Turkish Journal of Botany
http://journals.tubitak.gov.tr/botany/
Research Article

Turk J Bot
(2013) 37: 859-871
© TÜBİTAK
doi:10.3906/bot-1209-6

Comparative anatomy of elaiophores and oil secretion in the genus *Gomesa* (Orchidaceae)

Natalia Elva GOMIZ[1], **Juan Pablo TORRETTA**[1,3], **Sandra Silvina ALISCIONI**[1,2,3,*]
[1]Faculty of Agronomy, Buenos Aires University, Buenos Aires, Argentina
[2]Institute of Botany Darwinion, San Isidro, Buenos Aires, Argentina
[3]CONICET, Argentina

Received: 04.09.2012 • **Accepted:** 21.02.2013 • **Published Online:** 06.09.2013 • **Printed:** 30.09.2013

Abstract: Oils are atypical floral rewards in Orchidaceae and are produced by epidermal glands called elaiophores. Flowers of many members of the subtribe Oncidiinae either produce oil or mimic oil-producing flowers of Malpighiaceae. We focus our research on 3 species of *Gomesa*, namely, *G. flexuosa*, *G. riograndensis*, and *G. varicosa* in order to investigate the presence of elaiophores, their micromorphology and internal structure, and the process of oil secretion. Elaiophores were located using Sudan III and their internal and external features examined using light and scanning electron microscopy, respectively. The presence of elaiophores was confirmed for regions of calli that are prominent and exposed. In all 3 species the secretory tissue comprised a single layer of epidermal cells, together with some parenchymatous, subsecretory cells. Epidermal cells were isodiametric or palisade-like, with dense cytoplasm, small vacuoles, and prominent nuclei. *Gomesa flexuosa* differs from the other species investigated in that it bears intermediate elaiophores (i.e. epithelial and possessing unicellular, secretory trichomes). Based on the absence of a distended cuticle, we propose that oil may pass through the wall and cuticle as small lipid moieties. In addition, some evolutionary trends in the elaiophores in the genus *Gomesa* are suggested.

Key words: *Gomesa*, micromorphology, oil secretion, Oncidiinae, Orchidaceae

1. Introduction

Many species of orchids produce floral rewards, such as nectar, fragrances, oils, edible epidermal hairs, pseudopollen, or resin-like substances. These rewards play an important role in attracting pollinators, nectar being the most common food-reward found in Orchidaceae. However, for most orchids a lack of reward predominates. Indeed, relatively few Oncidiinae produce nectar, fragrances, or oil (Singer et al., 2006; Davies & Stpiczyńska, 2008).

In oil-producing species oil is produced by specialised glands called elaiophores that are located on the labellum, usually on the callus. The presence of elaiophores has been confirmed for representatives of several genera of Oncidiinae, such as *Gomesa* R.Br., *Lockhartia* Hook., *Oncidium* Sw., *Ornithocephalus* Hook., *Phymatidium* Lindl., and *Trichocentrum* Poepp. & Endl. (Singer & Cocucci, 1999; Flach et al., 2004; Reis et al., 2000, 2006; Stpiczyńska et al., 2007; Stpiczyńska & Davies, 2008; Pacek & Stpiczyńska, 2007; Aliscioni et al., 2009; Pansarin & Pansarin, 2011; Pacek et al., 2012).

The most recent generic circumscription of subtribe Oncidiinae is based on a number of monophyletic, strongly supported clades and recognises 61 genera. The genus *Gomesa* includes the third largest number of species, after *Oncidium* Sw. and *Telipogon* Kunth (Neubig et al., 2012).

Gomesa originally comprised 11 species endemic to Brazil (Pabst & Dungs, 1977), but following molecular phylogenetic analyses based on nuclear and plastid DNA data, this was increased to encompass most of the Brazilian species previously included in *Oncidium* (Chase, 2009; Chase et al., 2009). According to the most recent circumscription, *Gomesa* includes 125 species that are distributed very widely in the Neotropics (Neubig et al., 2012).

Vogel (1974) described 2 types of elaiophores; trichomatous and epithelial. Some authors have suggested that elaiophores may be derived from nectaries (Stpiczyńska et al., 2007) and that the resemblance of the epithelial elaiophores of several Oncidiinae species to those of Malpighiaceae is probably due to evolutionary convergence (Singer and Cocucci, 1999; van der Cingel,

---

* Correspondence: aliscion@agro.uba.ar

2001; Singer et al., 2006; Stpiczyńska et al., 2007; Davies and Stpiczyńska, 2008). However, in other species of Oncidiinae, oil is produced in poorly defined areas and may merely mimic the spectral reflection of Malpighiaceae flowers rather than provide an actual reward (Chase et al., 2009). Nevertheless, current understanding of elaiophore structure and oil secretion in *Gomesa*, given the size of this genus, is still somewhat limited and based on a relatively small number of species.

Neubig et al. (2012) commented that a large percentage of Oncidiinae possess flowers that either produce an oil reward or mimic oil-producing flowers of Malpighiaceae in terms of their colour and morphology. These latter species produce oil-deceit flowers that attract oil-collecting bees but present no reward whatsoever. In recent years several anatomical investigations have demonstrated the presence of elaiophores in certain species of Oncidiinae (Singer & Cocucci, 1999; Pacek & Stpiczyńska, 2007; Stpiczyńska et al., 2007; Stpiczyńska & Davies, 2008; Aliscioni et al., 2009; Davies & Stpiczyńska, 2009; Pacek et al., 2012). Thus, the number of species of Oncidiinae that are known to produce an oil reward is steadily increasing.

The floral morphology of members of subtribe Oncidiinae is highly diverse and related to pollination strategies (Dressler, 1993). Consequently, these orchids provide a very attractive group for evolutionary studies. In order to understand the evolution of oil-offering flowers in Oncidiinae, both a robust phylogenetic framework and detailed morphological studies of flowers covering a large number of species are necessary.

Several well-resolved phylogenies for the subtribe Oncidiinae based on a large number of sampled species were recently published, thereby providing a frame of reference for evolutionary studies of floral morphology (Chase et al., 2009; Neubig et al., 2012). In addition, Renner and Schaefer (2010) provided a list detailing the distribution of oil rewards amongst flowering plants and added a historical context to the study of elaiophore evolution. They noted that the oil flower syndrome, especially that of tropical Orchidaceae, where it is very diverse, is poorly known.

