



## Colleters in monocots: New record for Orchidaceae

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### ABSTRACT

Colleters are widely occurring in eudicots showing relevant taxonomic importance in several families. Nevertheless, there are few records in monocots, restricted to only one description of these glands in Orchidaceae. The genus *Oncidium* is polyphyletic, currently the subject of taxonomic studies. In this context, the secretory structures can be an important diagnostic character that may help in the delineation of this group. *O. flexuosum* Sims presents colleters in vegetative – leaf primordium of protocorms, apical and axillary buds in the mature rhizomes – and reproductive organs – at the base of bracts, bracteoles and sepals. All the colleters observed are finger-like trichomes, composed of two uniseriated cells, where the apical one is elongated and possesses dense cytoplasm. The exsudate accumulates in a subcuticular space, causing displacement of the cuticle. Histochemical tests indicate the presence of mucilage in association with lipophilic and proteinic compounds inside the secretory cell. Secretion is abundant, hyaline and slightly viscous. The localization of the trichomes and their exsudate indicate the involvement of these colleters with the protection of meristematic regions in vegetative and reproductive organs. These results can be useful in the taxonomy of the genus *Oncidium* and for future studies about colleters in monocots.

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### Introduction

Colleters are trichomes or more complex appendages which secrete a sticky substance containing carbohydrate mucilage or a lipophilic substance or both that permeate and cover the buds entirely (Esau, 1977; Fahn, 1979). The functions attributed to this exsudate are to protect the young developing organs against desiccation (Kronstedt-Robards and Robards, 1991), to promote symbiotic associations with bacteria (Lersten, 1975) and to facilitate the sliding of one surface over another during growth (Uphof, 1962). The use of the term ‘colleter’ has varied with different authors because of the criteria used to define these secretory structures, such as morphology, location and the chemical nature of the secretion (Leitão and Cortelazzo, 2008). Nevertheless, the term ‘colleter’ is better used in a functional concept, since only the composition and function of the exsudate are preconditions to their characterization. Structurally, they can be not only trichomes but glandular vascularized structures (Appezzato-da-Glória and Estelita, 2000; Foster, 1949; Rio et al., 2002), and their occurrence is not limited to vegetative buds, occurring also in reproductive organs and seedlings (De-Paula and Oliveira, 2007). According to Radford et al. (1974) for the classification of secretory tissue it is important to use histochemical tests or the chemical analysis of

the secretory product to supplement morphological, structural and developmental characters.

Anatomical data concerning colleters in monocots are scarce. In fact, registers refer to “axillary squamules” that consist on intravaginal or axillary, non-vascularized glands that are small scale-, gland- or finger-like trichomes occurring in pairs or larger numbers in the axils of vegetative leaves that apparently secrete protective mucilage (Dahlgren and Clifford, 1982; and references therein). In Orchidaceae, there is only one record of colleters in bracts of *Rodriguezia venusta* (Lindl.) Rchb.f. (Leitão and Cortelazzo, 2008).

The genus *Oncidium* Sw. *sensu lato* possesses more than 400 species (Chase et al., 2009), including natural and artificial hybrids, which have economic relevance as ornamental flowers (Chen and Chang, 2000). The genus *Oncidium* is clearly polyphyletic, and its delimitation is controversial (Chase, 1986, 1987; Chase and Palmer, 1992; Chase et al., 2009; Félix and Guerra, 2000). Chase et al. (2009) proposed changes to the genus, including *Oncidium flexuosum* Sims in genus *Gomesa*. However, different proposals for grouping have been submitted and inclusion of the species in a new genus is still uncertain (Rosa and Affonso, 2009). We decided to maintain the name of the species as *Oncidium flexuosum* Sims.

*O. flexuosum* (Epidendroideae) is an epiphytic orchid native to Brazil and Argentina (Pabst and Dungs, 1977). In this paper, the colleters of *Oncidium flexuosum* on both vegetative and reproductive organs are considered, in relation to distribution, morphology and the histochemistry of the main secretion compounds. This work presents new records of colleters in different organs of *O. flexuo-*

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sum; the results will be useful in the taxonomy of this genus and in future studies about colleters in monocots.

