative colleters of *Odontadenia lutea*. 1. General view. 2. Axillary colleters. 3. Inter-petiolar (intra-stipular) colleters. 4-5. Internodal region showing median and apical portions of the inter-petiolar (intra-stipular) and axillary colleters. Bar = 100 µm (1); 50 µm (2-3); 200 µm (4-5).

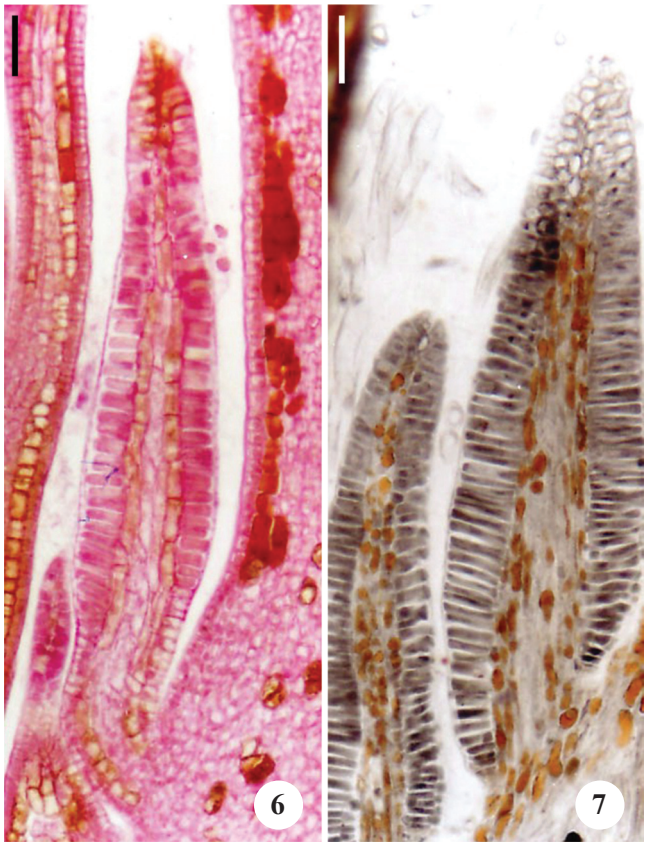
node (figure 1); the origin and type of colleter were similar on all the nodes. Each node (figures 1-5) had 68 to 80 colleters. Each foliar primordium had one axillary colleter, 3-5 intra-petiolar colleters, and 12-16 inter-petiolar (intra-stipular) colleters (figures 2-5). The numbers of intra-petiolar and inter-petiolar (intra-stipular) colleters were found to vary at different nodes along the same apex and among apices of the same individual.

Four types of colleters were seen (table 2): standard (figure 2), bipartite standard, sessile (figure 3), and bipartite sessile (figure 4), and they occupied either axillary (figure 2), intra-petiolar, or inter-petiolar (intra-stipular) positions (figures 3-4). All of the colleter types were composed of a central nucleus of parenchyma cells that was covered by a uniseriate palisade secretory epidermis (figures 2-3). Crystals were observed in the parenchyma of the axillary colleter. Vascularization was only observed in standard type axillary colleters. The bipartite colleter divided after reaching two thirds of its total length, giving rise to two segments that differed only in terms of their dimensions (figure 4).

The palisade secretory epidermal cells had thin cuticle, relatively large nuclei, and dense cytoplasm. The dense cytoplasm in the parenchymatous axis of the colleters and the secretory idioblasts stained strongly with safranin.

The axillary colleter was the largest encountered (standard type) and was approximately 800 µm long. The bipartite and standard intra-petiolar and inter-petiolar (intra-stipular) colleters were at most 420 µm long.

Tests undertaken using Ruthenium red (figure 6) and Mayer's reagent (figure 7) indicated the presence of mucilage within the cells of the palisade epidermis.



Figures 6-7. Longitudinal sections of the vegetative colleters of *Odontadenia lutea*. 6. Ruthenium red. 7. Mayer's reagent. Bar = 200 µm.