Similarities in floral oil secretion in both Malpighiaceae and certain Oncidiinae are thought to be due to parallelism (Neubig et al., 2012). Many clades of Oncidiinae also exhibit considerable variations in pollination-related traits. However, there is insufficient anatomical information available to test this hypothesis. Elaiophores occur in different clades of Oncidiinae, where they are thought to have arisen at least 7 times (Renner & Schaefer, 2010), being particularly well represented in *Gomesa*. In order to understand the evolution of elaiophores in *Gomesa*, we focused our research on representative species of this genus. In this first report, we studied 3 species: *Gomesa flexuosa* (Lodd.) M.W.Chase & N.H.Williams, *G. riograndensis* (Cogn.) M.W.Chase & N.H.Williams, and *G. varicosa* (Lindl.) M.W.Chase & N.H.Williams. Our aim was to identify the presence of elaiophores in these species, to describe their structure (both internal structure and external micromorphology), and to investigate how oil is secreted. Moreover, we propose a hypothesis for the origin and evolution of elaiophores in the genus *Gomesa*.

2. Materials and methods

Fresh flowers of *Gomesa flexuosa*, *G. riograndensis*, and *G. varicosa* were obtained from plants cultivated at the Botanical Garden Lucien Hauman at the Faculty of Agronomy, University of Buenos Aires, Argentina. The original plants were derived from natural populations growing at Montecarlo, Misiones, Argentina. Reference vouchers were deposited in the Herbario Gaspar Xuarez of the Faculty of Agronomy, University of Buenos Aires (BAA). Identification of investigated specimens was corroborated by comparison with herbarium specimens deposited at BAA and the Institute of Botany Darwinion (SI).

Fresh, entire flowers of the 3 species were examined using a Wild M5 stereomicroscope and subsequently submerged in saturated alcoholic Sudan III solution in order to detect the presence of lipids. Labellar fragments showing positive reactions with Sudan III were sectioned by hand and the location of elaiophores confirmed using light microscopy (LM).

Transverse and longitudinal sections of elaiophores were examined under a light microscope. Fresh material was fixed in F.A.A. [ethyl alcohol 70%:glacial acetic acid:formaldehyde 40%; 90:5:5] for 48 h and stored in 70% ethanol. The samples were then dehydrated in an ethanol series, transferred to xylene, embedded in paraffin (58 °C), and sectioned at a thickness of 6–7 µm on a rotary microtome (Leitz Wetzlar) using conventional methods. Histological samples were stained with safranin-fast green and mounted in Canada balsam (D'Ambrogio de Argüeso, 1986). Observations were made using a Wild M20 optical light microscope; polarised light was used to detect any crystalline structures present. Photomicrographs and measurements were taken using Axio Vs40 V 4.8.2.0 (Carl Zeiss).

For scanning electron microscopy (SEM), portions of the labellum were dehydrated and subjected to critical-point drying using liquid CO_2. The material was then sputter-coated with gold and examined using a Philips XL 30 TMP microscope at an accelerating voltage of 80 kV.

3. Results

3.1. Morphology

All 3 species have paniculate inflorescences measuring about 20–80 cm in length and bearing 10–20 flowers. The flowers lack fragrance and are approximately 1.3–2.3 cm in diameter. Sepals and petals are yellow with brown to red-brown spots or transverse markings; the lateral sepals are partly fused.

The labellum is 3-lobed with a large mid-lobe (usually yellow and emarginate) and 2 small, auriculate, lateral lobes in *Gomesa flexuosa* and *G. varicosa*. In *G. riograndensis*, the mid-lobe is smaller and often brown, while the lateral lobes vary from quadrangular to acute and are horizontally curved.

The calli are located on the base of the mid-lobes, between the lateral lobes, but are morphologically strikingly different for each of the 3 species investigated. The callus of *Gomesa flexuosa* is subdivided into a basal, pulvinular section and an apical area bearing protuberances (Figure 1). In *G. riograndensis* the callus is more prominent, accounting for more than half of the surface of the mid-lobe, and 3 areas can be recognised: a basal, transverse, crinkled platform; a central, flat, inverted heart-shaped section; and an apical, irregularly verrucose area (Figure 1). *Gomesa varicosa* possesses a callus formed by a basal, tri-lobulate platform and an apical, central keel, both surrounded by several minor lateral protuberances (Figure 1).

