



Comparative survey of secretory structures and floral anatomy of *Cohniella cepula* and *Cohniella jonesiana* (Orchidaceae: Oncidiinae). New evidences of nectaries and osmophores in the genus

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Abstract

The morpho-anatomical structure of nectaries, osmophores, and elaiophores, and the anatomical and micromorphological features of floral pieces of *Cohniella cepula* Hoffmans. and *Cohniella jonesiana* Rchb.f. were comparatively analyzed. In both species, bracteal and sepal nectaries are structured, i.e., they present a secretory epidermis, secretory parenchyma, and vascular bundles. Nectar secretion is released through stomata. The anatomical and micromorphological traits are similar in both nectaries, which can be detected only if the nectar drops are secreted. Considering the location of these nectaries, the secreted nectar would not be a reward to pollinators. Osmophores are located at the base of both callus and laterals lobes, and consist of a layer of secretory epidermis composed of quadrangular cells and papillae. Elaiophores are found on the callus of the labellum and are of the epithelial type. The anatomical features of floral pieces are similar in both species. The anatomical analysis of sepals and petals showed a few differences, which could be of potential taxonomic value. Our results contribute valuable and novel information for the knowledge of these species and the genus, which will be useful in future taxonomic evaluations.

Keywords Anatomy · *Cohniella* · Elaiophore · Micromorphology · Nectary · Osmophore

Introduction

Cohniella Pfitzer belongs to the Epidendroideae subfamily (considered the most advanced in the family), Cymbidieae tribe, and Oncidiinae subtribe (Dressler and Dodson 1960). Oncidiinae is one of the most diverse subtribes of Orchidaceae, with a wide range of floral and vegetative

morphologies. The subtribe includes the greatest diversity of pollination systems and the widest range of chromosome numbers known for Orchidaceae (Neubig et al. 2011). *Lophiarella* Szlach., Mytnik and Romowicz, *Lophiaris* Raf., *Trichocentrum* Poepp. and Endl., and *Cohniella* form the *Trichocentrum* clade (Cetzal-Ix et al. 2013a).

Cohniella includes 22 species in five complexes and two natural hybrids (Cetzal-Ix et al. 2016) that are distributed from northern Mexico to southeastern Brazil and northern Argentina (Cetzal-Ix and Carnevali Fernández-Concha 2010). The species of the genus *Cohniella* differ from other members of Oncidiinae in the presence of relatively small and sub-spherical pseudobulbs with succulent and terete leaves, and *Oncidium*-like flowers (Carnevali Fernández-Concha et al. 2010).

Orchid flowers attract their pollinators using visual or olfactory cues, or through rewards such as nectar, oils, and scents (van der Pijl and Dodson 1966; Dressler 1990). However, very often these plants offer no reward and are pollinated by deceit (van der Pijl and Dodson 1966; Nilsson 1992; Singer and Cocucci 1999; Soliva and Widmer 2003; Cozzolino and Widmer 2005).

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Secretory structures

Nectar is the floral food-reward most frequently found in orchids (van der Cingel 2001; Davies and Stpiczyńska 2008). However, the presence of nectar was reported in a few representatives of Oncidiinae, such as the genus *Leochilus* Knowles and Westc. (Chase 1986), *Comparettia falcata* Poepp. and Endl. (Rodríguez-Robles et al. 1992), *Rodriguezia bahiensis* Rchb.f. (Carvalho and Machado 2006), *Symphylglossum sanguineum* (Rchb. f.) Schltr. (Stpiczyńska and Davies 2006), *Capanemia therezae* Bar. Rodr. (Buzatto et al. 2012), *Rodriguezia venusta* Rchb. f. (Leitão et al. 2014), and *Comparettia coccinea* Lindl. (Pansarin et al. 2015). In this subtribe, nectar is usually found in a nectariferous spur. However, nectar deceit is more common and the presence of a spur does not always ensure the presence of nectar (Neubig et al. 2011), as in some members of *Trichocentrum* s.s. that have a non-functional hollow spur (van der Cingel 2001).

Along the years, there have been numerous criterias to classify nectaries. Caspary (1848) proposed a topographical distinction, recognizing floral nectaries (on the flowers) and extrafloral ones (on vegetative organs). Delpino (1868–1875) established a functional classification, grouping them into nuptials (related to the pollination process) and extranuptials (not related to pollination). In a more recent classification, Schmid (1988) followed a positional criteria and recognized reproductives and extra-reproductives nectaries. The first placed in parts of inflorescences, flowers, or fruits, and the second on vegetative organs. This author also distinguished a secondary category considering the organ in which the nectaries are located in the flower (Bernardello 2007). According to Bernardello (2007), the most practical scheme to categorize nectaries is that proposed by Schmid (1988).

Some flowers emit a fragrance that may or may not be associated with pollinator attraction. The fragrance is produced by glands called osmophores and is mainly composed of volatile oils (Vogel 1963, 1990). According to Proctor et al. (1996), floral odor is responsible for long-distance attraction, whereas nectar is the principal floral reward. Osmophores may be located on the adaxial surface of sepals, petals, or part of the lip (Dressler 1993), and are usually composed of a single layer of epidermal cells, secretory papillae, or trichomes (Curry et al. 1991; Endress 1994; Ascensão et al. 2005; Cseke et al. 2007; Melo et al. 2010; Kowalkowska et al. 2015). In Oncidiinae, the presence of osmophores has been recorded only in a few representatives, such as *Capanemia* Bar. Rodr. Buzatto et al. 2012), *Ionopsis* Kunth (Aguilar 2014), and *Gomesa* R. Br. (Pansarin et al. 2016).

According to Neubig et al. (2011), most Oncidiinae species have flowers that produce oil as a reward. Floral oils are secreted by structures known as elaiophores. Vogel (1974) distinguished two types of elaiophores: trichomal and epithelial.

Trichomal elaiophores are composed of secretory hairs and epithelial elaiophores consist of one layer of secretory epidermis and layers of sub-secretory parenchyma, and frequently occur on the callus of the labellum (Singer and Cocucci 1999; Singer et al. 2006). The presence of elaiophores has been confirmed in representatives of several genera of Oncidiinae, such as *Gomesa*, *Lockhartia* Hook., *Oncidium* Sw., *Ornithocephalus* Hook., *Phymatidium* Lindl., and *Trichocentrum* (Singer and Cocucci 1999; Flach et al. 2004; Reis et al. 2000, 2006; Stpiczyńska et al. 2007; Stpiczyńska and Davies 2008; Pácek and Stpiczyńska 2007; Aliscioni et al. 2009; Pansarin and Pansarin 2011; Pácek et al. 2012). Recently, Gomiz et al. (2017) have described the presence of elaiophores and their structure in *Trichocentrum jonesianum* (Rchb. F.) M. W. Chase and N. H. Williams (= *Cohniella jonesiana*) and *Trichocentrum cebolleta* (Jacq.) M. W. Chase and N. H. Williams (= *Cohniella cepula*), along with three *Gomesa* species and *Grandiphyllum divaricatum* (Lindl.) Docha Neto.

Despite all this evidence, the general consensus is that many Oncidiinae offer no reward, but that they deceive their pollinators (Chase et al. 2009). However, as already mentioned, different research works have shown that several members of Oncidiinae produce some kind of substance to reward their pollinator, such as oils and nectar, or fragrances to attract it.

Floral anatomy

Some studies focused on foliar anatomy within Oncidiinae (Sandoval et al. 2003; Stern and Carlswald 2006; Sandoval et al. 2010; Cetzal-Ix et al. 2013b), which include representatives of *Cohniella*. However, there are no records about the floral anatomy of this genus. Within Oncidiinae, this kind of studies is mainly related to floral pieces involved with secretory structures such as elaiophores and nectaries (Stpiczyńska 2003; Stpiczyńska et al. 2005; Buzatto et al. 2012; Gomiz et al. 2013; Stpiczyńska et al. 2013; Leitão et al. 2014; Gomiz et al. 2017).

Anatomical features may be important to understand phylogenetic relationships. Some authors have considered that morpho-anatomical features are not informative because they present high levels of homoplasy and generate low support for the clades in the phylogenetic analyses (Sibley and Ahlquist 1987; Scotland et al. 2003). However, other authors use these characters and combine them with DNA sequences to elucidate relationships at the generic level (Sandoval et al. 2010; Noguera-Savelli and Jáuregui 2011).

The aims of this comparative study between *C. cepula* and *C. jonesiana* were to: (a) analyze the morpho-anatomical structure of nectaries; (b) confirm the presence of osmophores and their location, and to describe their structure and anatomy; and (c) describe the micromorphology and anatomy of floral pieces in these species, identifying their individual features.

Moreover, the study of elaiophores was included in order to contemplate all secretory structures present in both species. The data about secretory structures will make an important contribution to *Cohniella*, since very little is known about these structures for this genus. In addition, these epiphytic orchids are the only natives species in Argentina (Zuloaga and Belgrano 2018) and widely used as ornamental.

Materials and methods

Plant material

Fresh flowers of *C. jonesiana* and *C. cepula* were obtained from plants of the first author's own collection, and from plants cultivated at the orchid's greenhouse at the Faculty of Agricultural Sciences, Northeastern National University, and at the "Orquisan" orchid nursery from Villa Ocampo, Santa Fe (National Route N° 11, Km 873). The original plants were derived from "in vitro" culture and from a specimen located in the locality of Leandro N. Alem, Misiones. Flowers at anthesis stage were fixed in formalin, acetic acid, and alcohol (FAA) for anatomical and scanning electron microscopy (SEM) examination. Reference vouchers were deposited in the Herbarium of the Institute of Botany of the Northeast, Corrientes (CTES).

***Cohniella cepula* ARGENTINA.** Corrientes province, Capital department, locality of Corrientes, specimen cultivated on a greenhouse, 4/01/2017, *Kettler* 1.

***Cohniella jonesiana* ARGENTINA.** Corrientes province, Capital department, locality of Corrientes, specimen cultivated on the greenhouse of Ing. Agr. E. Flachsland (FCA-UNNE), 26/01/2017, *Kettler* 2. Santa Fe province, General Obligado department, locality of Villa Ocampo, young specimen cultivated at "Orquisan Orquídeas" nursery. 7/03/2018, *Kettler* 3.

Light microscopy

For preparing permanent microscope slides, the fixed material was processed by dehydration through an ethanol series with a pre-impregnant rinsing of tertiary butyl alcohol (Gonzalez and Cristóbal 1997) and infiltration in paraffin Histoplast® (Biopack, Buenos Aires, Argentina) according to Johansen (1940). Floral pieces were sectioned transversely and longitudinally (15–18 µm thickness) with a rotary microtome; the sections were stained with Astra Blue-Safranin (Luque et al. 1996) and mounted with synthetic Canada balsam (Biopur, Buenos Aires, Argentina). Slides were examined under a Leica DMLB2 (Leica, Wetzlar, Germany) binocular microscope equipped with a digital camera.

Scanning electron microscopy

Floral pieces were first dehydrated through a series of increasing ethanol solutions. Then, critical point dried with solvent-substituted liquid carbon dioxide and coated with a thin layer of gold-palladium. Finally the samples were examined with a JEOL 5800 LV SEM, operating at 20 kV.

Histochemical tests

To localize osmophore areas, fresh flowers of both species were submerged by 10 min in Neutral Red dye, which reacts with tissue and stains it pink to red (D'Ambrogio de Argueso 1986). The material was examined under Leica MZ6 (Leica, Wetzlar, Germany) binocular stereomicroscope equipped with a digital camera.

To detect the presence of lipids, fresh flowers of both species were processed in the same way with the saturated alcoholic Sudan III solution. Oils were identified by contact with dye, which is specific for lipids and gives a reddish coloration to oils if present (D'Ambrogio de Argueso 1986).