Calycine colleters (figures 8-32)

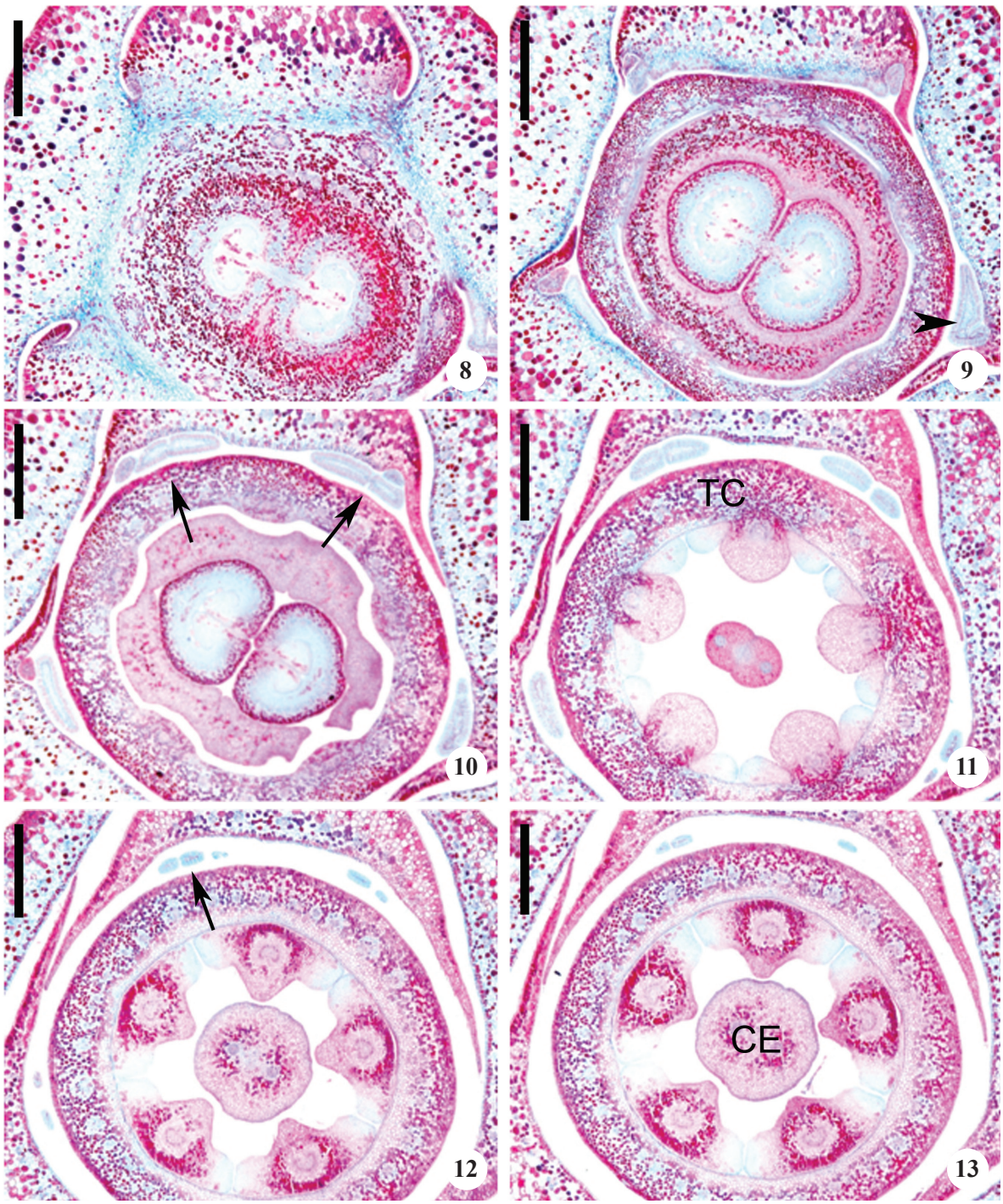
The descriptions and illustrations of the calycine colleters were derived from 15 mm and 30 mm long floral buds. These colleters originate at the base of the calyx. In *O. lutea*, the calyx has five unequal sepals, two

Table 2. Origin, position, and types of vegetative and calycine colleters in *Odontadenia lutea* (Apocynaceae – Apocynoideae – Odontadenieae).

Organ	Origin	Position	Type
Vegetative apex	Axillary	Axillary	Standard (figures 1-2)
	Marginal	Intra-petiolar	Sessile
			Bipartite sessile
			Bipartite standard
		Inter-petiolar	Sessile (figure 3)
		(intra-stipular)	Bipartite sessile
			Bipartite standard (figures 4-5)
Reproductive apex	Marginal	Marginal	Sessile (figures 15 and 23)
			Reduced sessile (figures 14 and 23)
			Tripartite laminar sessile (figures 16 and 24-26)
			Asymmetrical (figures 17 and 21-22)

external, two internal, and one imbricate, characterizing a sinistrorse quincuncinal preflowering. The colleters are found on the edges of the internal sepals and only one colleter was observed on the internal portion of the imbricate sepal. Due to their position on the edges of the sepals, the colleters can be considered alternate-sepalous (figures 8-13).