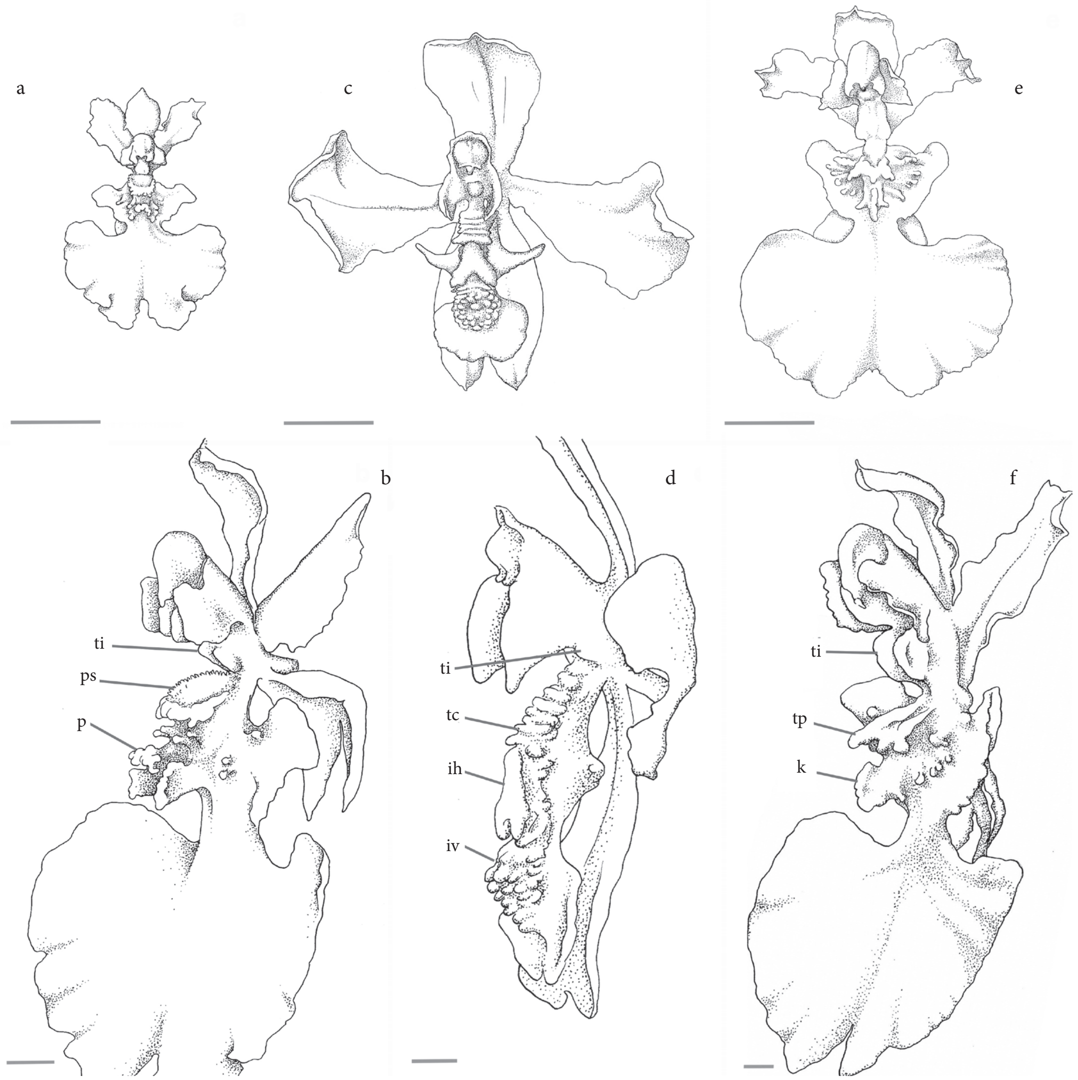

Figure 1. General aspect of flowers. *Gomesa flexuosa*: a- in frontal view, b- in lateral view; *G. riograndensis*: c- in frontal view, d- in lateral view; *G. varicosa*: e- in frontal view, f- in lateral view. References = **ti**- tabula infrastigmatica, **ps**- basal, pulvinular section, **p**- apical area with protuberances, **tc**- basal, transverse, crinkled area, **ih**- central, inverted heart-shaped section, **iv**- apical, irregularly verrucose area, **tp**- basal, tri-lobulate platform, **k**- apical, central keel. Scale bars: a, c, e = 5 mm; b, d, f = 1 mm.

A tabula infrastigmatica, located between the base of the column and the callus, is obvious in all 3 species and is more developed in *G. flexuosa* and *G. varicosa* than in *G. riograndensis* (Figure 1).

3.2. Sudan III reaction

In all 3 species the callus reacted more strongly to saturated, alcoholic Sudan III solution than did any other part of the flower, indicating that this is the site of the elaiophore. However, the most intensely stained section varied among species: the central, flat, inverted heart-shaped region in *Gomesa riograndensis* (Figure 2); the basal, pulvinular area and tips of the callus protuberances in *G. flexuosa* (Figure 2); and the basal, tri-lobulate platform, central keel, and tips of the lateral protuberances in *G. varicosa* (not shown).

3.3. Scanning electron microscopy (SEM)

The callus of *Gomesa flexuosa*, when viewed with SEM, revealed that the basal, pulvinular surface is entirely coated with long trichomes that gradually diminish in length from the centre of this region towards the periphery. These trichomes are unicellular and cylindrical with dome-shaped tips and a smooth or pulverulent cuticle. This trichomatous area is rounded by a slightly faveolate, semicircular region of smooth epidermal cells with few stomata. The apical area of the callus has protuberances or finger-like projections covered with smooth epidermal cells and an irregular cuticle, while in deeper areas the surface is papillose. These pyriform papillae become progressively conical to globose as they approach the lateral lobes (Figure 3).

SEM observations revealed that each of the 3 regions of the callus of *G. riograndensis* has a different texture. The basal platform is slightly raised to very obviously transversely corrugated, depending on the specimen, and the epidermal cells are somewhat inflated with a pulverulent cuticle. The central, inverted heart-shaped section consists of flat epidermal cells with a smooth cuticle. The apical area is irregularly verrucose, with some epidermal cells having a small, central papilla. Stomata are rare or absent for all 3 regions (Figure 4).

The basal, tri-lobulate platform, the apical keel, and the tips of the lateral protuberances of the callus of *Gomesa varicosa* are clothed by flat cells with a slightly striate

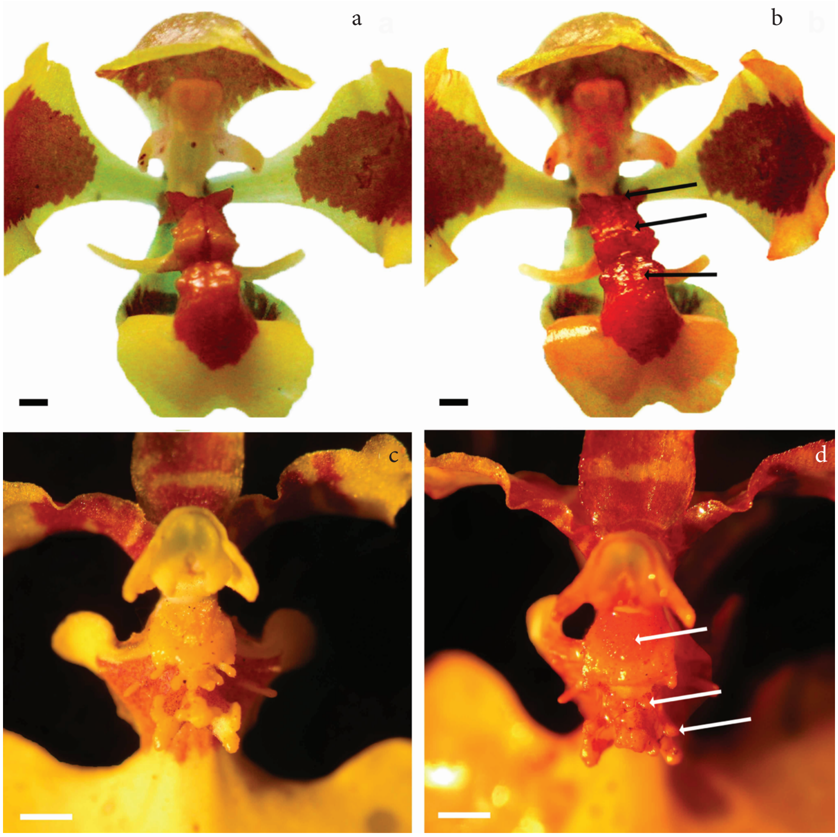

Figure 2. Elaiophores in flowers of *Gomesa* prior to (a and c) and following (b and d) staining with Sudan III, indicated by arrows. **a** and **b**- *Gomesa riograndensis*, **c** and **d**- *G. flexuosa*. Scale bars: a, b, c, d = 3 mm.