Analysis of nectar

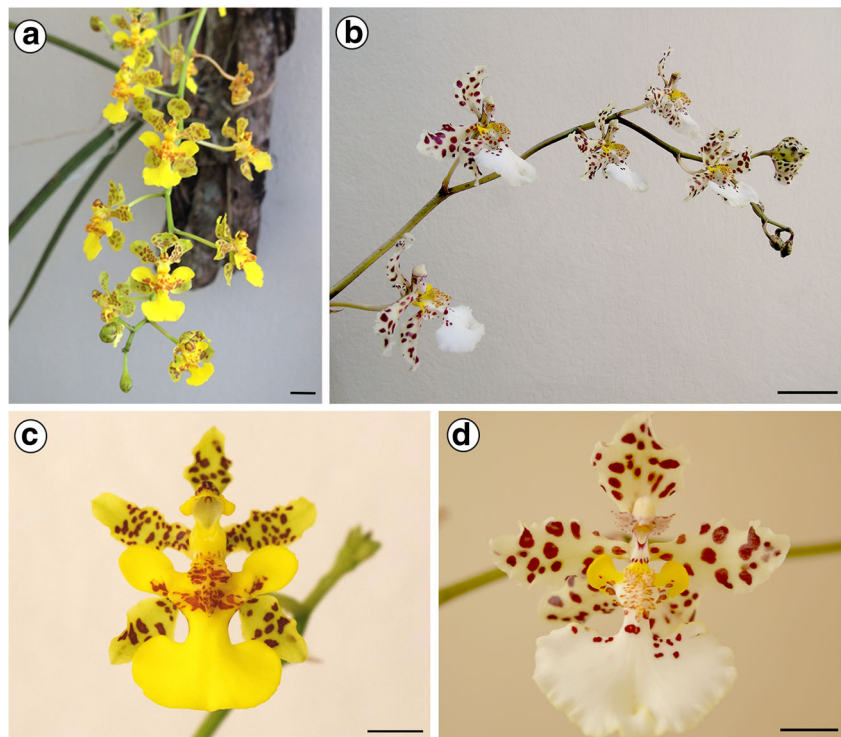
In order to confirm that the drops secreted at the base of the bracts and sepals corresponded to nectar, we measured the sugar concentration (percent sucrose w/w) with a hand refractometer Arcano, REF 103 (range 0–32%). Samples of nectar of FN and EFN were obtained of different flowers in both species and in different times of the day. Dilutions were performed as necessary to keep the concentration readings within the range of the refractometer.

Results

Floral morphology

Inflorescences of both species are larger than leaves, racemiform, and bear resupinate flowers, which open more or less simultaneously (Fig. 1a, b). Flowers in *C. cepula* are yellow and small, of 2–2.5 cm in length. The labellum is three-lobed, with the mid-lobe a little larger than the two lateral ones. Sepals and petals are shorter than the labellum, and are pale yellow with red-brown spots. Callus is composed of three smooth keels or teeth, without protrusions. The column is solid, with two lateral wings, perpendicular to its axis. Tabula infrastigmatica is well developed, deeply bilobed, cleft, and shiny, grooved at the base of the stigma and ventrally flat (Fig. 1c). Flowers in *C. jonesiana* are showy and large, 3.5–4 cm in length. The labellum is three-lobed with a mid-lobe larger, white with reddish spots and two small, yellow lateral ones. Sepals and petals are almost as long as the labellum, pale

Fig. 1 Floral morphology. **a, c** *Cohniella cepula*. **b, d** *C. jonesiana*. **a, b** Detail of inflorescences. **c, d** Detail of flowers. Scale bars: **a, b** = 2 cm; and **c, d** = 5 mm



yellow-green with widely separated red-brown spots. Callus is composed of five keels or teeth, and a denticulate surface. Column is elongated with two lateral wings, parallel to its axis. Tabula infrastigmatica is well developed, shiny, ventrally grooved, and slightly elongated (Fig. 1d). Both species share the pollinarium with two pollinia (Fig. 6a–c).

Nectaries

General aspects

Following Schmid's scheme, *C. cepula* and *C. jonesiana* present two types of reproductive nectaries: bracteal nectaries (RBNs) and floral nectaries (RFNs). According to Delpino, these nectaries correspond to extranuptial type (not related to pollination). The RBNs are located at the base of the bracts, which subtend the flowers. The base of the bract is thicker and the remaining part is thinner and somewhat transparent in both species. The presence of a drop of nectar allows us to identify the nectary; without a drop, the nectary would be indistinguishable (Fig. 2a). The RFNs are located at the base of the sepals, on the abaxial surface (Fig. 2b, c), so they may be called sepal nectaries (hereafter, we will refer to them as RSNs). As in the RBNs, the presence of the RSNs may be detected only with the drop secretion; otherwise, it would go unnoticed. Nectar secretion on RBNs occurs very early in the development of the inflorescence, as in *C. cepula*, where the drop secretion can be observed even before the floral buds begin to appear. On RSNs, nectar secretion starts between

the third and fifth day before anthesis. In both nectaries, the visit of small ants feeding on the drops was often observed (Fig. 2d).

Light microscopy

RBNs

The nectary in both *C. cepula* and *C. jonesiana* has a similar anatomical structure. A secretory tissue was evidenced in the cross section, and it is composed of a layer of epidermal cells and several layers of underlying secretory parenchyma consisting of cells with a dense and granular cytoplasm, an evident nucleus, and one or two nucleoli. Secretory parenchyma and subjacent parenchyma contain idioblasts with raphidies (Fig. 2e).

The nectary epidermal cells are more or less isodiametric and have a thin cuticle. The secretory activity on the epidermis is present only at the base of the bract, with the rest of the bract presenting epidermal cells without granular content. The presence of modified stomata (nectarostomata) covered by a thick cuticle is noticeable. The substomatal chamber is small and the cells close to it limit intercellular spaces that are wider than those between the more distant cells (Fig. 2f). The vascular bundles that supply the bract consist of phloem and xylem. Some of them were found in direct contact with the secretory parenchyma (Fig. 2g). In *C. jonesiana*, the vascular bundles associated with nectary parenchyma appear surrounded by fibers.

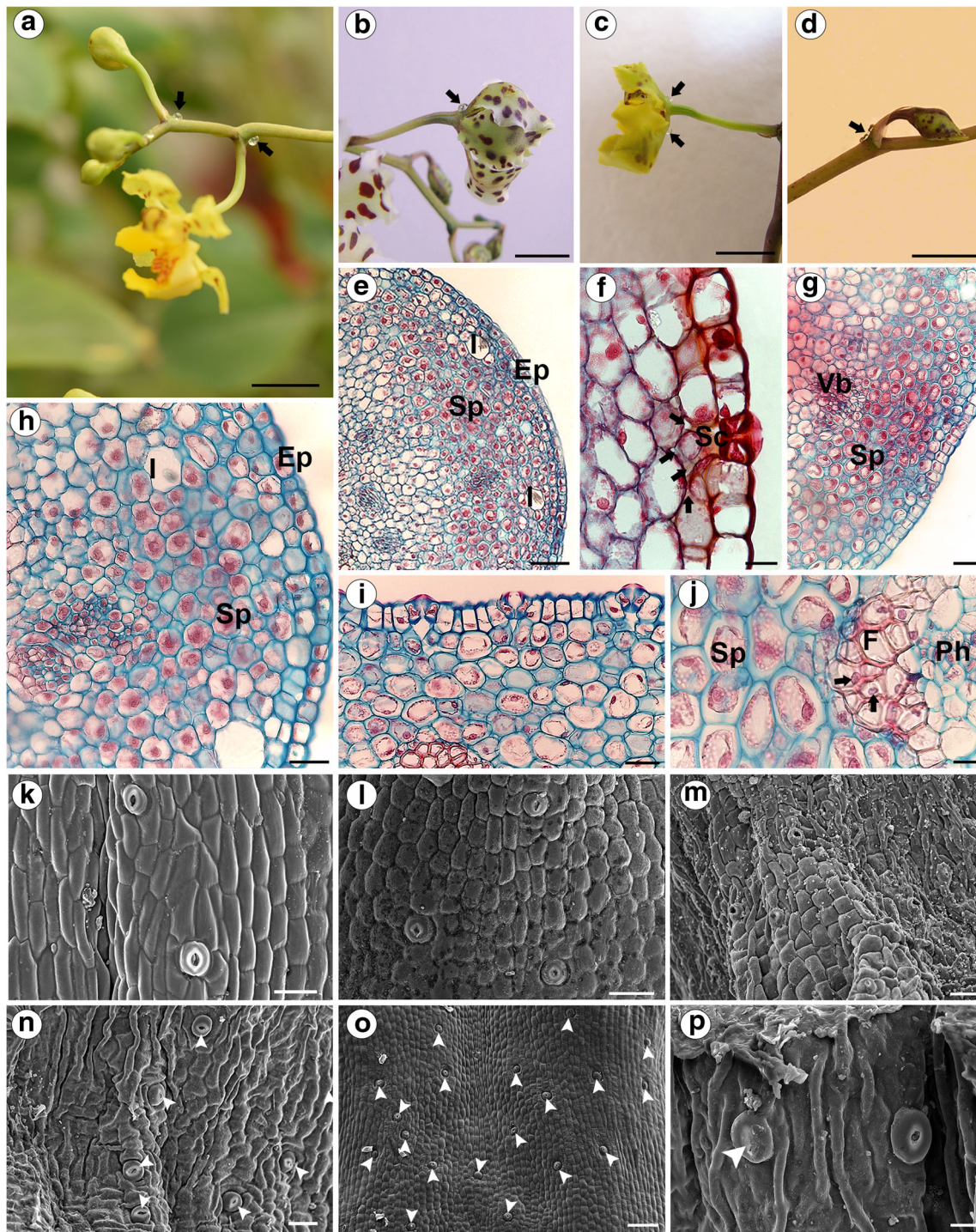


Fig. 2 Morphoanatomy and micromorphology of RBNs and RSNs. **a, c, e, g, h, i, l, o** *C. cepula*. **b, d, f, j, k, m, n, p** *C. jonesiana*. **a-c** BRNs (**a**) and FRNs (**b, c**) with the presence of drops of nectar (arrows). **d** Detail of small ants (arrow) feeding of nectar on the BRNs. **e-j** Light photomicrographs of BRNs (**e-g**) and RSNs (**h-j**). **e** Cross section showing the secretory tissue and idioblasts with raphidies. **f** Detail of the nectarostoma with the small substomatal chamber and the wide intercellular spaces (arrows) between the surrounding cells. **g** Detail of a vascular bundle in contact with secretory parenchyma. **h** Cross section showing details of the secretory tissue. **i** Nectarostomata associates to nectar secretion on the base of sepal. **j** Detail of alive fibers, note the

evident nuclei (arrows). **k-p** SEM photomicrographs of RBNs (**k-m**) and RSNs (**n-p**). **k, l** General aspect of the epidermis with subrectangular (**k**) and polygonal (**l**) cells. **m** Detail of residual of nectar secretion above epidermal cells. **n, o** Nectarostomata (arrowheads) distribute all over the surface of the nectaries. **p** Detail of nectarostoma with pore completely blocked by the cuticle (arrowhead). *Ep* epidermis, *F* fiber, *I* idioblast, *Ph* phloem, *Sc* substomatal chamber, *Sp* secretory parenchyma, *Vb* vascular bundle. Scale bars: **a** = 5 mm; **b-d** = 1 cm; **e, o** = 100 μ m; **f** = 25 μ m; **g, h, l, k, l, m, n** = 50 μ m; **j** = 15 μ m; and **p** = 20 μ m

RSNs

The anatomical structure is similar in both species, and is comparable to the anatomical structure of RBNs. Epidermal cells and the underlying parenchyma cells present dense granular cytoplasm, large nuclei, and evident nucleoli (Fig. 2h). The presence of idioblasts containing raphidies is frequent. The remainder of the mesophyll is homogenous, with irregularly shaped cells. As in the RBNs, we have frequently observed nectarostomata that would be associated with nectar secretion (Fig. 2i). The vascular bundles found in the nectary correspond to sepal vascularization. In *C. jonesiana*, the vascular bundles have fibers below the phloem and in some cases above the xylem. In addition, some of these fibers are alive and their nuclei can be observed (Fig. 2j).