## Materials and methods

For light microscopy and scanning electron microscopy (SEM), five samples of protocorms, apical and axillary buds of rhizomes of adult plants and inflorescences of *O. flexuosum* were collected. The voucher of the specimen (150303) is deposited in the UEC, Herbarium of the Universidade Estadual de Campinas, Brazil.

In order to obtain the protocorms, mature capsules were maintained in a 2.5% sodium hypochlorite solution for 20 min and washed in sterile distilled water. The capsules were opened in a laminar flow hood and seeds inoculated directly in culture medium containing half-strength macro- and micro-elements of MS salts (Murashige and Skoog, 1962) supplemented with 30 g L<sup>-1</sup> sucrose [mg L<sup>-1</sup>]: myo-inositol (100), niacin (0.5), pyridoxine HCl (0.5), thiamine HCl (0.1) and glycine (2.0). The pH of the media was adjusted to 5.8 with 1 M KOH or HCl prior to autoclaving. Cultures were maintained at 25 ± 2 °C, under irradiance of 40 μmol m<sup>-2</sup> s<sup>-1</sup> and 16 h photoperiod.

For the anatomical study, samples of *O. flexuosum* protocorms, apical and axillary buds of rhizomes of adult plants and inflorescences were fixed in Karnovsky fixative (Karnovsky, 1965; modified by phosphate buffer pH 7.2) for 24 h, dehydrated in a graded ethanol series, and embedded in Leica histo-resin® (Heraeus Kulzer, Hanau, Germany). Serial sections (5 μm thick) were cut on a rotation microtome and stained with toluidine blue O (Sakai, 1973). Permanent slides were mounted in synthetic resin. To determine the chemical nature of the substances found in the colleters, hand-made cuts from fixed and non-fixed material were examined using the following histochemical tests: periodic acid-Schiff's (PAS) reaction for 1,2-glycol groups present in total polysaccharides (McManus, 1948); 5% tannic acid–3% ferric chloride and methylene blue for mucilage (Johansen, 1940; Pizzolato and Lillie, 1973); coriophosphine for pectic materials (Ueda and Yoshioka, 1976); copper acetate–rubeanic acid for fatty acids (Ganter and Jollés, 1969/1970); Sudan black B to identify the aliphatic compounds (Pearse, 1985); Nadi reagent for terpenoids (David and Carde, 1964); 1% Xylidine Ponceau (pH 2.5) for total proteins (Vidal, 1970); Lugol's iodine solution for starch (Jensen, 1962); 10% ferric chloride for phenolic compounds (Johansen, 1940). Standard control procedures were performed simultaneously. Photomicrographs were taken with a Leica® DM LB photomicroscope equipped with a Leica® DC 300F camera. For analysis of Coriophosphine, the microscope was equipped for epi-illumination with an HBO 50 mercury lamp and a Leica® I3 filter, providing excitation (Bandpass filter 450–490 nm) and suppression (Long-pass filter 515 nm).

For scanning electron microscope analysis, samples of *O. flexuosum* protocorms, apical and axillary buds of rhizomes of adult plants and inflorescences were fixed in Karnovsky fixative (Karnovsky, 1965; modified by phosphate buffer pH 7.2) for 24 h, dehydrated in a graded ethanol series and critical point-dried with CO<sub>2</sub> (Horridge and Tamm, 1969). Samples were attached to aluminium stubs and coated with gold (30–40 nm). Then, the samples were examined under a LEO VP 435 scanning electron microscope at 20 kV.

## Results

### Distribution and morphology of the colleters

The colleters of *Oncidium flexuosum* appear as finger-like trichomes (Figs. 2, 4, 6–8) found only during the development of

**Table 1**

Histochemistry of the colleters on vegetative and reproductive organs of *Oncidium flexuosum* Sims.