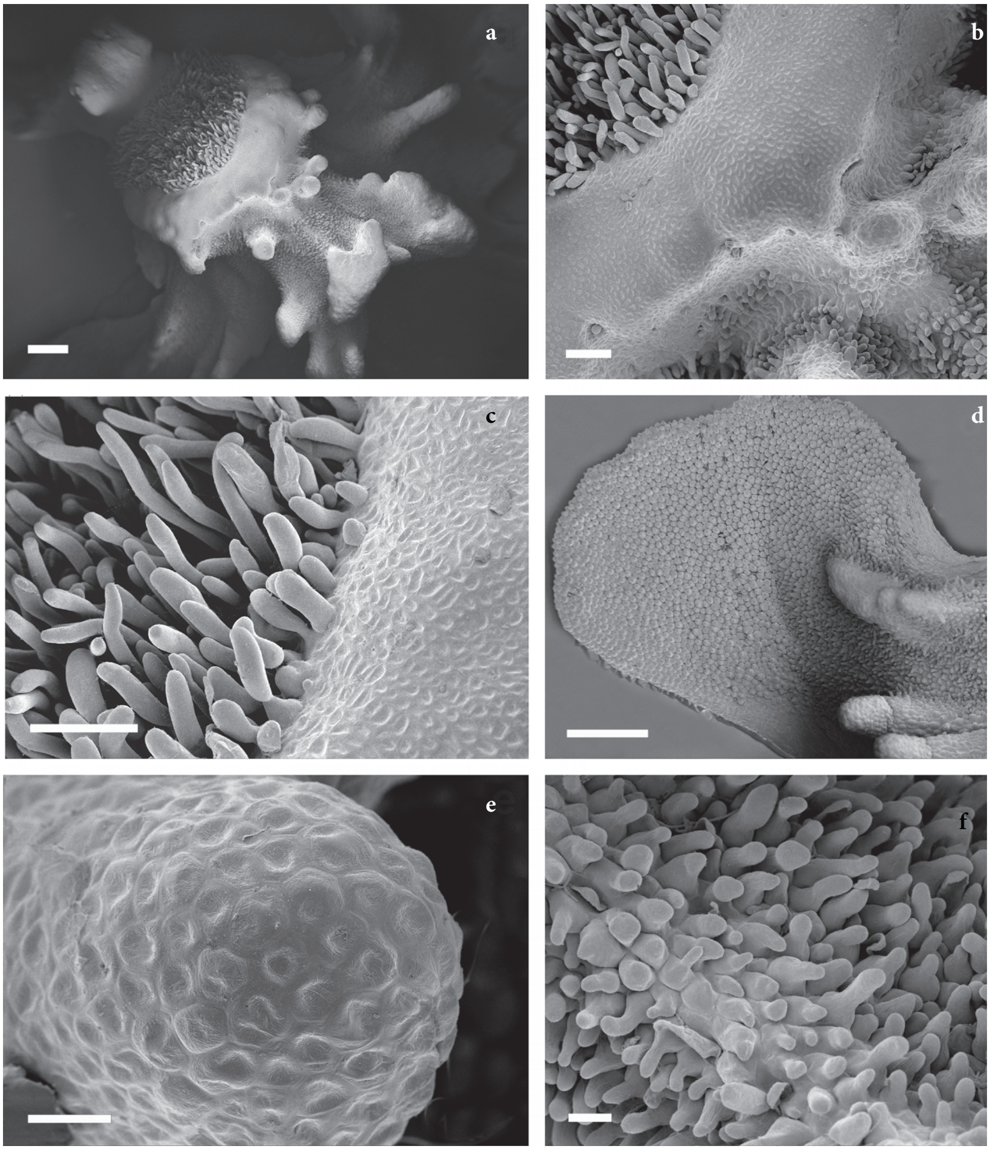

Figure 3. Scanning electron micrographs of the callus of *Gomesa flexuosa*. a- general view of the callus, b- border of the pulvinular area, c- unicellular trichomes of pulvinular area, d- lateral lobes of labellum and lateral protuberances of callus, e- detail of cells at the tips of callus protuberances, f- papillae between callus protuberances. Scale bars: a = 300 µm; b = 100 µm; c = 100 µm; d = 300 µm; e = 30 µm; f = 30 µm.

cuticle. The epidermis of the deeper areas of the callus and the region between the tabula infrastigmatica and the basal crest consist of pyriform papillae. Stomata are present and are mainly located on the upper part of the central keel. The lateral lobes bear globose epidermal cells (Figure 5).

3.4. Light microscopy (LM)

Observations of the internal structure of the elaiophores of all 3 species using light microscopy confirmed that the principal secretory tissue in each case is the epidermis of the callus.

The basal, pulvinular section of the callus of *Gomesa flexuosa* is densely covered with unicellular trichomes (100–350 µm long) that contain dense cytoplasm and obvious nuclei. A thick cuticle (0.9–1.1 µm) is present. In some trichomes, small crystals were observed. The cells of the epidermis and the first few layers of subepidermal cells around the pulvinular area are more or less isodiametric (20–27 µm) with small vacuoles, centrally located nuclei (some containing 2–3 nucleoli), and very dense cytoplasm. Similar features were observed for cells at the tips of protuberances on the apical area of the callus. The papillae