Scanning electron microscopy of RBNs and RSNs

Although the features are similar in both types of nectaries, there are some differences between species. Epidermal cells are subrectangular in *C. jonesiana* (Fig. 2k) and polygonal in *C. cepula* in the surface view (Fig. 2l). In both species, the cuticle is smooth and residuals of nectar secretion on the surface are observed (Fig. 2m). Nectarostomata distributed all over the surface of the nectaries are present in both species (Fig. 2n, o). Their pores are normally open and some of them are blocked by the cuticle (Fig. 2p). Stomata are situated at the same level of neighboring cells in both species.

Analysis of nectar

Sugar concentration (percent sucrose w/w) was similar in both species and did not show important variations at the different moments of the day and between nectaries (personal observation). Average nectar concentration was 23.1 and 18.56% for RBNs and RSNs, respectively, in *C. cepula* and 17.6 and 20.12% for RBNs and RSNs, respectively, in *C. jonesiana*.

Osmophores

Neutral red reaction

The flowers of both species present a softly perceptible, sweetish fragrance, which is quite softer in *C. cepula*. Tests with neutral red were positive, indicating the presence of a secretory tissue of volatile substances in the upper portion of the lateral lobes in *C. cepula* (Fig. 3a) and at the base of the callus and of lateral lobes in *C. jonesiana* (Fig. 3b). Such areas stained pale pink. In addition, the callus of both species also reacted and stained pale pink.

Light microscopy

In the longitudinal section, at the base of the callus and at the proximal adaxial surface of lateral lobes, papillae and epidermal isodiametric cells with dense, granular cytoplasm and obvious nuclei were observed (Fig. 3c). In *C. cepula*, the presence of a secretory tissue is less evident. This trait would support the less intense neutral red reaction than in *C. jonesiana* and the almost imperceptible fragrance of flowers of *C. cepula*. The secretion of a volatile substance would occur through the epidermis. The abaxial epidermis of the lateral lobes and the adaxial epidermis of the middle lobe of lip are also covered by papillae, but these lack a dense, granular cytoplasm; therefore, they would not have secretory activity (Fig. 3d).

Elaiophores

Sudan III reaction

In *C. cepula* and *C. jonesiana*, the callus reacted more strongly to saturated, alcoholic Sudan III solution than did any other parts of the flower, revealing the elaiophores. Organ surface, originally yellowish in *C. cepula* and white-cream in *C. jonesiana*, stained intense red, evidencing the presence of an oil-secreting tissue (Fig. 3e, f). In addition, the proximal portion of the lateral lobes also reacted, but less intensely. However, in *C. cepula*, this reaction was more noticeable than in *C. jonesiana*.

Light microscopy

The anatomical structure is very similar in both species. The principal secretory tissue of elaiophores is the epidermis of the callus, which consists of epidermal palisade-like cells with a dense granular cytoplasm and a large nucleus. The subjacent parenchyma cells have a large nuclei but their cytoplasm is not as dense as in the epidermal cells (Fig. 3g, h).

Anatomical and micromorphological study of floral pieces

Light microscopy

The anatomical features of the different floral pieces are very similar in both species, showing only a few differences. In most cases, the observations correspond to the cross section.

Sepals In *C. cepula*, the middle portion of the sepal has cells with straight outer tangential walls in the adaxial epidermis and cells with convex outer tangential walls in the abaxial epidermis (Fig. 4a). In *C. jonesiana*, the epidermis of the middle portion shows quadrangular to subrectangular cells in the

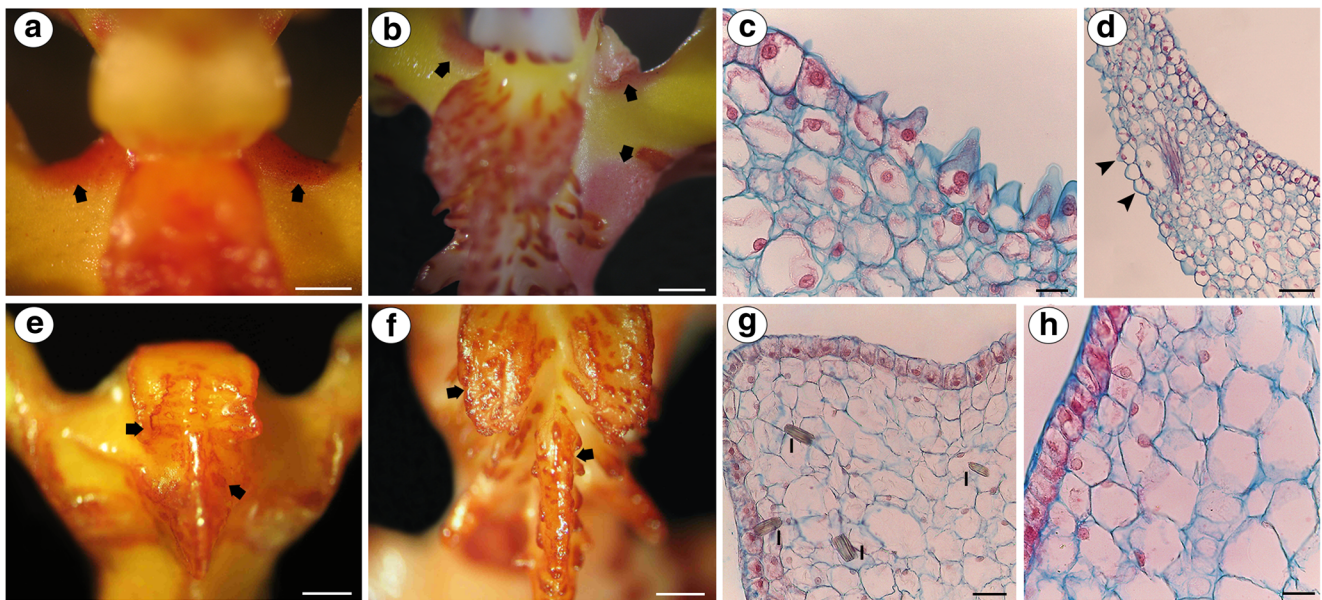


Fig. 3 Osmophores and elaiophores. **a, e, g** *C. cepula*. **b, c, d, e, h *C. jonesiana*. **a, b** Flowers showing the areas of osmophores colored pink (arrows), after staining them with Neutral Red. **c, d** Light photomicrographs of lateral the lobe in long section. **c** Detail of the secretory epidermis consisting of papillae and epidermal isodiametric cells. **d** Abaxial epidermis of the lateral lobe covered by non-secretory**

papillae (arrowheads). **e, f** Flowers showing the areas of elaiophores colored dark red (arrows), after staining them with Sudan III. **g, h** Light photomicrographs of callus showing the secretory epidermis, corresponding to epithelial elaiophores. *I* idioblast. Scale bars: **a, b, e, f** = 1 mm; **c** = 100 μ m; **d, h** = 25 μ m; and **g** = 50 μ m

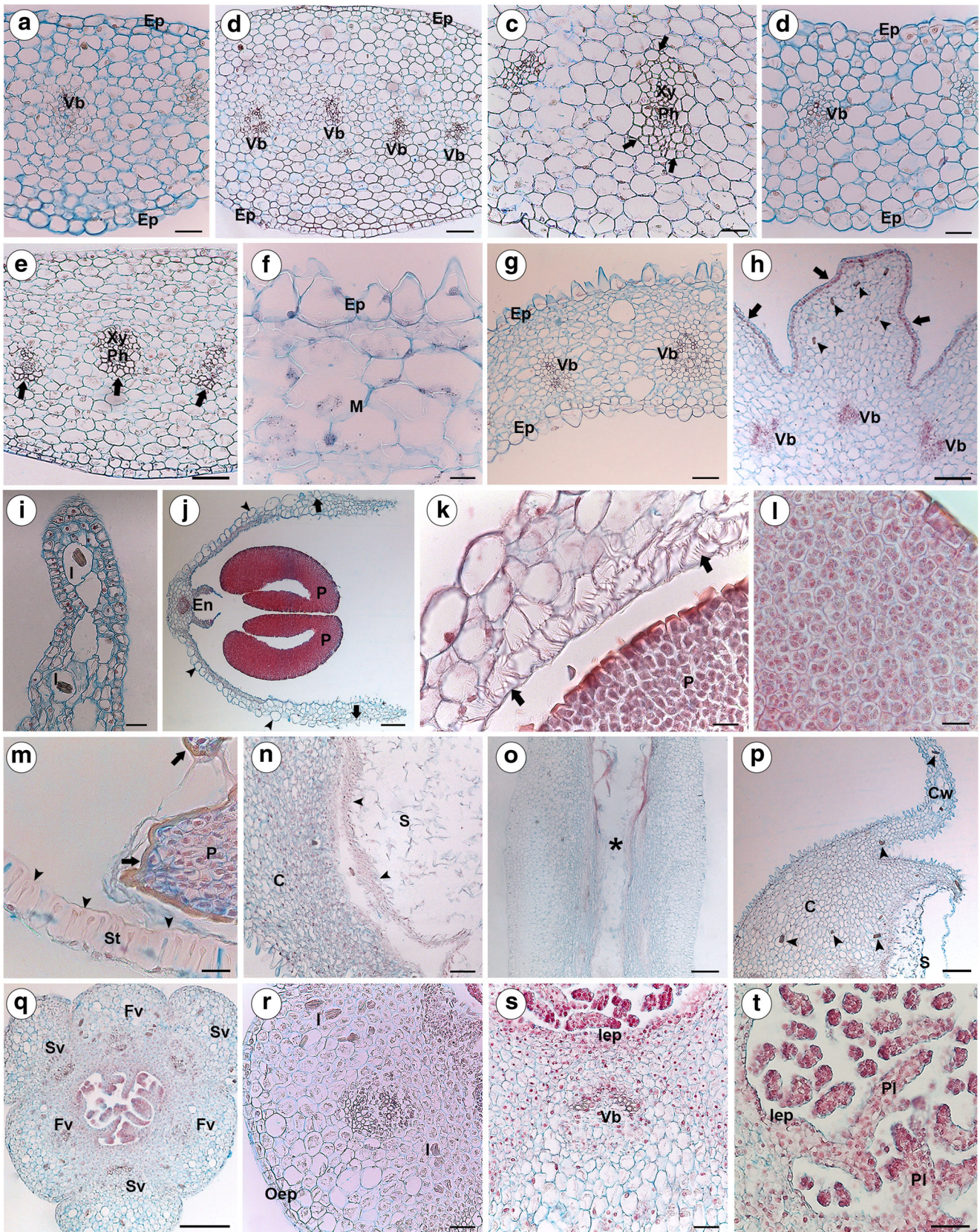
cross section in both abaxial and adaxial surfaces (Fig. 4b). In both species, the cells on the adaxial surface are larger than in the abaxial one, and on the extremes of sepals the cells present convex outer tangential walls. The mesophyll is homogenous, with irregularly shaped parenchyma cells. Some are larger and contain raphidies. Vascular bundles are composed of xylem and phloem. In *C. jonesiana*, the vascular bundles of the dorsal sepal are surrounded by sclerenquimatic cells (Fig. 4c), whereas in *C. cepula* these cells are absent.

Petals As in the sepals, epidermal cells in the petals of *C. cepula* present straight outer tangential walls on the basal portion and convex outer tangential walls in the rest of the petal (Fig. 4d). The epidermis of *C. jonesiana* has quadrangular to subrectangular cells in the cross section of both abaxial and adaxial surfaces, with no differences between them. The mesophyll shows the same features as in the sepals. However, the presence of fibers below the phloem on the vascular bundles of *C. jonesiana* is observed (Fig. 4e).