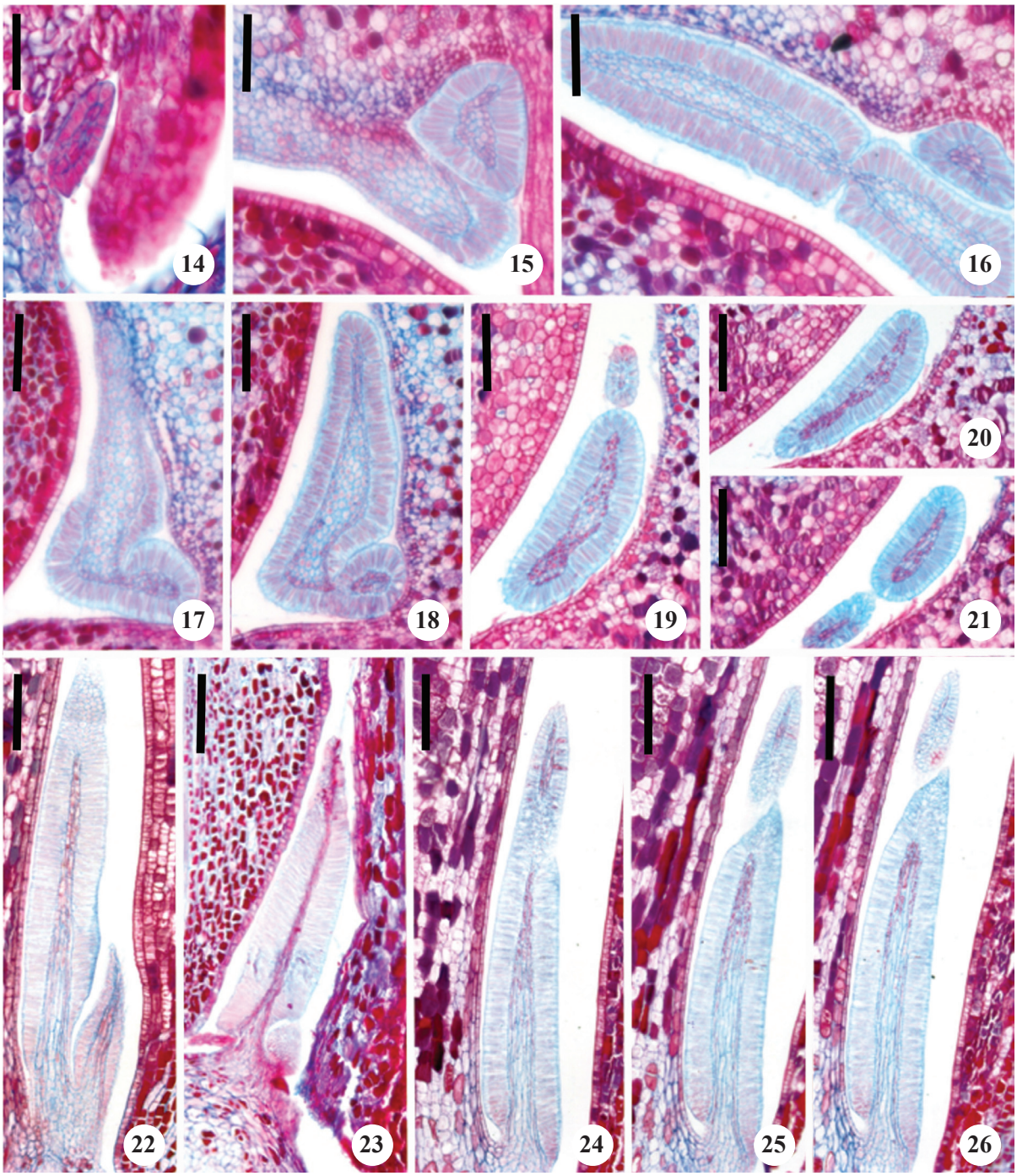
Four types of colleters (table 2) were observed on the calyx, with three types occurring on the internal sepals, and one on the internal portion of the imbricate