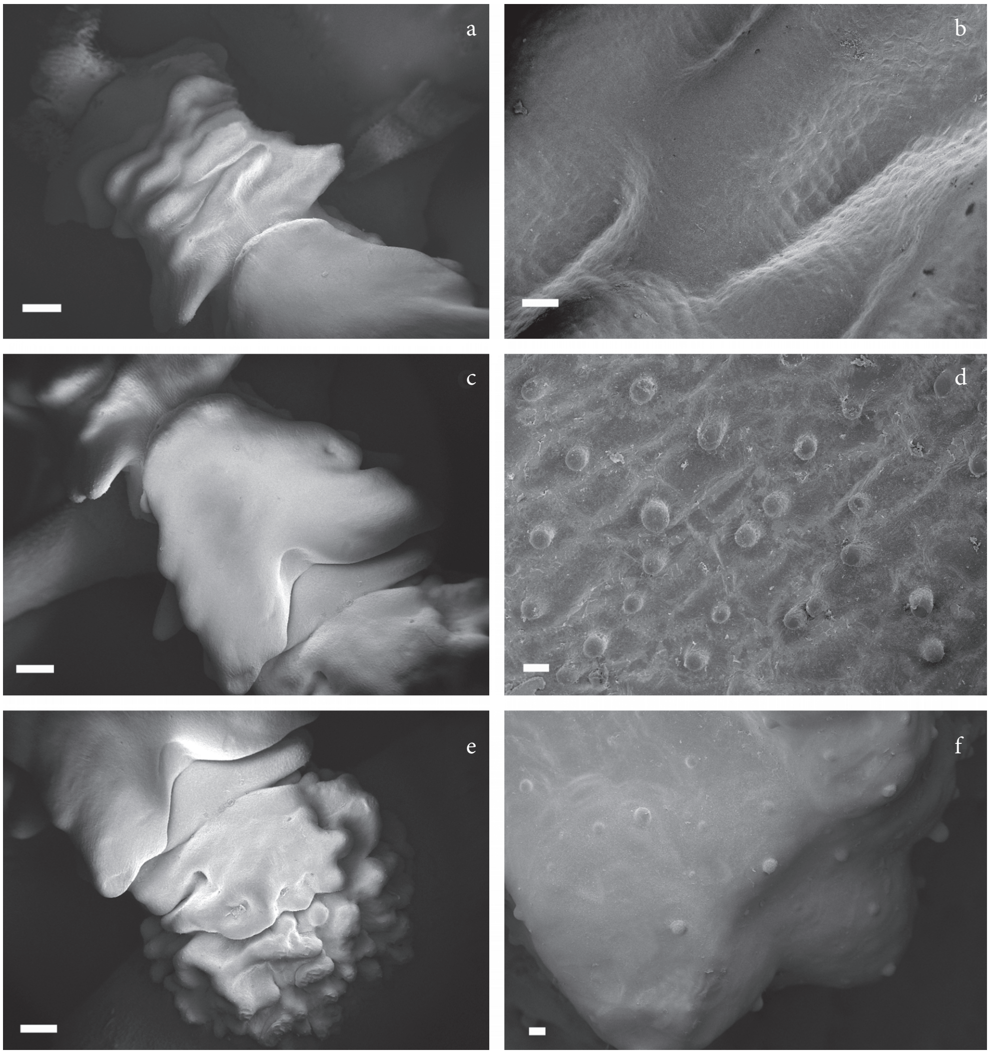

Figure 4. Scanning electron micrographs of the callus of *Gomesa riograndensis*. a- aspect of the transversely corrugate, basal platform; b- detail of the transversely corrugate, basal platform; c- central, inverted heart- shaped section; d- detail of the lateral lobes; e- apical, irregularly verrucose area; f- detail of the apical area. Scale bars: a = 300 µm; b = 40 µm; c = 300 µm; d = 10 µm; e = 300 µm; f = 10 µm.

of the deeper areas between the protuberances and the lateral lobes contained translucent cytoplasm that did not stain deeply. Polarised light microscopy revealed the presence of small, intravacuolar crystals within trichomes and subepidermal cells of the pulvinular area. Numerous idioblasts with raphides and plastids were also present in the subjacent parenchyma of both regions of the callus (Figure 6).

In *G. riograndensis* the epidermal cells along the entire callus contained dense cytoplasm that stained intensely, large basal nuclei, often containing several nucleoli, and small, parietal vacuoles. Throughout the callus, epidermal cells were radially elongated in the central, inverted heart-shaped section. These measured 50–80 µm × 7–10 µm but were more isodiametric towards the apical and basal zones of the callus. The external tangential walls were thick and cutinised with an obvious cuticle (1.1–2.0 µm). The subjacent tissue was composed of isodiametric, parenchymatous cells with plastids and numerous idioblasts with raphides. The adaxial surface of the central, inverted heart-shaped section of the callus of some specimens possessed a channel consisting of epidermal cells with dense cytoplasm (Figure 7).

The callus of *Gomesa varicosa* showed that the epidermal cells and some subjacent cells stained more intensely than the inner parenchyma. The epidermal cells of the basal, tri-lobulate platform, the central keel, and the tips of callus protuberances were somewhat elongate, measuring 30–50 µm × 15–20 µm and contained dense cytoplasm, an apical vacuole, and a large nucleus with an obvious nucleolus.