Labellum In both species, the epidermis on the adaxial surface has papillae (Fig. 4f). In *C. cepula*, papillae are larger than in *C. jonesiana* (Fig. 4g). Epidermal cells on the abaxial surface are conical and smaller than the adaxial ones. The portion of lateral lobes close to the callus presents papillae with a dense cytoplasm, which are associated with the presence of osmophores (Fig. 3c). Mesophyll is homogenous, with irregularly shaped parenchyma cells. Large idioblasts containing raphidies are also observed.

Callus The epidermis is formed by a layer of quadrangular cells with dense granular cytoplasm and large nucleus. Parenchyma is homogenous, compact, and is formed by irregularly shaped cells delimiting intercellular spaces. Vascular bundles consist of xylem and phloem (Fig. 4h). Idioblasts containing raphidies are frequently observed (Fig. 4i).

Column The anatomical structure of the column is similar and complex in both species. On the anther region, the outer epidermis is composed of large cells, with convex outer tangential walls and papillae in some areas are observed (Fig. 4j). The cells of the endothecium present fibrous wall thickenings (Fig. 4k). Pollinia show the pollen grains aggregated in tetrads and a thick exine is observed covering the external ones (Fig. 4l). In the longitudinal section, pollinia are supported on a stipe, which is formed by two layers of cells, one of them is very thin, composed by flattened and evident nucleated cells, the other presents thickened u-shaped walls (Fig. 4m). The epidermis of the stigma and a few subjacent layers of parenchyma cells detach and disintegrate, and are part of the stigmatic matrix that has a viscous aspect (Fig. 4n). The stylar canal is hollow and, as the stigma, epidermis and a few layers of parenchyma cells detach from its surface (Fig. 4o). This can be observed both in longitudinal and cross sections. The column wings have an epidermis composed of papillae and a thin mesophyll with small parenchyma cells and idioblasts with raphidies (Fig. 4p). The region surrounding the stigma presents epidermis with papillae and the remainder of the column has subrectangular cells, homogenous parenchyma, and idioblasts with raphidies.



◀ **Fig. 4** Light photomicrographs of floral pieces. **a, d, g, h, j, k, l, p, q, r** *C. cepula*. **b, c, e, f, i, m, n, o, s, t** *C. jonesiana*. **a, b** General aspect of the cross section of dorsal and lateral sepals, respectively. **c** Detail of the dorsal sepal with the vascular bundle surrounding by sclerenquimatic cells (arrows). **d, e** General aspect of the cross section of petals. Note the sclerenquimatic cells below the phloem (arrows) in *C. jonesiana* (**e**). **f, g** General aspect of the labellum. **h** Cross section of the callus showing the epidermal cells with dense cytoplasm, corresponding to elaiophores (arrows) and the compact parenchyma with vascular bundles and several idioblasts with raphidies (arrowheads). **i** Detail of a section of a tooth with two raphidies. **j-p** Column. **j** Cross section of an anther region showing the large cells on the outer epidermis with circular cells (arrowheads) and papillae (arrows). Is observed the endothecium in the inner side, and pollinia. **k** Detail of cells of the endothecium (long section) with the fibrous wall thickening (arrows). **l** Detail of a cross section of pollinia showing the pollen grain aggregates in tetrads. **m** Long section of pollinia showing the thick exine covering the external pollen grains (arrows) and the stipe with the walls cellular thickened u-shaped (arrowheads). Note below the layer of flattened cells. **n** Section of stigma, with layers of detached cells (arrowheads). **o** Long section of the hollow stylar canal (asterisk). **p** Cross section of the column with a column wing. Note an important presence of raphidies (arrowheads). **q-t** Ovary. **q** General aspect in the cross section showing the fertile and sterile valves. **r** Detail of a fertile valve showing the outer epidermis, the compact parenchyma, and a high number of raphidies. **s** Section of the sterile valve with a vascular bundle. **t** Detail of placentas showing the projections and cells with dense cytoplasm. Note the absence of fully developed ovules. *C* column, *Cw* column wing, *En* endothecium, *Ep* epidermis, *Fv* fertile valve, *I* idioblast, *Iep* inner epidermis, *M* mesophyll, *Oep* outer epidermis, *P* pollinia, *Ph* phloem, *Pl* placenta, *S* stigma, *St* stipe, *Sv* sterile valve, *Vb* vascular bundle, *Xy* xylem. Scale bars: **a, c, e, i, k, r-t** = 50 μ m; **b, d, g, h, n** = 100 μ m; **f, m** = 25 μ m; **j, o-q** = 200 μ m; and **l** = 15 μ m

Ovary The ovary has similar features in both species. In the cross section, the ovary presents three carpels divided into six valves: three are fertile, with the presence of a placenta region, and the other three are sterile (Fig. 4q). The outer epidermis is single-layered and cells are polygonal. The fundamental tissue consists of compact, more or less isodiametric parenchyma cells, and a high number of idioblasts with raphidies (Fig. 4r). The valves present one vascular bundle composed of phloem and xylem, and are located near the inner epidermis (Fig. 4s). The latter consists of small cells with a dense and granular cytoplasm. The placentation is parietal, and many ovule primordia are observed (Fig. 4t).

SEM

The features of the different floral pieces are very similar in both species, showing only a few differences.

Sepals and petals In both faces, *C. cepula* presents irregular epidermal cells, polygonal to subrectangular (Fig. 5a, c), whereas in *C. jonesiana* they are subrectangular (Fig. 5b, d). The cuticle that covers the epidermal cells is smooth to

slightly striate. Abaxially, at the base of sepals, there is a high number of nectarostomata, which are irregularly distributed across a delimited area and associated with nectar secretion (Fig. 2n, o).

Labellum The adaxial epidermis of both lateral and middle lobes consists of papillose cells. On the middle lobe, these cells are conical and become thinner at the distal extreme (Fig. 5e, f). In *C. cepula*, the papillae seem to be grouped in higher density. On the lateral lobes, papillae are less prominent and have a rounded distal extreme (Fig. 5g). They are covered by a striate cuticle. In both lateral and middle lobes, the abaxial epidermis consists of spherical papillae that have a slightly striate cuticle (Fig. 5h).

Callus In the surface view, a high number of non-functional stomata with wide pores distributed across the surface are observed in both species (Fig. 5i, j). The epidermal cells are more or less polygonal in *C. cepula*, and subrectangular in *C. jonesiana*. The guard cells of stomata are covered by a smooth cuticle and the pores of some stomata are almost completely covered. In *C. cepula*, the guard cells appear to be slightly sunken (Fig. 5k), whereas in *C. jonesiana* they are elevated (Fig. 5l).

Column The distal extreme of the column (i.e., the anther region and the column wings) presents papillae and the rest of the column exhibits subquadrangular to subrectangular cells (Fig. 5m). These cells have a smooth cuticle, whereas the papillae show a striate cuticle (Fig. 5n, o). However, the papillae surrounding the stigma present a strongly striate cuticle (Fig. 5p). The surface of the stigma is smooth.

Pollinarium The pollinarium of both species consists of two pollinia, which present a partial division in the dorsal side, a semi-transparent stipe that supports them, and a sticky terminal viscidium. A smooth exine covers the pollen grains (Fig. 6d–f). The stipe is covered by a smooth cuticle with ridges that correspond to the u-shaped thickenings observed in the cross section (Fig. 6g).

Discussion

This survey reveals for the first time the presence of nectaries and osmophores in *C. cepula* and *C. jonesiana* and contributes novel information about their anatomical structure and micromorphology. The anatomy and micromorphology of floral pieces are also described for both species, providing interesting data of taxonomic value.

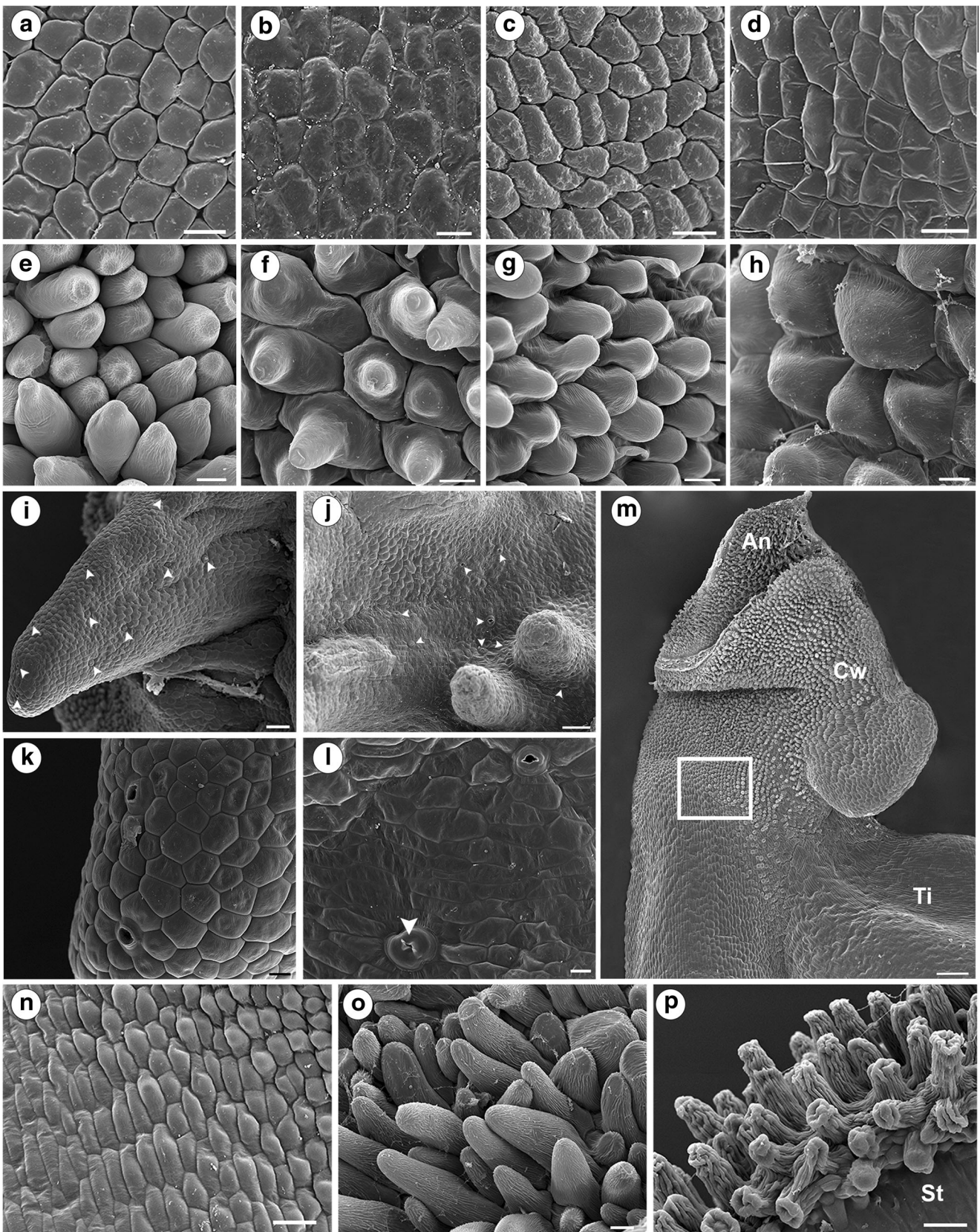


Fig. 5 SEM micrographs of floral pieces. **a, c, e, g, h, i, k, m, n, p** *C. cepula*. **b, d, f, j, l, o** *C. jonesiana*. **a-d** General aspect of sepals and petals. Abaxial (**a**) and adaxial (**b**) surface of sepals and adaxial (**c**) and abaxial (**d**) surface of petals. **e-h** Labellum. **e, f** Adaxial surface of the middle lobe showing papillae with the striate cuticle. **g, h** Adaxial and abaxial surface of the lateral lobe, respectively. **i-l** Callus. **i, j** General aspect of the surface. Note the high number of modified stomata (arrowheads). **k, l** Detail of stomata slightly sunken and elevated, respectively. Note the pore almost covered by the cuticle (arrowhead). **m-p** Column. **m** General aspect of a lateral side of the column where observed is the epidermis of distal extreme covered with papillae and the rest of the column with subquadrangular to subrectangular cells. **n** Detail of epidermal cells with the cuticle smooth to slightly striate. **o** Detail of papillae showing the striate cuticle. **p** Detail of papillae surrounding the stigma. Note the cuticle strongly striate. *An* anther, *Cw* column wing, *St* stigma, *Ti* tabula infrastigmatica. Scale bars: **a-d, n** = 50 μ m; **e-h, o, p** = 20 μ m; **i, j** = 100 μ m; **k, l** = 20 μ m; and **m** = 200 μ m

Nectaries

We detected two types of reproductive nectaries, according to their position: RBNs located at the base of the bracts, which subtend the flowers, and RSNs located at the base of the sepals, on the abaxial surface. The nectaries situated on the flowers (frequently known as floral nectaries) are usually

related to the pollination process, since they secrete nectar in order to reward the pollinators. However, in this case, the RSNs would not be related to the pollination process nor would nectar be considered a reward; these assumptions are based, on the one hand, on location of the nectary and, on the other hand, on the fact this species would be pollinated by oil-collecting bees, as the case of other Oncidiinae species (Pansarin and Pansarin 2011; Torretta et al. 2011; Gomiz et al. 2014; Pansarin et al. 2016).