Figures 8-13. Transversal sections of the calycine colleters of *Odontadenia lutea*. 8. Colleters in the inner portion of the imbricate sepal. 9. Colleters at the bases of the internal parts of the calyx, asymmetrical colleters. 10-11. Tripartite laminar colleters, first portion. 12. Tripartite laminar colleters, second portion. 13. Terminal portion of a tripartite colleter. (arrow = divisions of the colleters; arrowhead = asymmetrical colleters; CE = style head; TC = corolla tube). Bar = 100 µm.

sepal. Reduced sessile colleters were observed on the edge of the internal sepal (figure 14). The second colleter type was morphologically similar to the first (although larger) and thus designated as sessile (figure 15); the third type of colleter was tripartite laminar sessile (figure 16). The fourth type of colleter occurred on the inner portion of the imbricate sepal and did not have a regular shape, thus being considered asymmetrical (figures 17-21).

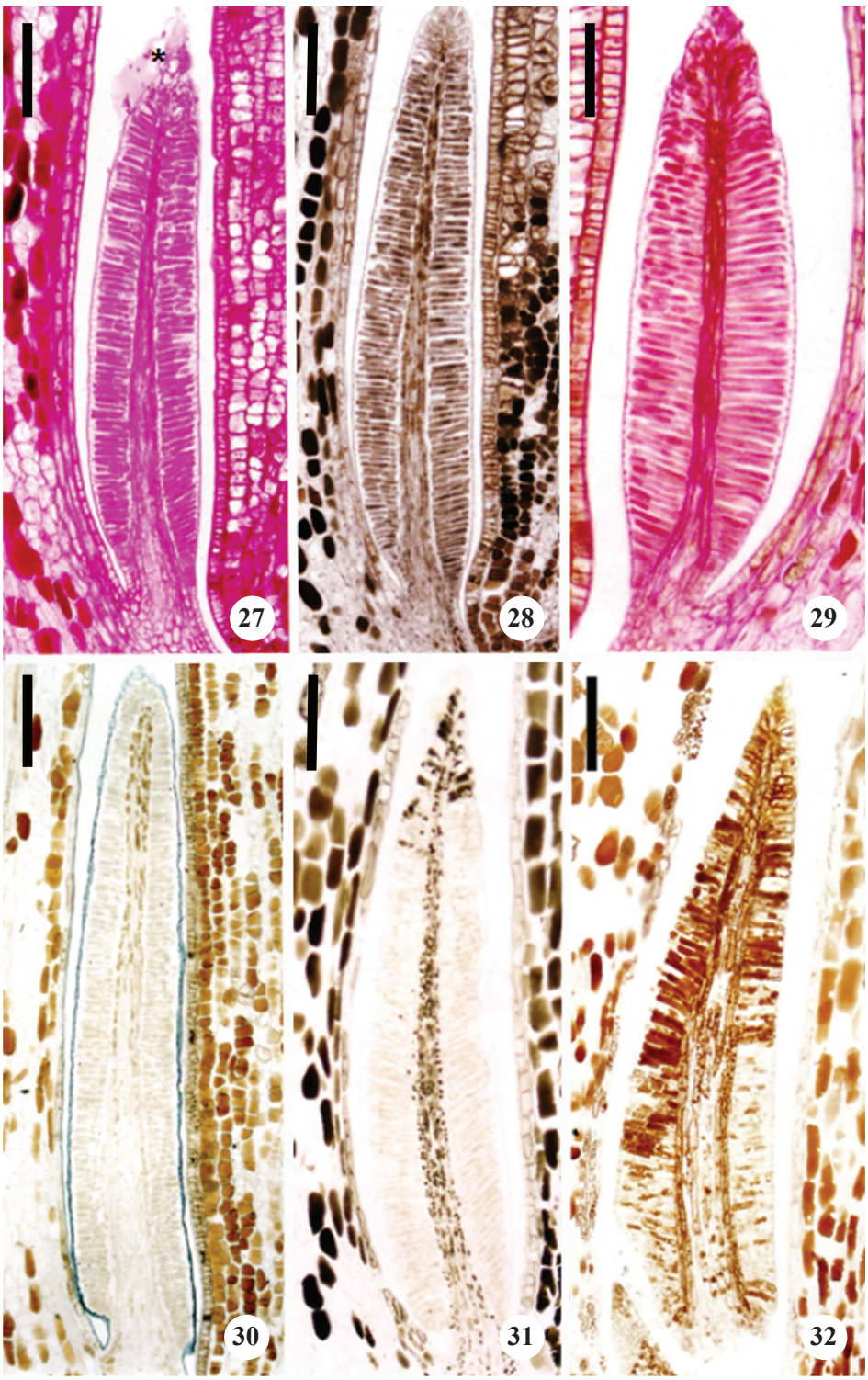
All of the calycine colleters were formed from a nucleus of parenchymatous cells surrounded by a uniseriate secretory palisade epidermis and covered by a cuticle (figures 22-26). The secretory cells had thin