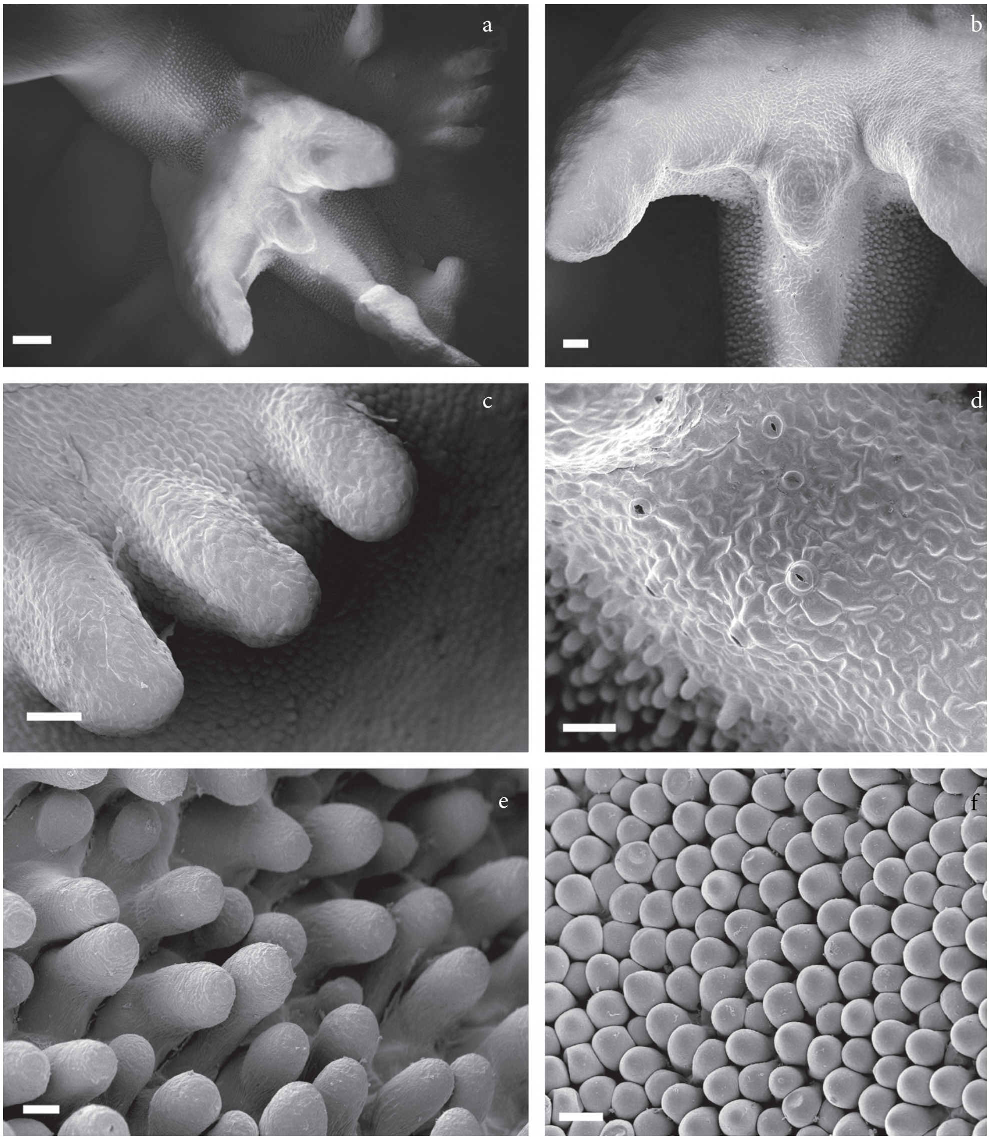

Figure 5. Scanning electron micrographs of callus of *Gomesa varicose*. a- general view of the callus; b- basal, tri-lobulate platform; c- lateral protuberances; d- detail of epidermal cells and stomata between apical, central keel; e- detail of nonsecreting papillae; f- lateral lobes. Scale bars: a = 300 µm; b = 100 µm; c = 100 µm; d = 50 µm; e = 10 µm; f = 30 µm.

This tissue was restricted only to regions of the epidermis having flat cells, whereas in deeper sections of the callus and on the lateral lobes, the epidermal cells were papillose, and their cytoplasm remained relatively unstained. Small idioblasts with raphides and plastids were present in the subjacent parenchyma (Figure 8).

4. Discussion

Our results confirm the presence of elaiophores in *Gomesa flexuosa*, *G. riograndensis*, and *G. varicosa*. Although the external morphology of the elaiophores varied between the species studied, their general anatomy was similar to that described previously for other species of Oncidiinae (Singer & Cocucci, 1999; Pacek & Stpiczyńska, 2007; Stpiczyńska et al., 2007; Stpiczyńska & Davies, 2008; Aliscioni et al., 2009; Davies & Stpiczyńska, 2009; Pacek et al., 2012). In the 3 species investigated here, elaiophores were located on the callus, in those areas that are most prominent and exposed. The whole elaiophore of *G. riograndensis* and *G. varicosa* but only the apical area of the callus of *G. flexuosa* comprised a single layer of epidermal secretory cells together with some layers of parenchymatous subsecretory tissue. These epidermal cells were isodiametric or palisade-like with dense cytoplasm, small vacuoles, and obvious nuclei that