As already mentioned, nectar secretion has been reported in a few cases within Oncidiinae. Within *Trichocentrum* clade, nectar secretion would be associated with the genus *Trichocentrum s.s.*, which is characterized by the presence of a spur. However, this structure is non-functional (van der Cingel 2001) and is not able to secrete nectar.

The morpho-anatomical structure of RBNs and RSNs is very similar in both *C. cepula* and *C. jonesiana*; in turn, both nectaries also present similarities with the foliar and bracteal nectaries of *Rodriguezia venusta* (Leitão et al. 2014). The nectaries placed in the flowers of members of Oncidiinae, such as *Comparettia falcata* (Rodríguez-Robles et al. 1992), *Symphoglossum sanguineum* (Stpiczyńska and Davies 2006),

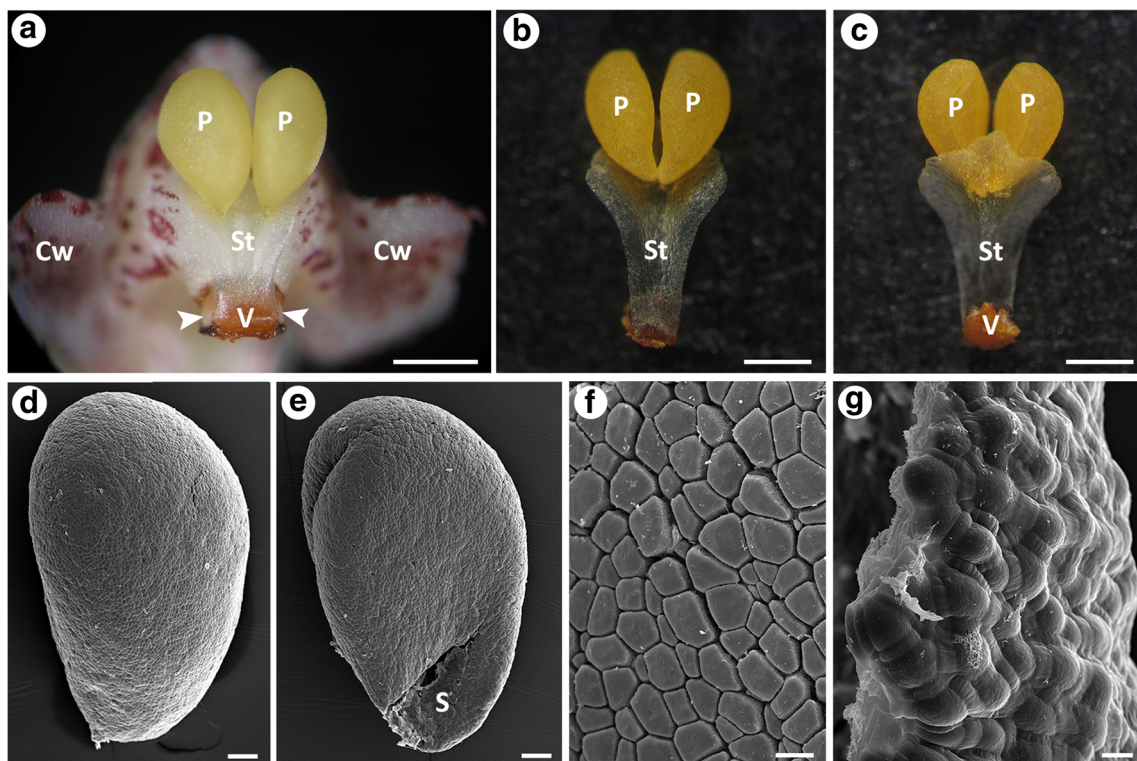


Fig. 6 Macromorphology and micromorphology of the pollinarium. **b, c, d, e** *C. cepula*. **a, f, g** *C. jonesiana*. **a-c** Pollinarium. **a** General aspect. **b, c** Frontal and dorsal views, respectively. **d-g** SEM micrographs. **d, e** Frontal and dorsal views of pollinia, respectively. **f** Detail of a section of pollinia

showing the smooth exine. **g** Detail of a section of the dorsal side the stipe. *Cw* column wing, *P* pollinia, *S* scar of the stipe, *St* stipe, *V* viscid. Scale bars: **a** = 1 mm; **b, c** = 0.5 mm; **d, e** = 100 μ m; **f** = 20 μ m; and **g** = 10 μ m

Capanemia therezae (Buzatto et al. 2012), and *Rodriguezia venusta* (Leitão et al. 2014), show a very diverse structural pattern. Since the literature about nectaries in Oncidiinae is scarce, we could assume that the structure of RSNs of *C. cepula* and *C. jonesiana* is another particular case within the subtribe. However, in order to understand the evolutionary trend of nectaries in Oncidiinae, it is necessary to explore a greater number of species.

Following the classification presented by Bernardello (2007), both RBNs and RSNs of *Cohniella* species correspond to the structural type. We observed the three typical histological components according to Nepi (2007): epidermis, specialized parenchyma, and vascular system. The anatomical features analyzed here are similar to those described in other Orchidaceae (Stpiczyńska et al. 2003; Teixeira et al. 2004; Davies et al. 2005; Stpiczyńska et al. 2005; Melo et al. 2010; Leitão et al. 2014).

We detected a high number of stomata distributed across the surface of both RBNs and RSNs, which suggests that nectar secretion is released through them. Additionally, the presence of secretory tissue (epidermis and parenchyma) around stomata was observed in the cross section. Similar traits were described in *R. venusta* by Leitão et al. (2014), who also remarked the presence of wide intercellular spaces near the substomatal chamber and propose that this feature would allow a more efficient flow of nectar through stomata. We also observed this particular trait in both nectaries in transverse sections.

According to Endress (1995), the modified stomata are apparently absent from monocotyledons. In these plants, nectar is secreted by trichomes or by diffusion through the epidermis, as in many Orchidaceae (Stpiczyńska et al. 2003, 2005; Leitão et al. 2014; Neubig et al. 2015). However, there are few records about nectar secretion through stomata in the family, as in the case of *Maxilaria anceps* Ames & C. Schweinf. (Davies et al. 2005) and the bracteal and foliar nectaries of *R. venusta* (Leitão et al. 2014).

Osmophores

The flowers of *C. cepula* and *C. jonesiana* emit a soft fragrance, which is almost imperceptible in *C. cepula*. The presence of osmophores is not common within the Oncidiinae subtribe, and was recorded only in a few representatives, such as *Capanemia adelaidae* Porto & Brade, *Capanemia micromera* Barb. Rod. and *Capanemia superflua* (Rchb. f.) Garay (Buzatto et al. 2012), *Ionopsis satyrioides* (Sw.) Rchb. f. and *Ionopsis utricularoides* (Sw.) Lindl. (Aguiar 2014) and *Gomesa montana* (Barb. Rodr.) M.W. Chase & N.H. Williams, and *Gomesa varicosa* (Lindl. & Paxton) M.W. Chase & N.H. Williams (Pansarin et al. 2016). However, the presence of osmophores in *G. varicosa* would be uncertain, because Pansarin et al. (2016) locate these structures in the

central lip callus, whereas Gomiz et al. (2013) confirm the presence of elaiophores in that position (reason why it was not included in Table 1). Within the *Trichocentrum* clade, Neubig et al. (2011) mentioned that *Trichocentrum tigrinum* Linden & Rchb. f. has an intense fragrance that attracts fragrance-collecting male euglossine bees (van der Pijl and Dodson 1966). To our knowledge, there are no records in the literature about osmophores in the *Cohniella* species.

The anatomical structure of osmophores in the different orchid species is similar and consists of a layer of epidermal cells with dense granular cytoplasm and one to several layers of sub-epidermal secretory parenchyma. However, their location varies and they may be present in different parts of the perianth, such as the adaxial surface of the labellum in *Capanemia* and *Ionopsis* species (Buzatto et al. 2012; Aguiar 2014), adaxial surface of sepals in *Acianthera* Scheidw. species (Melo et al. 2010), multicellular epidermal protuberances on the labellum of *Vanilla edwallii* Hoehne (Pansarin et al. 2013), and appendages in dorsal sepal and petals in *Bulbophyllum wendlandianum* (Kraenzl.) Dammer (Kowalkowska et al. 2015).

In most Orchidaceae, the cells responsible for secreting volatile substances are normally papillose (Ascensão et al. 2005; Melo et al. 2010; Buzatto et al. 2012; Aguiar 2014; Kowalkowska et al. 2015), although osmophores with no papillose cells occur, as in *Ionopsis satyrioides* (Aguiar 2014), *Cirrhaea* Lindl. species (Pansarin et al. 2006, 2014), and *Vanilla edwallii* (Pansarin et al. 2013). However, the presence of both papilla and no papillose cells has not been described before. Thus, this will be the first record of osmophores with and without papillae reported in the same species.

The emission of volatile compounds may occur in different ways in Orchidaceae. Cuticular diffusion has been recorded in some species of *Scaphosepalum* Pfitzer (Pridgeon and Stern 1985), *Stanhopea* Frost ex Hook. (Stern et al. 1987), and *Cirrhaea* (Pansarin et al. 2014). In other species, such as *Bulbophyllum weberi* Ames (Kowalkowska et al. 2017), *Anacamptis pyramidalis* f. *fumeauxiana* Marg. & Kowalk. (Kowalkowska et al. 2012), *Bulbophyllum wendlandianum* (Kowalkowska et al. 2015), and African *Bulbophyllum* species (Stpiczyńska et al. 2015), the exudation of fragrances is released through microchannels formed by cuticular reticulation. The secretion of volatile compounds also occurs through pores such as in *Restrepia* Kunth (Pridgeon and Stern 1983) or stomata such as in *Acianthera* (Melo et al. 2010). In the species here studied, we could assume that the secretion of volatile substances occurs through the cuticle, since the presence of stomata is not observed. However, it would be necessary to explore the areas of osmophores in more detail to determine how the fragrances are emitted.