Metabolites	Staining procedures	Reactivity (Fig.) <sup>a</sup>
Total polysaccharides	PAS reaction	+ (15, 16)
Mucilage	Tannic acid–ferric chloride; methylene blue	+ (17, 18)
Pectic compounds	Coriophosphine	+ (19)
Fatty acids	Copper acetate–rubeanic acid	+ (20)
Aliphatic compounds	Sudan black B	+ (21)
Terpenoids	Nadi reagent	+ (22)
Total proteins	Xylidine Ponceau	+ (23)
Starch	Lugol	–
Phenolic compounds	10% Ferric chloride	–

<sup>a</sup> Positive signs indicate the presence and negative signs the absence of reaction.

young organs (Figs. 1–8) in all organs analyzed here. In the extracellular space, an abundant slightly viscous secretory product is visible by SEM (Figs. 4 and 6).

### Vegetative organs

Thirty to forty days after the inoculation of seeds *in vitro*, the protocorms showed meristematic differentiation in the upper pole. In this region, the colleters were observed on the adaxial side, where they start the secretory phase before the expansion of foliar lamina (Figs. 1–2 and 9–10). In the mature rhizome the colleters were found on leaf primordia of the apical and axillary buds (Figs. 3–4 and 11).

### Reproductive organs

Colleters are present on the adaxial side of bracts and bracteoles (Figs. 5–7 and 12, 13) and sepals (Figs. 8 and 14).

The colleters are composed of two cells, in line, of equal diameter and different length, where the apical cell is longer; a distinction between head and stalk cells is clear (Figs. 9–14). The apical cell of the trichomes presents thin cell walls and the content inside has an emulsion-like appearance (Figs. 10, 11 and 13, 14).

The apical secretory cell develops a subcuticular space formed between the cell wall and the detached cuticle, where the exudate accumulates (Fig. 11). Cuticle rupture was not observed. The exudate can be found on the surface (Figs. 4, 6, 11–12).