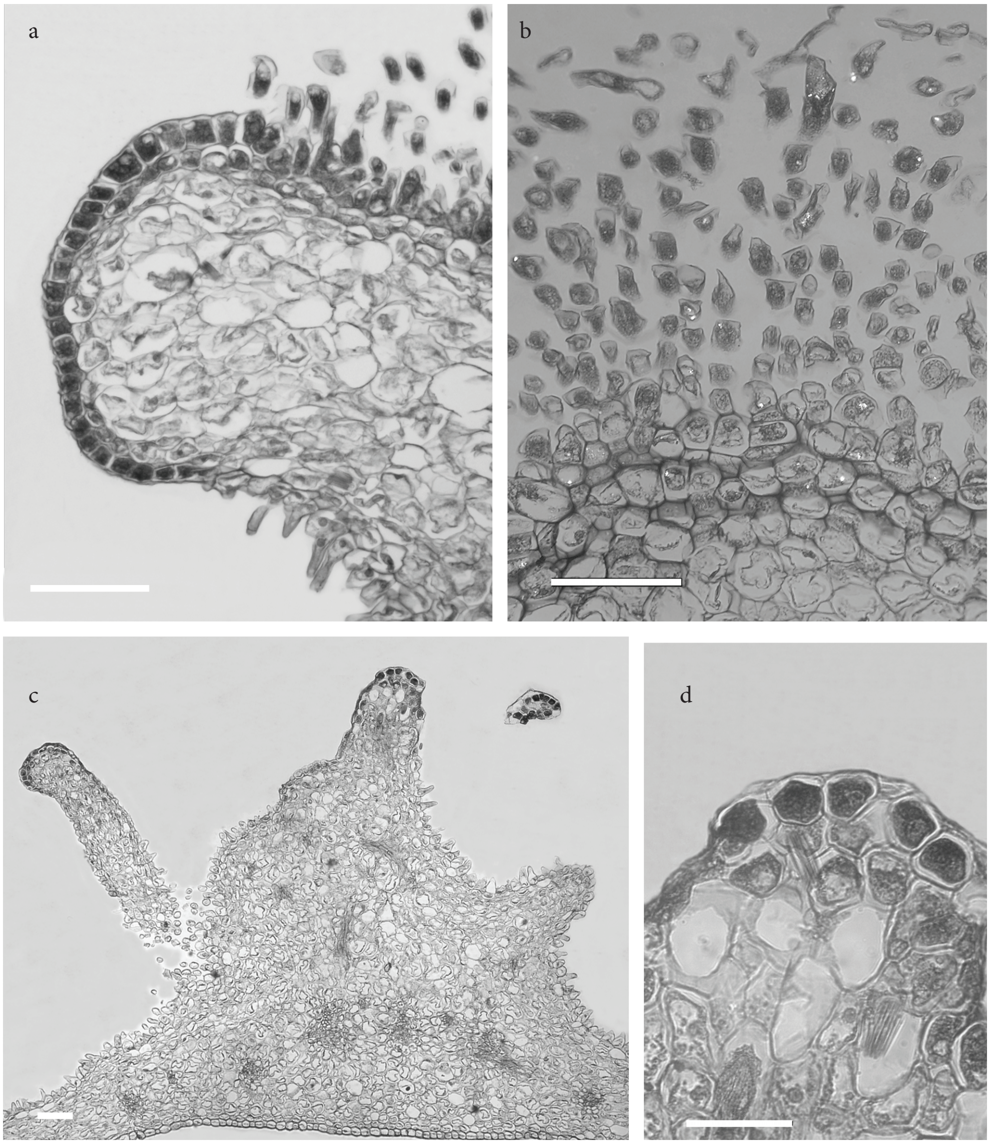

Figure 6. Light micrographs of *Gomesa flexuosa.* a- longitudinal section through border of the pulvinular area; b- longitudinal section of the pulvinular area with trichomes. Note small crystals visible when using polarised light; c- transverse section through the apical area, with protuberances; d- detail of tip of callus protuberance. Note idioblasts with raphides. Scale bars: a = 100 µm; b = 100 µm; c = 200 µm; d = 50 µm.

indicated a high degree of cellular metabolic activity. The parenchymatous subsecretory tissue also showed similar features, together with plastids, also indicating high metabolic activity. Since living tissue in these regions of the labellum stained with Sudan III, it is probable that their main function is the secretion of oil.

Following the classification of Vogel (1974), the elaiophores of *Gomesa riograndensis* and *G. varicosa* belong to the epithelial type, but *G. flexuosa* showed both epithelial and trichomatous regions. This intermediate type of elaiophore is described here for first time in the genus *Gomesa*; it has previously been described for *Ornithocephalus gladiatus* Hook. (Pacek et al., 2012), also in Oncidiinae. Pacek and co-workers (Pacek and Stpiczyńska, 2007; Pacek et al., 2012) also described trichomatous elaiophores in other members of the *Ornithocephalus* clade, including *O. ciliatus* Lindl. (as *O. kruegeri* Rchb.f.), *Phymatidium falcifolium* Lindl., *Zygostates grandiflora* (Lindl.) Mansf., and *Z. lunata* Lindl., species not closely related to *Gomesa*.

Numerous hypotheses have been proposed for the role of crystals in plants, including calcium regulation, plant

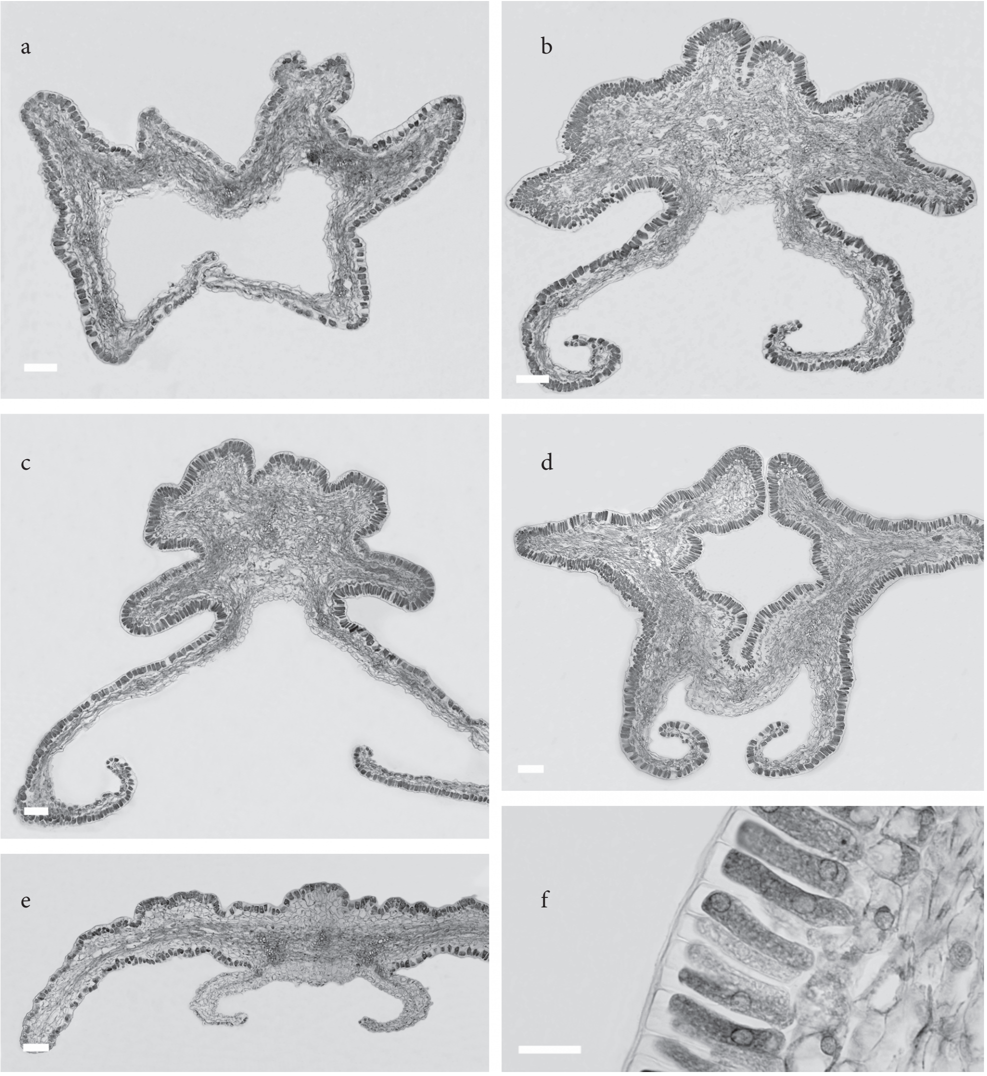

Figure 7. Light micrographs of *Gomesa riograndensis.* Transverse sections of the callus through: a- basal, transverse, crinkled area; b- central, inverted heart-shaped area; c- apical, irregularly verrucose area; d- central, inverted heart-shaped area in specimens with channel consisting of secretory cells; e- terminal portion of the callus in the apical, irregularly verrucose area; f- detail of secretory cells from the apical, irregularly verrucose area. Scale bars: a, b, c, d, e = 100 µm; f = 50 µm.

protection, detoxification, ion balance, tissue support/plant rigidity, and even the gathering and reflection of light (Franceschi & Nakata, 2005). Coté and Gibernau (2012) tested the function of calcium oxalate crystals in floral organs of Araceae relative to pollination strategy and concluded that these crystals protect the plant against insect predation of gametes and embryos and may possibly limit direct feeding by pollinators of some species.