Table 1 Secretory structures recorded to the Oncidiinae subtribe

Secretory structure	Taxa	Reference
Nectaries	<i>Capanemia therezae</i>	Buzatto et al. 2012
	<i>Cohniella cepula</i>	This study
	<i>C. jonesiana</i>	This study
	<i>Comparettia coccinea</i>	Pansarin et al. 2015
	<i>C. falcata</i>	Rodríguez-Robles et al. 1992
	<i>Ionopsis multiflora</i>	Pupulin 1998
	<i>Leochilus</i> spp. ^a	Chase 1986
	<i>Rodriguezia bahiensis</i>	Carvalho and Machado 2006
	<i>R. venusta</i>	Leitão et al. 2014
	<i>Symphlyglossum sanguineum</i>	Stpiczyńska and Davies 2006
Osmophores	<i>Capanemia adelaidae</i>	Buzatto et al. 2012
	<i>C. carinata</i>	Buzatto et al. 2012
	<i>C. gehrtii</i>	Buzatto et al. 2012
	<i>C. mircomera</i>	Buzatto et al. 2012
	<i>C. superflua</i>	Buzatto et al. 2012
	<i>Cohniella cepula</i>	This study
	<i>C. jonesiana</i>	This study
	<i>Ionopsis satyrioides</i>	Aguiar 2014
	<i>I. utricularoides</i>	Aguiar 2014
	<i>Gomesa montana</i>	Pansarin et al. 2016
Elaiophores	<i>Cyrtochilum meirax</i>	Davies et al. 2014
	<i>Gomesa bifolia</i>	Aliscioni et al. 2009
	<i>G. cornigera</i>	Gomiz et al. 2017
	<i>G. echinata</i>	Stpiczyńska et al. 2013
	<i>G. flexuosa</i>	Gomiz et al. 2013
	<i>G. herzogii</i>	Gomiz et al. 2017
	<i>G. longicornu</i>	Gomiz et al. 2017
	<i>G. longipes</i>	Davies et al. 2014
	<i>G. ranifera</i>	Stpiczyńska et al. 2013
	<i>G. recurva</i>	Stpiczyńska et al. 2007
	<i>G. riograndensis</i>	Gomiz et al. 2013
	<i>Grandiphyllum divaricatum</i>	Gomiz et al. 2017
	<i>Lockhartia</i> spp. ^a	Blanco et al. 2013
	<i>Oncidium amazonicum</i>	Stpiczyńska et al. 2013
	<i>O. cheiophorum</i>	Pacek and Stpiczyńska 2007
	<i>O. heteranthum</i> var. <i>album</i>	Davies et al. 2014
	<i>O. loefgrenii</i>	Stpiczyńska et al. 2007
	<i>O. ornithorhynchum</i>	Davies et al. 2009
	<i>O. oxyceras</i>	Stpiczyńska et al. 2013
	<i>O. paranaense</i> (= <i>G. paranaensis</i>)	Singer and Cocucci 1999
<i>O. trulliferum</i>	Stpiczyńska and Davies 2008	
<i>Ornithocephalus gladius</i>	Pacek et al. 2012	
<i>O. kruegeri</i>	Pacek and Stpiczyńska 2007	
<i>Ornithophora radicans</i>	Stpiczyńska and Davies 2008	
<i>Phymatidium falcifolium</i>	Pacek et al. 2012	
<i>Trichocentrum cebolleta</i> (= <i>Cohniella cepula</i>)	Gomiz et al. 2017	
<i>T. cavendishianum</i>	Stpiczyńska et al. 2007	

Table 1 (continued)

Secretory structure	Taxa	Reference
	<i>T. jonesianum</i> (= <i>C. jonesiana</i>)	Gomiz et al. 2017
	<i>T. pumilum</i>	Pansarin and Pansarin 2011
	<i>Vitekorchis excavata</i>	Davies et al. 2014
	<i>Zygostates alleniana</i>	Gomiz et al. 2014
	<i>Z. grandiflora</i>	Pacek et al. 2012
	<i>Z. lunata</i>	Pacek et al. 2012

^a spp. is used in cases of authors who cite numerous species

Elaiophores

C. cepula and *C. jonesiana* present elaiophores on the callus of the labellum. The secretory epidermis consists of cells with a dense granular cytoplasm and evident nuclei, and a layer of little differentiated sub-secretory parenchyma. Our results agree with the data reported by Gomiz et al. (2017). However, our SEM observations revealed the presence of a high number of modified stomata over the surface of the callus, unlike findings reported by Gomiz et al. (2017), who mentioned that stomata were scattered or absent. Based on the classification of Vogel (1974), the elaiophores of both *Cohniella* species belong to the epithelial type. Several studies have shown that epithelial elaiophores are the most common within the Oncidiinae (Singer and Cocucci 1999; Reis et al. 2006; Pacek and Stpiczyńska 2007; Stpiczyńska et al. 2007; Stpiczyńska and Davies 2008; Aliscioni et al. 2009; Pansarin and Pansarin 2011; Pacek et al. 2012; Gomiz et al. 2013).

The presence of both odor and oil-reward in the same flower could increase the range of visiting pollinator species, since visitors will collect the oils as reward whereas the sweet scent attract them. Although there are no studies about reproductive biology of *Cohniella* species, the few research studies in Oncidiinae show that species that have elaiophores are pollinated by oil-collecting bees (Pansarin and Pansarin 2011; Torretta et al. 2011; Gomiz et al. 2014; Pansarin et al. 2016). Therefore, it is expected that *C. cepula* and *C. jonesiana* will be pollinated by these kinds of bees.

Finally, as a summary, an update of the secretory structure recorded to the different taxa of the Oncidiinae subtribe (Table 1) is presented.

Floral pieces

Although no studies have described floral pieces completely in the family, their anatomical analysis usually accompanies studies about secretory structures (Melo et al. 2010; Buzatto et al. 2012; Kowalkowska et al. 2015) or mechanisms of pollinator attraction (Teixeira et al. 2004; Ascensão et al. 2005). Moreover, some authors have analyzed the development of particular floral pieces, such as anther (Freudenstein et al.

2002), pollinarium (Mosquera-Mosquera and Valencia-Barrera 2009; Mosquera-Mosquera 2012), gynostemium/pistil (Figuroa et al. 2012; Mosquera-Mosquera 2012), and pericarp and seed (Mayer et al. 2011).

The anatomy of the studied floral pieces of *Cohniella* species is similar within Orchidaceae. Mesophyll of sepals, petals, and lip is generally formed by parenchymal cells of different sizes, and idioblasts containing raphidies are frequently observed. Epidermis is frequently uniseriate, with the shape of epidermal cells varying according to species and function. The presence of papillae or trichomes is always associated with some mechanism of pollinator attraction or system of rewards.

Adaxial surface of the lip is often composed of papillae whose size and function vary among species. *Ophrys fusca* Link and *Ophrys lutea* (Gouan) Cav. present four types of epidermal cells on the lip, represented by papillae and trichomes, each of them occupying a specific position and function, e.g., the long trichomes located on the basal part of the labellum produce a tactile stimulation and orient male bees during pseudocopulation, whereas the short trichomes of the speculum with cuticular striations would be involved in the brightness of this region (Ascensão et al. 2005). Some *Bulbophyllum* species have papillae and trichomes acting as osmophores on the lip and others have them in the dorsal sepal and petals (Teixeira et al. 2004; Kowalkowska et al. 2015, 2017).

When the epidermis presents secretory activity, a dense cytoplasm usually composed of starch grains is observed, as in *Bulbophyllum* species (Teixeira et al. 2004; Kowalkowska et al. 2015, 2017), *Acianthera* species (Melo et al. 2010), and *Capanemia therezae* (Buzatto et al. 2012). However, sepals or lip are not the only floral pieces with secretory activity in Orchidaceae. Like *C. cepula* and *C. jonesiana*, several Oncidiinae usually present oil-secreting tissue on the callus of the labellum. Therefore, epidermal cells present a dense cytoplasm and some sub-epidermal layers of secretory parenchyma, as in *Gomesa* species (Stpiczyńska et al. 2007; Gomiz et al. 2013; Pansarin et al. 2016), *Trichocentrum cavendishianum* (Bateman) M.W. Chase & N.H. Williams (Stpiczyńska et al. 2007), *Oncidium* species (Pacek and Stpiczyńska 2007; Stpiczyńska et al. 2007; Stpiczyńska and Davies 2008), and *Ornithocephalus* clade (Pacek et al. 2012).

We found notorious differences in sepals and petals between *C. cepula* and *C. jonesiana*. The presence of sclerenchymal cells around vascular bundles in the dorsal sepal and below the phloem in the petals of *C. jonesiana* would have potential taxonomic value and might be useful to characterize the species.

Features of column and anther vary among species. In *C. cepula* and *C. jonesiana*, the anatomical structure is very similar. Characters of the anther have been considered the most important for systematics in Orchidaceae since the beginning of the nineteenth century (Freudenstein et al. 2002). Some of the characters studied are related to the orientation of the anther, the nature of pollinia, and the differences in numbers and packaging (Freudenstein et al. 2002). The pollinarium consisting of two pollinia, stipe and viscidium, as in *C. cepula* and *C. jonesiana*, is considered the typical pollinarium of Oncidiinae (Dressler 1993). The pollen grains are covered by a smooth exine, a typical feature of the most advanced members of Epidendroideae (Dressler 1993). In *C. cepula* and *C. jonesiana*, the stipe consists of two layers of cells, the second one with thickened u-shaped walls. This trait was similarly described by Gomiz et al. (2014) for the stipe of *Zygostates alleniana* Kraenzl. Apparently, the presence of thickened u-shaped walls would be associated with the reconfiguration of pollinarium during the pollination process (Gomiz et al. 2014).

According to Heslop-Harrison and Shivanna (1977) and Heslop-Harrison (1981), the stigma in Orchidaceae is characterized as “wet, with low to medium papillae”. However, Calder and Slater (1985) and Slater and Calder (1988) described a new type of stigma in *Dendrobium speciosum* Sm.: the wet detached cells. Slater and Calder (1990) studied the structure of this kind of stigma in *D. speciosum* and found that the detached cells are completely isolated from each other and suspended in the stigmatic mucilage. The authors also mentioned that these cells present secretory activity. In *C. cepula* and *C. jonesiana*, we have observed both in longitudinal and cross sections that the stigma presents several layers of detached cells and a viscous content on the stigmatic cavity. However, under SEM, we observed that stigmatic surface is slightly smooth and could be covered by mucilage.

Papillae surrounding the stigma have been reported by Clifford and Owens (1990) in several species of Oncidiinae. According to observations of Brown (1833), Cruger (1864), and Darwin (1904), the orchid stigma is composed of detach cells, which are separated by a thick, sweet, mucilaginous secretion at maturity. This secretion was also involved in promoting pollen germination and tube growth (Slater and Calder 1990).

Anatomical studies about column are scarce in the literature. Only a few research works about development of the gynostemium (Figueroa et al. 2012) and its external traits (Bonatti et al. 2006; Figueroa et al. 2012) were reported. However, the organ that has received the greatest attention was the ovary. The number of carpels has been widely

discussed. Some authors considered that the ovary of Orchidaceae is composed of six carpels: three with a placenta and three without it (Lindley 1830–1840, Lindley 1847; Saunders 1923; Arber 1925). On the other hand, the ovary was found to be composed of three carpels and shows a pattern of six valves: three fertile, with parietal placentas, and three sterile (Brown 1831; Duncan and Curtis 1943; Swamy 1949; Cribb 1999; Rasmussen and Johansen 2006). According to the scheme proposed by Rasmussen and Johansen (2006), the sterile valves are composed of the bases of the sepals, whereas the fertile valves correspond to a base of a petal and two carpel-halves. Our observations follow the latter scheme and agree with findings reported by Mayer et al. (2011), who analyzed the anatomical development of the pericarp of *Oncidium flexuosum* (Kunth) Lindl. and described the anatomical structure of the ovary. We have observed only one vascular bundle in both fertile and sterile valves, whereas the latter authors observed two vascular bundles in the sterile valves.

We have not observed fully developed ovules at anthesis. Swamy (1943) suggested that the developmental stage at which orchid ovules become arrested depends on the plant habit. In epiphytic species, the archesporial cell differentiates only after pollination, when it is well differentiated in terrestrial taxa (Arditti and Pridgeon 1997). In most studied orchids, the ovules are rudimentary previous to pollination. The orchid ovary consists of three placental ridges and is often bilobed. Following successful pollination, the placental ridges begin to divide and form branches. At the tip of each branch, an ovular primordium is generated. After several divisions, a massive number of ovules are produced (Arditti and Pridgeon 1997).