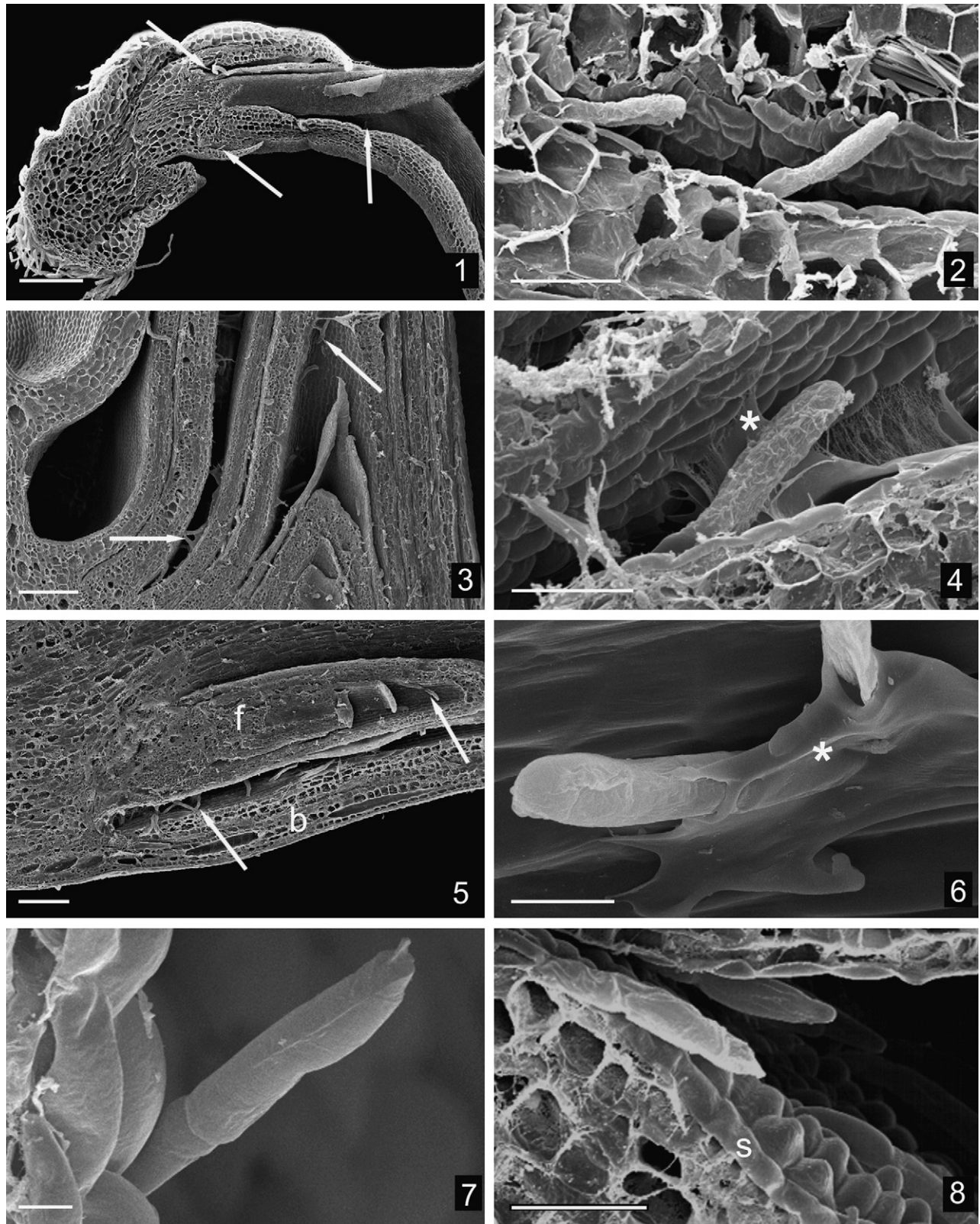
### Histochemistry

The exudate detected on the surface (Fig. 15) consists of hydrophilic substances only (Figs. 15–17) while inside the secretory cell there is mucilage in association with lipophilic and protein compounds (Fig. 16–23). Granules present in the cytoplasm have a positive reaction with lipophilic dye and reagents (Fig. 21). Histochemical test results are summarized in Table 1.

## Discussion

The location, the precocious secretory activity, and the mucilaginous nature of the exudates support the classification of the glandular trichomes of *Oncidium flexuosum* as colleters as these were mentioned by Leitão and Cortelazzo (2008) in *Rodriguezia venusta*.