Figures 14-26. Transversal (14-21) and longitudinal (22-26) sections of the calycine colleters of *Odontadenia lutea*. 14. Reduced sessile colleter. 15. Sessile colleter. 16. Tripartite laminar sessile colleter. 17-21. Asymmetrical colleter, note separation of the larger portion into three sections. 22. Asymmetrical colleter. 23. Sessile colleter and reduced sessile colleter. 24-26. Detail of the sections of a tripartite laminar sessile colleter. Bar = 200 µm.

walls, evident nuclei, and dense cytoplasm. Vascular tissue was only observed in laminar colleters.

Histochemical tests indicated that the cuticle did not rupture during secretion (figures 27-32). It was also possible to verify that the cuticle did not become distended, with the secretion apparently accumulating in this space between the external periclinal wall and the protoplast (figure 27). Secretion was visible in the



Figures 27-32. Histochemical tests of the calycine colleters of *Odontadenia lutea* in longitudinal sections. 27. PAS reaction. 28. Mayer's reagent. 29. Ruthenium red. 30. Sudan black B, note a positive reaction only in the cuticle. 31. Ferric chloride. 32. Potassium dichromate. Bar = 200 µm.

interior of the secretory epidermal cells of buds that were 15 mm long, as well as accumulated outside these structures and lubricating the buds in the external medium of the colleters on buds that were 30 mm long.

The results of the histochemical tests designed to detect the presence of water-soluble and lipid-soluble substances are presented in table 3. Mucilage was identified in 15 mm and 30 mm buds by its reactions with PAS (figure 27), Mayer's reagent (figure 28), and Ruthenium red (figure 29). Mucilage is less evident in more developed buds, indicating a decrease (or even a cessation) of its secretion in a later phase. Phenolic compounds were observed using ferric chloride and potassium dichromate (figures 31-32) only in the colleters of buds that were 30 mm long. Calycine colleters thus demonstrated two distinct secretory phases, the first with synthesis of mucilage and a second phase in which phenolic compounds are produced.

Table 3. Results of the histochemical tests applied to calycine and floral bud (15 mm and 30 mm long) colleters in *Odontadenia lutea* (Apocynaceae – Apocynoideae – Odontadenieae) (– = negative; + = positive).

Treatments	Substances to be detected	Results	
		15 mm	30 mm
PAS reaction	Total polysaccharides	+ (figure 27)	+
Mayer`s reagent	Mucilage	+ (figure 28)	+
Ruthenium red	Acidic mucilage	+ (figure 29)	–
Alcian blue	Acidic mucopolysaccharides	–	–
Lugol reagent	Starch	–	–
Coomassie blue	Total proteins	–	–
Bromophenol blue	Total proteins	–	–
Aniline blue-black	Total protein	–	–
Sudan black B	Total lipids	– (figure 30)	+
Sudan IV	Total lipids	+	+
Nile blue sulfate	Acidic and neutral lipids	–	–
Copper acetate/ rubeanic acid	Fatty acid	–	–
Nadi reagent	Terpenes	–	–
Ferrous sulfate in formaldehyde	Total phenolic compound	–	+
Ferric chloride	Phenolic compounds	–	+ (figure 31)
Potassium dichromate	Phenolic compounds	–	+ (figure 32)
Wagner reagent	Alkaloids	–	–

DISCUSSION

Colleters are frequently seen on vegetative and reproductive structures in Apocynaceae (Thomas 1991) and have been reported in 25 genera of the Apocynoideae (Endress & Bruyns 2000).