Conclusions

Given the overall great diversity that exists within Oncidiinae and Orchidaceae, a great number of species should be included in this kind of surveys in order to contribute with information that allows us to understand the floral evolution in the family. It is also important to increase our knowledge about secretory structures and mechanisms of pollination reward in order to improve the characterization of species. This survey contributes with novel and interesting data about two species that have received scarce attention within the *Trichocentrum* clade. We also provide important knowledge on the morphological and anatomical features of the secretory structures. We consider this information could be the basis for future studies about reproductive biology in these species and within the genus. On the other hand, this comparative study will add evidence about anatomical and micromorphological features that could have potential taxonomic value and may be an additional tool to identify these species. Moreover, when combined with other data, such as DNA-sequences, foliar

anatomy, and ultrastructure, among others, this information may be useful to help understand phylogenetic relationships within the subtribe or the *Trichocentrum* clade.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Aguiar JMRBV (2014) Biología reproductiva das *Ionopsis* Kunth (Orchidaceae) do Brasil. Tese de Mestrado, Universidade de São Paulo, Brasil
- Aliscioni SS, Torretta JP, Bello ME, Galati BG (2009) Elaiophores in *Gomesa bifolia* (Sims) M. W. Chase and N. H. Williams (Oncidiinae: Cymbididae: Orchidaceae): structure and oil secretion. *Ann Bot* 104: 1141–1149
- Arber A (1925) *Monocotyledons: a morphological study*. Cambridge University Press, London
- Arditti J, Pridgeon AM (1997) *Orchid biology: review and perspectives*, VII. Springer Science + Business Media, Dordrecht
- Ascensão L, Francisco A, Cotrim H, Pais MS (2005) Comparative structure of the labellum in *Ophrys fusca* and *O. lutea* (Orchidaceae). *Am J Bot* 92:1059–1067
- Bernardello G (2007) A systematic survey of floral nectaries. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and nectar*. Springer, Dordrecht, pp 19–128
- Blanco MA, Davies KL, Stpiczynska M, Carlswald BS, Ionta GM, Gerlach G (2013) Floral elaiophores in *Lockhartia* Hook. (Orchidaceae: Oncidiinae): their distribution, diversity and anatomy. *Ann Bot* 112:1775–1791
- Bonatti PM, Sgarbi E, Del Prete C (2006) Gynostemium micromorphology and pollination in *Epipactis microphylla* (Orchidaceae). *J Plant Res* 119:431–437
- Brown R (1831) *Observations on the organs and mode of fecundation in Orchideae and Asclepiadeae*. Richard Taylor, London
- Brown R (1833) On the organs and mode of fecundation in Orchideae and Asclepiadeae. In: Brown R (ed) *The miscellaneous botanical works of Robert Brown*. The Ray Society, London, pp 487–543
- Buzatto CR, Davies KL, Singer RB, Pires Dos Santos R, Van Den Berg C (2012) A comparative survey of floral characters in *Capanemia* Barb. Rodr. (Orchidaceae: Oncidiinae). *Ann Bot* 109:135–144
- Calder DM, Slater AT (1985) The stigma of *Dendrobium speciosum* Sm. (Orchidaceae): a new stigma type comprising detached cells within a mucilaginous matrix. *Ann Bot* 55:297–307
- Carnevali Fernández-Concha G, Cetzal-Ix WR, Balam Narváez R, Romero-González GA (2010) A synopsis of *Cohniella* (Orchidaceae, Oncidiinae). *Brittonia* 62(2):153–177
- Carvalho R, Machado IC (2006) *Rodriguezia bahiensis* Rchb.f.: biología floral, polinizadores e primeiro registro de polinização por moscas Acroceridae em Orchidaceae. *Rev Bras Bot* 29:461–470
- Caspary R (1848) *De nectariis*. Adolphum Marcum, Bonn
- Cetzal-Ix WR, Carnevali Fernández-Concha G (2010) A revision of *Cohniella* Pfitzer (Orchidaceae) in Mexico. *J Torrey Bot Soc* 137(2–3):180–213
- Cetzal-Ix WR, Carnevali G, Noguera-Savelli E, Romero-González GA (2013a) What is *Cohniella cebolleta*? Recircumscription and new reinstated species and combinations (Orchidaceae). *Syst Bot* 38(3):606–623
- Cetzal-Ix WR, Noguera-Savelli E, Jáuregui D, Carnevali G (2013b) Anatomía foliar comparada y sistemática del clado *Trichocentrum* con énfasis en *Cohniella* (Asparagales: Orchidaceae). *Rev Biol Trop* 61:1841–1858
- Cetzal-Ix WR, Carnevali G, Romero González G (2016) Synopsis of the *Trichocentrum*-clade (Orchidaceae, Oncidiinae). *Harv Pap Bot* 21(2):141–169
- Chase MW (1986) A monograph of *Leochilus* (Orchidaceae). *Syst Bot Monogr* 14:1–97
- Chase MW, Williams NH, Faria AD, Neubig KM, Amaral MCE, Whitten WM (2009) Floral convergence in Oncidiinae (Cymbididae; Orchidaceae): an expanded concept of *Gomesa* and a new genus *Nohawilliamsia*. *Ann Bot* 104(3):387–402
- Clifford SC, Owens SJ (1990) The stigma, style, and ovarian transmitting tract in the Oncidiinae (Orchidaceae): morphology, developmental anatomy, and histochemistry. *Bot Gaz* 151:440–451
- Cozzolino S, Widmer A (2005) Orchid diversity: an evolutionary consequence of deception? *Trends Ecol Evol* 20:487–494
- Cribb PJ (1999) Morphology. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) *Genera Orchidacearum: vol. 1: General Introduction, Apostasioideae, Cypripedioideae*. Oxford University Press, Oxford, pp 13–23
- Cruger H (1864) A few notes on the fecundation of orchids and their morphology. *J Proc Linn Soc, Bot* 8(31):122–135
- Cseke LJ, Kaufman PB, Kirakosyan A (2007) The biology of essential oils in the pollination of flowers. *Nat Prod Commun* 2:1317–1336
- Curry KJ, McDowell LM, Judd WS, Stern WL (1991) Osmophores, floral features, and systematics of *Stanhopea* (Orchidaceae). *Am J Bot* 78: 610–623
- D’Ambrogio De Argueso A (1986) *Manual de técnicas en histología vegetal*. Facultad de Agronomía U.B.A. Hemisferio Sur, Buenos Aires
- Darwin CR (1904) *The various contrivances by which orchids are fertilized by insects*. Murray, London
- Davies KL, Stpiczynska M (2008) The anatomical basis of floral, food-reward production in Orchidaceae. In: Teixeira Da Silva JA (ed) *Floriculture, ornamental and plant biotechnology: advances and topical issues*, vol V. Global Science Books, London, pp 392–407
- Davies KL, Stpiczynska M, Gregg A (2005) Nectar-secreting floral stomata in *Maxillaria anceps* Ames & C. Schweinf. (Orchidaceae). *Ann Bot* 96:217–227
- Davies KL, Stpiczynska M, Gregg A (2009) Comparative histology of floral elaiophores in the orchids *Rudolfiella picta* (Schltr.) Hoehne (Maxillariinae *sensu lato*) and *Oncidium ornithorhynchum* H.B.K. (Oncidiinae *sensu lato*). *Ann Bot* 104:221–234
- Davies KL, Stpiczynska M, Rawski M (2014) Comparative anatomy of floral elaiophores in *Vitekorchis* Romowicz & Szlach., *Cyrtochilum* Kunth and a florally dimorphic species of *Oncidium* Sw. (Orchidaceae: Oncidiinae). *Ann Bot* 113:1155–1173
- Delpino F (1868–1875) Ulteriori osservazione e considerazione sulla dicogamia nel regno vegetale. *Atti Della Società Italiana di Scienze Naturali e dei Museo Civico di Storia et Naturale di Milano*, 11:265–332; 12:21–141, 179–233; 13:167–205; 16:151–349; 17:266–407
- Dressler RL (1990) *The orchids: natural history and classification*. Harvard University Press, Cambridge
- Dressler RL (1993) *Phylogeny and classification of the orchid family*. Press Syndicate of the University of Cambridge, Cambridge
- Dressler RL, Dodson CH (1960) Classification and phylogeny in the Orchidaceae. *Ann Mo Bot Gard* 47:25–67
- Duncan RE, Curtis JT (1943) Growth of fruits in *Cattleya* and allied genera in the Orchidaceae. *Bull Torrey Bot Club* 70:104–119
- Endress PK (1994) *Diversity and evolutionary biology of tropical flowers*. Cambridge University Press, Cambridge