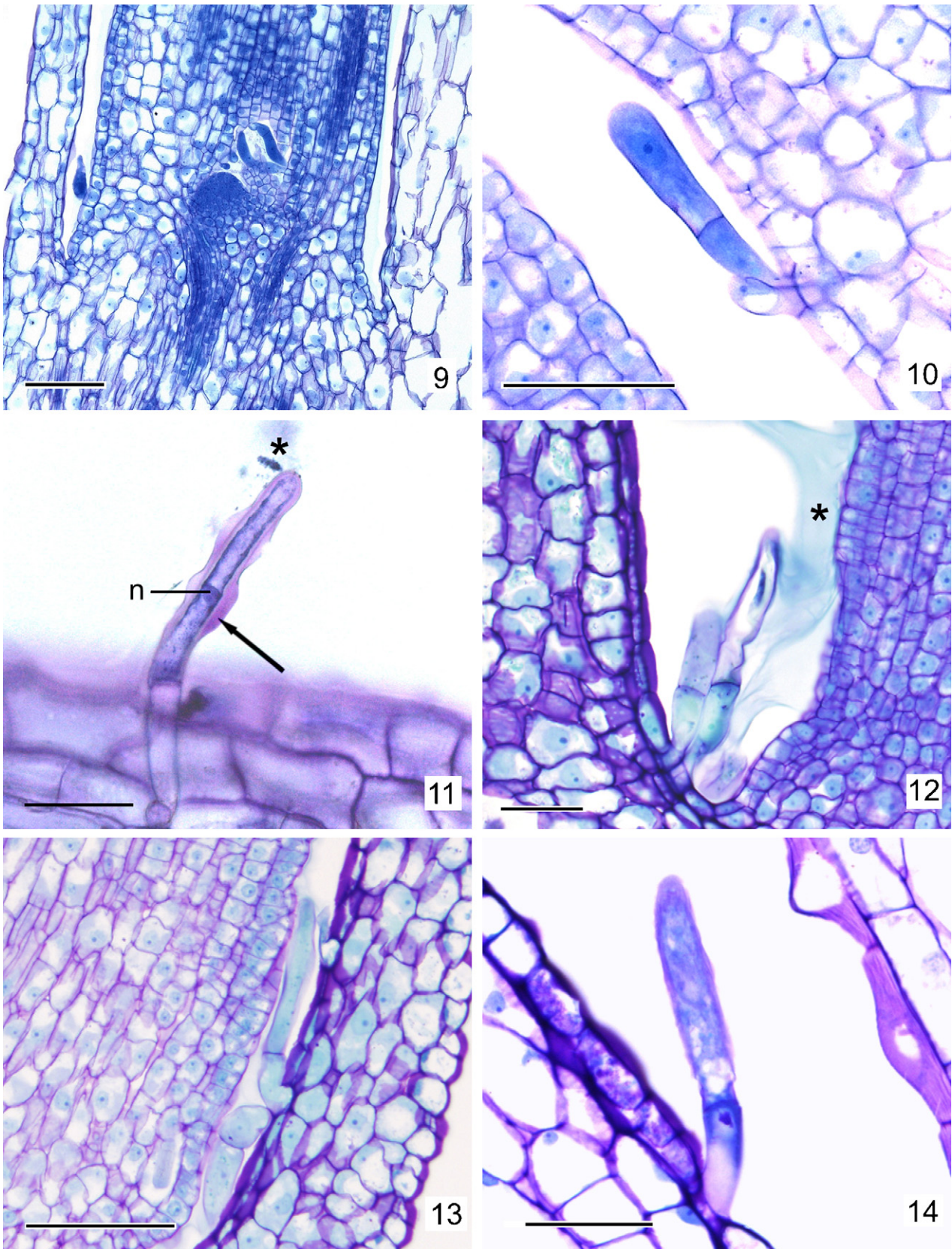
The protocorm arises after the germination of orchid seeds and is considered an intermediary structure between embryo and seedling (Leroux et al., 1997). The occurrence of colleters in this structure is original in monocot; in eudicot there are few records of colleters in seedlings, occurring in the plumule in three species of *Chamaecrista* (De-Paula and Oliveira, 2007), *Nerium oleander* L. (Williams et al., 1982), *Mandevilla illustris* (Vell.) Woodson and *M.*