The genus *Odontadenia* demonstrates a common characteristic for the family – the presence of inter-petiolar stipules – and large numbers of colleters have been observed on these structures. Woodson & Moore (1938) proposed that colleters are derived from stipules in many species of Apocynaceae and they considered colleters as a category of stipules – with the retention of a vascular system, such as seen in *Mandevilla illustris* and *M. pohliana* (Stadelm.) A.H.Gentry (= *M. velutina* (Mart. ex Stadelm. Woodson, Appezzato-da-Glória & Estelita 2000) and in *Prestonia coalita* (Rio et al. 2002). The numerous colleters in *O. lutea* originate on the adaxial face of an inter-petiolar stipule. The occurrence of these colleters on stipules has not been previously described in Apocynaceae and they are therefore denominated intra-stipular colleters.

Variations in the numbers of vegetative colleters have been observed, and occur quite frequently in Apocynaceae (Ramayya & Bahadur 1968, Fjell 1983, Thomas & Dave 1989a, Thomas 1991). According to Thomas (1991), the number of colleters in a single species can vary according to its geographic distribution, indicating that this character is plastic and of little taxonomic value. This large variation in the number of colleters is normal, and reflects the high number of colleters that can occur at a single node.

In terms of the size of the colleters, large variations were seen in their length independent of their origin or position (whether inter- or intra-stipular). Lersten (1975) studied more than 296 species of Rubiaceae from different

continents and often observed considerable differences in the size of colleters on the same species.

A noticeable character encountered in *Odontadenia lutea* was the occurrence of four types of colleters on the same floral bud that developed asynchronously. A similar ontogenetic study undertaken with *Blepharodon bicuspidatum* Fourn. identified the asynchronous initiation of vegetative colleters (Demarco 2005).

Bifurcated colleters have been described from the floral buds of *Temnadenia violacea* (Martins et al. 2010), *Prestonia coalita* (Rio 2001), *Forsteronia* (Rio 2006), *Mandevilla pycnantha* (Steud. ex A.DC.) Woodson, and *M. tenuifolia* (Simões et al. 2006). There is considerable confusion surrounding the terminology utilized to describe colleters, and in many cases tripartite and fimbriate colleters are synonymized. According to Simões et al. (2006), the terminology traditionally used to describe calycine colleters in Apocynaceae is confusing, and adjustments are needed to avoid equivocal interpretations. These authors proposed typologies derived from a standard colleter, using the fundamental mechanisms of cell separation, proliferation, and elongation to describe them. As such, bifurcate and fimbriate colleters originate from the separation of distinct portions of the colleter axis.

The first classification of colleters was undertaken by Lersten (1974), based on the typologies encountered in Rubiaceae – without contemplating any of the forms described for Apocynaceae – which resulted in equivocal interpretations for this and other families. In spite of the proximity of the families Apocynaceae and Rubiaceae, there is an enormous divergence in the types of colleters encountered in each; dendritic colleters are consistently observed in Rubiaceae (Lersten 1974) for example, but are completely absent in Apocynaceae.

The occurrence of different types of colleters on the reproductive and vegetative organs of the same species runs contrary to the theory proposed by Woodson & Moore (1938). These authors assumed that foliar and calycine colleters are structurally homologous and that the alternate-sepal positions of the calycine colleters are similar to those of the vegetative nodes in relation to the petiole. If the stipules of *O. lutea* do in fact correspond to calycine colleters, this would support the theory of Woodson & Moore (1938); however, it is difficult to explain the absence of colleters on the external portion of the imbricate sepal as a structural variation of calycine colleters. It is possible that the occurrence of asymmetrical colleters is related to the fusion of tripartite laminar colleters and reduced sessile colleters.

The presence of vascular tissue associated with colleters has been observed in various species of Apocynaceae and in many cases this is due to the retention of stipule vascularization, as in *Prestonia coalita* (Rio et al. 2002). Vascularized colleters have been reported in *Wattakaka volubilis* Stapf. (Arekal & Ramakrishna 1980), *Aganosma caryophyllata* G. Don (Dave et al. 1987), *Mandevilla illustris,* and *M. pohliana* (Appezzato-da-Glória & Estelita 2000). However, many species of Apocynaceae do not have any vascularized colleters, such as *Allamanda neriifolia* Hook*., Thevetia peruviana* Merr., *Vinca minor* Sm. (Fjell 1983), *Allamanda cathartica* Schrad. (Thomas & Dave 1989a), and *Roupelia grata* Wall. (Thomas et al. 1989). Vascularization in *Odontadenia lutea* occurs only in the axillary colleter of the stem apex and on the laminar colleter of the floral apex. According to Demarco (2005), the presence of vascular tissue can vary among the different types of colleters present on the same organ. Appezzato-da-Glória & Estelita (2000) reported that the inter-petiolar colleters of *Mandevilla* Lindl. were vascularized, while the foliar colleters could be associated with vascular tissue, or not. Woodson & Moore (1938) consider the absence of vascularization as a predominant characteristic of the calycine colleters of Apocynaceae species.