- Endress PK (1995) Major evolutionary traits of monocot flowers. In: Rudall PJ, Cribb PJ, Cutler DF, Humphries CJ (eds) *Monocotyledons: systematics and evolution*. Royal Botanic Gardens, Kew, pp 43–79
- Figueroa C, Salazar GA, Terrazas T, Dávila P (2012) Estructura y desarrollo del ginostemio en *Dichromanthus michuacanus* (Orchidaceae, Spiranthinae). *Rev Mex Biodiv* 83:73–82
- Flach A, Dondon RC, Singer RB, Koehler S, Amaral MCE, Marsaioli AJ (2004) The chemistry of pollination in selected Brazilian Maxillariinae orchids, floral rewards and fragrance. *J Chem Ecol* 30:1045–1056
- Freudenstein JV, Harris EM, Rasmussen FN (2002) The evolution of anther morphology in orchids: incumbent anthers, superposed pollinia, and the vandoid complex. *Am J Bot* 89(11):1747–1755
- Gomiz NE, Torretta JP, Aliscioni SS (2013) Comparative anatomy of elaiophores and oil secretion in the genus *Gomesa* (Orchidaceae). *Turk J Bot* 37:859–871
- Gomiz NE, Torretta JP, Aliscioni SS (2014) *Zygostates alleniana* (Orchidaceae: Epidendroideae: Cymbidieae: Oncidiinae): estructura floral relacionada con la polinización. *Anales Jar Bot Madrid* 7:1–9
- Gomiz NE, Torretta JP, Aliscioni SS (2017) New evidence of floral elaiophores and characterization of the oil flowers in the subtribe Oncidiinae (Orchidaceae). *Plant Syst Evol* 303:433–449
- Gonzalez AM, Cristóbal CL (1997) Anatomía y ontogenia de semillas de *Helicteres lhotzkyana* (Sterculiaceae). *Bonplandia* 9(3–4):287–294
- Heslop-Harrison Y (1981) Stigma characteristics and angiosperm taxonomy. *Nor J Bot* 1:401–420
- Heslop-Harrison Y, Shivanna KR (1977) The receptive surface of the angiosperm stigma. *Ann Bot* 41:1233–1258
- Johansen DA (1940) *Plant microtechnique*. Mc Graw-Hill Book Co. Inc, New York
- Kowalkowska AK, Margońska HB, Kozieradzka-Kiszkurno M, Bohdanowicz J (2012) Studies on the ultrastructure of a threespurred fumeauxiana form of *Anacamptis pyramidalis*. *Plant Syst Evol* 298:1025–1035
- Kowalkowska AK, Kozieradzka-Kiszkurno M, Turzyński S (2015) Morphological, histological and ultrastructural features of osmophores and nectary of *Bulbophyllum wendlandianum* (Kraenzl.) Dammer (B. section Cirrhopetalum Lindl., Bulbophyllinae Schltr., Orchidaceae). *Plant Syst Evol* 301:609–622
- Kowalkowska AK, Turzyński S, Kozieradzka-Kiszkurno M, Wiśniewska N (2017) Floral structure of two species of *Bulbophyllum* section Cirrhopetalum Lindl.: *B. weberi* Ames and *B. cumingii* (Lindl.) Rchb. f. (Bulbophyllinae Schltr., Orchidaceae). *Protoplasma* 254:1431–1449
- Leitão CAE, Heidi Dolderb MA, Cortelazzob AL (2014) Anatomy and histochemistry of the nectaries of *Rodriguezia venusta* (Lindl.) Rchb. f. (Orchidaceae). *Flora* 209:233–243
- Lindley J (1830–1840) *The genera and species of orchidaceous plants*. Ridgways, Piccadilly, London
- Lindley J (1847) *The vegetative kingdom*, 2nd edn. Bradbury and Evans, London
- Luque R, Sousa HC, Kraus JE (1996) Métodos de coloração de Roeser (1972) - Modificado - E Kropp (1972), visado a substituição do azul de astra por azul de alciano 8GS on 8GX. *Acta Bot Bras* 10:199–212
- Mayer JL, Carmello-Guerreiro SM, Appezzato-Da-Glória B (2011) Anatomical development of the pericarp and seed of *Oncidium flexuosum* Sims. (Orchidaceae). *Flora* 206:601–609
- Melo MC, Borba EL, Paiva EAS (2010) Morphological and histological characterization of the osmophores and nectaries of four species of *Acianthera* (Orchidaceae: Pleurothallidinae). *Plant Syst Evol* 286:141–151
- Mosquera-Mosquera HR (2012) Análisis palinológico y anatómico del pistilo en la familia Orchidaceae. Ph.D. thesis, Universidad de León, España
- Mosquera-Mosquera HR, Valencia-Barrera RM (2009) Morfología del polinario de la subtribu Laeliinae Benth (tribu Epidendreae I, Orchidaceae) de Colombia. *Polen* 19:49–71
- Nepi M (2007) Nectary structure and ultrastructure. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and nectar*. Springer, Dordrecht, pp 129–166
- Neubig KM, Whitten WM, Williams NH, Blanco MA, Endara I, Burleigh JG, Silvera K, Cushman JC, Chase MW (2011) Generic circumscriptions of Oncidiinae (Orchidaceae: Cymbidieae) based on maximum likelihood analysis of combined DNA datasets. *Bot J Linn Soc* 168:117–146
- Neubig KM, Carlswald BS, Whitten WM, Williams NH (2015) Nectary structure and nectar in *Sobralia* and *Elleanthus* (Sobralieae: Orchidaceae). *Lankesteriana* 15(2):113–127
- Nilsson LA (1992) Orchid pollination biology. *Tree* 7:255–259
- Noguera-Savelli E, Jáuregui D (2011) Anatomía foliar comparada y relaciones filogenéticas de 11 especies de Laeliinae con énfasis en *Brassavola* (Orchidaceae). *Rev Biol Trop* 59(3):1047–1059
- Pacek A, Stpicyńska M (2007) The structure of elaiophores in *Oncidium cheirophorum* Rchb. f. and *Ornithocephalus kruegeri* Rchb. f. (Orchidaceae). *Acta Agrobot* 60:9–14
- Pacek A, Stpicyńska M, Davies KL, Szymczak G (2012) Floral elaiophore structure in four representatives of the *Ornithocephalus* clade (Orchidaceae: Oncidiinae). *Ann Bot* 110:809–820
- Pansarin EM, Pansarin LM (2011) Reproductive biology of *Trichocentrum pumilum*: an orchid pollinated by oil-collecting bees. *Plant Biol* 13:576–581
- Pansarin ER, Bittrich V, Amaral MCE (2006) At dyebreak—reproductive biology and isolation mechanisms of *Cirrhaea dependens* (Orchidaceae). *Plant Biol* 8:494–502
- Pansarin ER, Aguiar JMRBV, Pansarin LM (2013) Floral biology and histochemical analysis of *Vanilla edwallii* Hoehne (Orchidaceae: Vanilloideae): an orchid pollinated by *Epicharis* (Apidae: Centridine). *Plant Species Biol* 29:242–252
- Pansarin LM, Pansarin ER, Sazima M (2014) Osmophore structure and phylogeny of *Cirrhaea* (Orchidaceae, Stanhopeinae). *Bot J Linn Soc* 176:369–383
- Pansarin EM, Pansarin LM, Alves Dos Santos I (2015) Floral features, pollination biology, and breeding system of *Compartmentia coccinea* (Orchidaceae: Oncidiinae). *Flora* 217:57–63
- Pansarin ER, Alves Dos Santos I, Pansarin LM (2016) Comparative reproductive biology and pollinator specificity among sympatric *Gomesa* (Orchidaceae: Oncidiinae). *Plant Biol* 19:147–155
- Pridgeon AM, Stern WL (1983) Ultrastructure of osmophores in *Restrepia* (Orchidaceae). *Am J Bot* 70:1233–1243
- Pridgeon AM, Stern WL (1985) Osmophores of *Scaphosepalum* (Orchidaceae). *Bot Gaz* 146:115–123
- Proctor M, Yeo P, Lack A (1996) *The natural history of pollination*. Harper Collins, London
- Pupulin F (1998) A new species of *Ionopsis* (Orchidaceae: Oncidiinae) and a reconsideration of the genus *Konantzia*. *Harv Pap Bot* 3:225–229
- Rasmussen FN, Johansen B (2006) Carpology of orchids. *Selbyana* 27:44–53
- Reis MG, De Faria AD, Bittrich V, Amaral MDE, Marsaioli AJ (2000) The chemistry of flower rewards—*Oncidium* (Orchidaceae). *J Braz Chem Soc* 11:600–608
- Reis MG, Singer RB, Goncalves R, Marsaioli AJ (2006) The chemical composition of *Phymatidium delicatulum* and *P. tillandsioides* (Orchidaceae) floral oils. *Nat Prod Commun* 1:757–761
- Rodríguez-Robles JA, Meléndez EJ, Ackerman JD (1992) Effects of display size, flowering phenology and nectar availability on effective visitation frequency in *Compartmentia falcata* (Orchidaceae). *Am J Bot* 79:1009–1017
- Sandoval E, Terrazas T, Vallejo A (2003) Análisis fenético de caracteres anatómico-foliales de *Trichocentrum* y géneros relacionados (Orchidaceae, Oncidiinae). *Lankesteriana* 7:51–53

- Sandoval E, García-Cruz J, Terrazas T, Villaseñor JL (2010) Relaciones filogenéticas de la subtribu Oncidiinae (Orchidaceae) inferidas a partir de caracteres estructurales y secuencias de ADN (ITS y matK): un enfoque combinado. *Rev Mex Biodiv* 81:263–279
- Saunders ER (1923) A reversionary character in the stock (*Matthiola incana*) and its significance in regard to the structure and evolution of the gynoeceum in the Rhoeadales, the Orchidaceae, and other families. *Ann Bot* 37:451–482
- Schmid R (1988) Reproductive versus extra-reproductive nectaries—historical perspective and terminological recommendations. *Bot Rev* 54:179–232
- Scotland RW, Olmstead RG, Bennett JR (2003) Phylogeny reconstruction: the role of morphology. *Syst Biol* 52(4):539–548
- Sibley CG, Ahlquist JE (1987) DNA hybridization evidence of hominid phylogeny: results from an expanded data set. *J Mol Evol* 26(1–2):99–121
- Singer RB, Cocucci AA (1999) Pollination mechanisms in four sympatric southern Brazilian Epidendroideae orchids. *Lindleyana* 14:47–56
- Singer RB, Marsaioli AJ, Flach A, Reis MG (2006) The ecology and chemistry of pollination in Brazilian orchids: recent advances. In: Teixeira Da Silva JA (ed) *Floriculture, ornamental and plant biotechnology*, vol IV. Global Science Books, London, pp 569–582
- Slater AT, Calder DM (1988) The pollination biology of *Dendrobium speciosum* smith: a case of false advertising? *Aust J Bot* 36:145–158
- Slater AT, Calder DM (1990) Fine structure of the wet, detached cell stigma of the orchid *Dendrobium speciosum* Sm. *Sex Plant Reprod* 3:61–69
- Soliva M, Widmer A (2003) Gene flow across species boundaries in sympatric, sexually deceptive *Ophrys* (Orchidaceae) species. *Evolution* 57:2252–2261
- Stem WL, Carlswald BS (2006) Comparative vegetative anatomy and systematics of the Oncidiinae (Maxillarieae, Orchidaceae). *Bot J Linn Soc* 152:91–107
- Stem WL, Curry KJ, Pridgeon AM (1987) Osmophores of *Stanhopea* (Orchidaceae). *Am J Bot* 74:1323–1331
- Stpiczyńska M (2003) Floral longevity and nectar secretion of *Platanthera chlorantha* (Custer) Rchb. (Orchidaceae). *Ann Bot* 92:191–197
- Stpiczyńska M, Davies KL (2006) Nectary structure in *Symphyglossum sanguineum* (Rchb. f.) Schltr. (Orchidaceae). *Acta Agrobot* 59:7–16
- Stpiczyńska M, Davies KL (2008) Elaiophore structure and oil secretion in flowers of *Oncidium trulliferum* Lindl. and *Ornithophora radicans* (Rchb.f.) Garay and Pabst (Oncidiinae: Orchidaceae). *Ann Bot* 101:375–384
- Stpiczyńska M, Davies KL, Gregg A (2003) Nectary structure and nectar secretion in *Maxillaria coccinea* (Jacq.) L. O. Williams ex Hodge (Orchidaceae). *Ann Bot* 93:87–95
- Stpiczyńska M, Davies KL, Gregg A (2005) Comparative account of nectary structure in *Hexisea imbricata* (Lindl.) Rchb.f. (Orchidaceae). *Ann Bot* 95:749–756
- Stpiczyńska M, Davies KL, Gregg A (2007) Elaiophore diversity in three contrasting members of Oncidiinae (Orchidaceae). *Bot J Linn Soc* 155:135–148
- Stpiczyńska M, Davies KL, Pácek-Bieniek A, Kamińska M (2013) Comparative anatomy of the floral elaiophore in representatives of the newly re-circumscribed *Gomesa* and *Oncidium* clades (Orchidaceae: Oncidiinae). *Ann Bot* 112:839–854
- Stpiczyńska M, Davies KL, Kamińska M (2015) Diverse labellar secretions in African *Bulbophyllum* (Orchidaceae: Bulbophyllinae) sections *Ptiloglossum*, *Oreanastes* and *Megaclinium*. *Bot J Linn Soc* 179:266–287
- Swamy BGL (1943) Embryology of Orchidaceae. *Curr Sci* 12:13–17
- Swamy BGL (1949) Embryological studies in the Orchidaceae. I. Gametophytes. *Am Midl Nat* 41:184–201
- Teixeira SP, Leite Borba E, Semir J (2004) Lip anatomy and its implications for the pollination mechanisms of *Bulbophyllum* species (Orchidaceae). *Ann Bot* 93:499–505
- Torretta JP, Gomiz NE, Aliscioni SS, Bello ME (2011) Biología reproductiva de *Gomesa bifolia* (Orchidaceae, Cymbidieae, Oncidiinae). *Darwiniana* 49:16–24
- van der Cingel NA (2001) An atlas of orchid pollination: America, Africa, Asia and Australia. A. A. Balkema, Rotterdam
- van der Pijl L, Dodson CH (1966) Orchid flowers: their pollination and evolution. University of Miami Press, Coral Gables, Florida
- Vogel S (1963) Das sexuelle Anlockungsprinzip der Catasetinen- und Stanhopeen-Blüten und die wahre Funktion ihres sogenannten Futtergewebes. *Österr Botsch Zagreb* 100:308–337
- Vogel S (1974) Ölblumen und ölsammelnde Bienen. *Trop Subtrop Pflanzenwelt* 7:285–547
- Vogel S (1990) Ölblumen und ölsammelnde Bienen. Dritte Folge: Momordica, Thaladiantha und die Ctenoplectridae. *Trop Subtrop Pflanzenwelt* 73:1–186
- Zuloaga FO, Belgrano MJ (2018) Catálogo de Plantas Vasculares del Cono Sur. <http://www.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm>. Accessed 15 Oct 2018