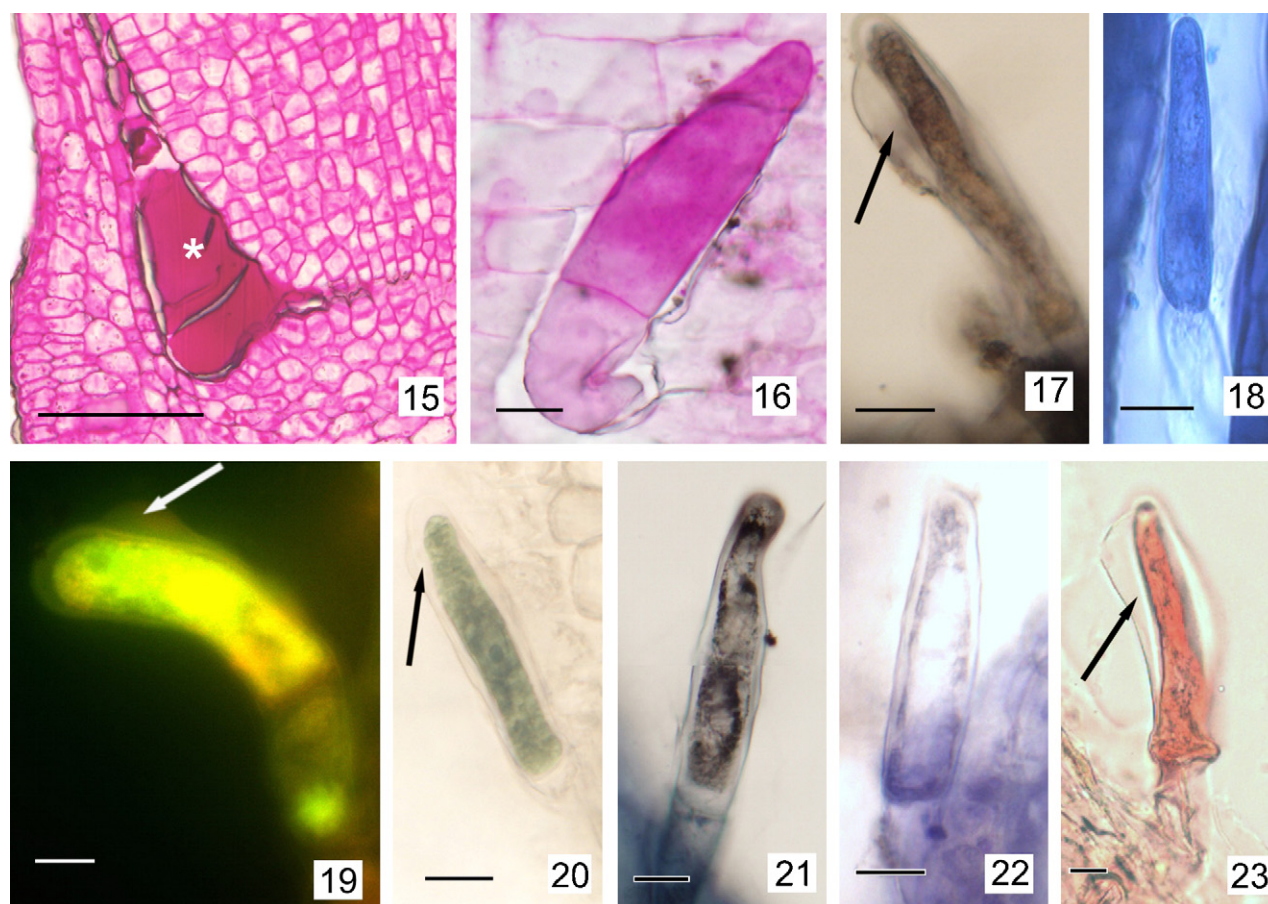
**Figs. 1–8.** Scanning electron micrographs showing distribution of colleters in *Oncidium flexuosum* Sims. (1, 3 and 5) Overview of colleters (arrows). (1, 2) Colleters in the leaf primordium of the protocorm, (3, 4) colleters in the axillary bud of mature rhizome and (5) colleters in the bract (b) and flower bud (f). (6, 7) Details of colleters located on the bract and (8) at the base of sepal (s). (4, 6) Note the secretion (\*) on the surface. Scale bars = 10  $\mu\text{m}$  (7), 20  $\mu\text{m}$  (6), 30  $\mu\text{m}$  (4), 50  $\mu\text{m}$  (2, 8), 200  $\mu\text{m}$  (3, 5) and 500  $\mu\text{m}$  (1).

*velutina* K. Schum. (Apezatto-da-Gloria and Estelita, 2000). The presence of colleters in seedlings or equivalent may constitute an adaptation to germination under adverse conditions (De-Paula and Oliveira, 2007).

According to Esau (1977), colleters may be trichomes or complex appendices. Trichomes have been described in *Hibiscus tiliaceus* L. and *Hibiscus pernambucensis* Arruda (Malvaceae) (Rocha et al., 2002), in *Hymenaea stigonocarpa* Mart. Ex Hayne (Fabaceae: Cae-



**Figs. 9–14.** Longitudinal sections of finger-like trichomes of *Oncidium flexuosum* Sims. (9, 10) Colleters on the leaf primordia of the shoot apex of the protocorm, (11) apical bud of the mature rhizome, (12) bract, (13) bracteoles and (14) sepal. (11, 12) Note the extracellular secretion (\*) and (11) cuticle displacement and subcuticular space filled with secretion (arrow). Scale bars = 25  $\mu\text{m}$  (12, 14), 50  $\mu\text{m}$  (10, 11, and 13) and 100  $\mu\text{m}$  (9). n = nucleus.