Vascularization was observed among the largest colleters of *O. lutea*. Carlquist (1969) noted that the vascular tissue associated with any structure is directly proportional to its size, and not necessarily related to its developmental state. According to Appezzato-da-Glória & Estelita (2000), the vascularization of petiolar or axillary colleters is independent of their size, while Martins et al. (2010) observed vascularization only among the largest vegetative colleters of *Temnadenia violacea*, corroborating the point of view of Carlquist (1969).

Idioblasts appear to be common features in Apocynaceae, and they have been observed in various organs of the same plant (Metcalfe & Chalk 1950, 1983). As such, the presence of idioblasts in vegetative and calycine colleters in this family is not surprising, as was seen in *O. lutea*. Crystal-containing idioblasts were observed in *Roupelia grata* (Thomas et al. 1989) and *Thevetia peruviana* (Fjell 1983) and tannin-containing idioblasts were reported in *Himatanthus* Willd. ex Roem. & Schult. (Barros 1988), *Mandevilla illustris,* and *M. pohliana* (Appezzato-da-Glória & Estelita 2000) as well as in the vegetative colleters of *Forsteronia glabrescens* Müll. Arg., *F. pubescens* DC. *A*nd *F. thyrsoidea* Müll. Arg. (Rio et al. 2005).

The liberation of accumulated secretions from the periplasmic space to the external environment most likely occurs through the cell wall and cuticle, as no

distention or rupturing of the cuticle was observed. This same manner of exudate liberation was attributed to the vegetative colleters of *Blepharodon bicuspidatum* (Demarco 2005).

Colleters are structures that produce viscose secretions of mucilage (Fahn 1979, Thomas 1991) or mixtures of mucilage and lipophilic substances (Fahn 1979). Thomas (1991) attributed colleters with the function of protecting plant meristems, which is supported by the nature of those secretions. The presence of mucilage on the developing meristem helps to prevent desiccation in a region with a very thin cuticle. Mucilage has been detected in the colleters of *Plumeria rubra* L. (Mohan & Inamdar 1986), *Roupelia grata* (Thomas et al. 1989), *Allamanda cathartica* (Thomas & Dave1989a), *Mandevilla illustris* and *M. pohliana* (Appezzato-da-Glória & Estelita 2000), *Prestonia coalita* (Rio 2001), *Blepharodon bicuspidatum* (Demarco 2005), *Forsteronia glabrescens* (Rio 2006), and *Temnadenia violacea* (Martins et al. 2010)

Mucilage, however, is also attractive to opportunistic organisms such as fungi and bacteria that can cause structural damage to the meristem. In various species of Rubiaceae, nodulation processes provoked by bacteria in apical meristems have been noted (Lersten 1974, 1975), which would indicate an intimate relationship between the host and nodule species, resulting in benefits for both through a symbiotic relationship. One way to avoid deleterious contamination would be to have a second secretion phase with exudates containing phenolic compounds. According to Harbone (1988), phenolic compounds aid in preventing infections in plants provoked by fungi and bacteria. The secretion of phenolic compounds during a second secretory phase has been observed in *Roupelia grata* (Thomas et al. 1989), *Forsteronia glabrescens* (Rio 2006), and *Temnadenia violacea* (Martins et al. 2010). The secretion of mucilage and phenolic compounds occur simultaneously in the foliar and calycine colleters of *Blepharodon bicuspidatum* (Demarco 2005).

The genus *Odontadenia* Benth. A. DC. was recently reassigned by Livshultz et al. (2007) to a new tribe composed of five genera, although only *Secondatia* has been anatomically studied (Simões 2004). This new organization was based on recent molecular information and floral morphological studies, without considering vegetative characteristics or their vegetative and calycine colleters. In describing the floral anatomy of *O. lutea* and *S. densiflora,* Martins (2008) observed morphological characteristics indicating similarities between these two species, although their vegetative and calycine colleters are quite divergent.

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