**Figs. 15–23.** Histochemical characterization of the secretions of *Oncidium flexuosum* Sims. colleters. (15, 16) Fixed material. (17–23) Fresh material. (15, 16) PAS reaction; note the extracellular secretion (\*). (17, 19–20 and 23) Colleters in the secretory stage; note the cuticle displacement and subcuticular space (arrows). Staining: (17) Tannic acid–ferric chloride. (18) Methylene blue. (19) Coriphosphine. (20) Copper acetate–rubeanic acid. (21) Sudan black B. (22) Nadi reagent. (23) Xylidine Ponceau. Scale bars = 10  $\mu\text{m}$  (16, 17, 19–23), 25  $\mu\text{m}$  (18) and 50  $\mu\text{m}$  (15).

salpinoideae) (Paiva and Machado, 2006b), in *Lithraea molleoides* Engl., *Tapirira guianensis* Aubl., *Spondias dulcis* Parkinson and *Anacardium humile* A. St.-Hil. (Anacardiaceae) (Lacchia, 2006) and in *Rodriguezia venusta* (Orchidaceae) (Leitão and Cortelazzo, 2008).

Cuticle rupture was not observed in the present study. The trichomes of *O. flexuosum* probably release the exsudate via micropores, as observed in capitate trichomes that produce polysaccharides in *Plectranthus ornatus* Codd (Ascensão et al., 1999).

The structural aspects of trichomes provide some information about the chemical nature of the gland, due to the phenomena of metachromasia attributed to the toluidine blue (Kiernan, 1990): in the trichomes present in the apical shoot bud of the rhizome (Fig. 11), the secretion found in the subcuticular space presents a purple color, evidence of mucilage; however, the extracellular secretion found in the bracteoles presents a blue color (Fig. 12), as found in the bracteal trichomes of *Rodriguezia venusta* by Leitão and Cortelazzo (2008). According to these authors, the metachromatic reaction with toluidin blue resulting in a blue color probably occurs due to small quantities of galacturonic acid and other pectins present in the exsudate. These results suggest that colleters can show a different composition of mucilage according to the organs of trichomes that are present. Furthermore, the differences in staining of the secretion may be also due to the stage of the colleters activity.

Exsudate composed of mucilage and lipophilic substances was described for other families of eudicots, like Apocynaceae (Thomas

and Dave, 1989), Rubiaceae (Barreiro and Machado, 2007) and Caryocaraceae (Paiva and Machado, 2006a). In *Mandevilla illustris* and *M. velutina* (Appezato-da-Gloria and Estelita, 2000), the colleters secrete mucilage during the greater part of secretory activity, and lipophilic compounds only just before senescence. The occurrence of heterogeneous exsudate may be explained by the function presented by colleters: the hydrophilic fraction lubricates the organs in development (Kronstedt-Robards and Robards, 1991), while the lipophilic fraction may act as a deterrent against microorganisms. The histochemical tests indicated the presence of mucilage, lipophilic substances and protein inside the secretory cell of the colleters of *O. flexuosum* and the presence of mucilage in the exsudate. Fahn (1979) defined that the concept of secretion should exclude substances stored as an energy source or for subsequent remobilization.

A protection mechanism can be attributed to the colleters of *O. flexuosum*, since the species is epiphytic, exposed to environmental variations, and the secretory phase occurs before the expansion of leaf primordia that cover the meristematic regions of protocorms, of apical and axillary buds of the mature rhizome and of bracts, bracteoles and base of the sepals. The number, distribution, morphology and structure of colleters presents taxonomic significance (Simões et al., 2006). Based on the current problems in the circumscription of the genus *Oncidium sensu lato*, we suggest the investigation of the presence of these glands in other species of this genus in order to use this structure as a taxonomic character.

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