Numerous idioblasts containing raphides, similar to those commonly found in elaiophores of other species of Oncidiinae, were observed in the taxa studied here. Although it is not possible to be certain about their function, possible roles include providing mechanical strength, thus protecting the flower from the foraging activities of the pollinator as it gathers oil (Aliscioni et al., 2009).

Areas between the callus protrusions, as well the lateral lobes of *Gomesa flexuosa, G. riograndensis*, and *G. varicosa*, consisted of papillose cells. These cells are not secretory but may have a mechanical role, facilitating the accumulation and retention of oils on these parts of the labellum, as proposed for *Gomesa bifolia* (Aliscioni et al., 2009).

At present, the secretion of oil in the genus *Gomesa* has been reported for 18 species, and of these, the detailed anatomy of the elaiophore has been described for 9 taxa,

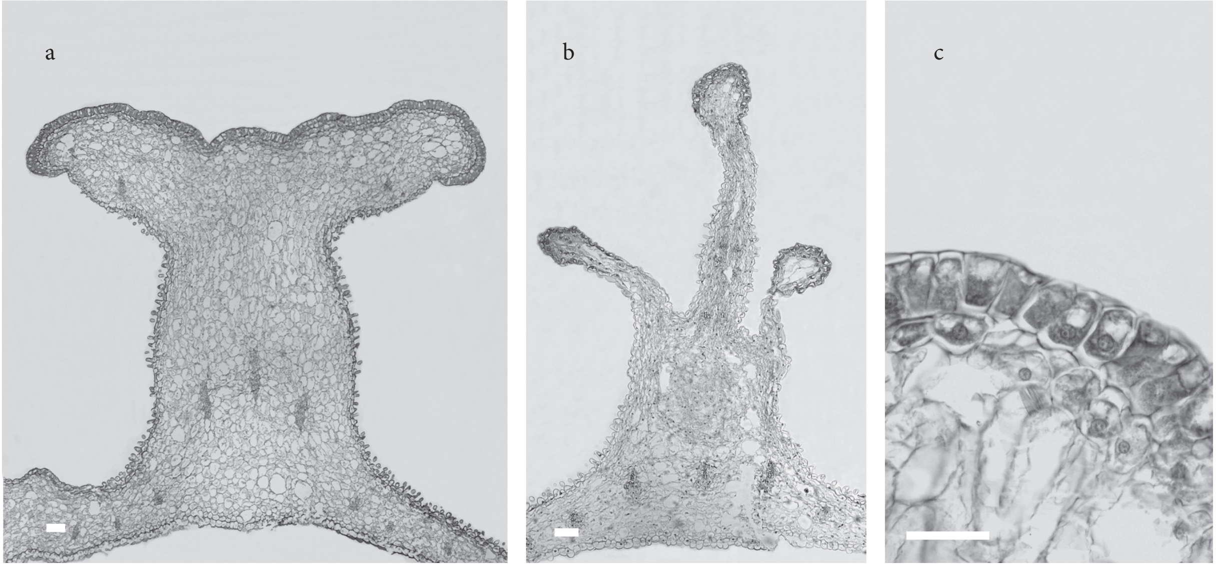

Figure 8. Light micrographs of *Gomesa varicosa.* Transverse sections of the callus through: a- basal, tri-lobulate platform; b- apical, central keel, and protuberances; c- detail of tip of protuberance. Scale bars: a = 100 µm; b = 100 µm; c = 50 µm.

including the 3 studied here (Table). In *Gomesa*, elaiophores are usually of the epithelial (occasionally intermediate) type and located on the callus. Only the secretory tissue of the elaiophore of *G. recurva* Lodd. was described as showing no obvious anatomical differentiation (Stpiczyńska et al., 2007). Comparative anatomical examination of the elaiophores of the species studied in this paper revealed that those of *G. varicosa* and *G. riograndensis* are similar to those of *G. bifolia* (Aliscioni et al., 2009) and *G. loefgrenii* (Stpiczyńska et al., 2007); however, unlike the last 2 species, their elaiophores lack obvious cavities in the outer tangential walls of the epidermis.

In several species of Oncidiinae, elaiophores display cuticular distension following the accumulation of oil between the outer tangential wall and the overlying cuticle. This has been reported for *G. radicans* (Stpiczyńska & Davies, 2008), *G. paranaensis* (Singer & Cocucci, 1999), *Trichocentrum cavendishianum* (Bateman) M.W.Chase & N.H.Williams (Stpiczyńska et al., 2007), *Oncidium cheirophorum* Rchb.f. (Pacek & Stpiczyńska, 2007), and *O. ornithorhynchum* Kunth (Davies & Stpiczyńska, 2009). However, in the species that form the subject of this paper, this character was not observed.

Although ultrastructural observations were not made for the present study, it is likely, based on the absence of cell wall cavities and distended cuticle, that the oil passes through the wall as small lipid moieties that then reassemble to form a continuous lipid layer that coats the labellar epidermal surface, as described for *G. bifolia* (Aliscioni et al., 2009).

Davies and Stpiczyńska (2009) showed that the proximal part of the labellum and the column of orchids that are pollinated by oil-gathering bees tend to lie more or less parallel to each other even though the distal part of the labellum may be more or less vertical. Although we do not have pollinator data for the species studied, some parts of the calli, such as the pulvinular area of *Gomesa flexuosa*; the basal, transverse, crinkled platform of *G. riograndensis*; and the basal, tri-lobulate platform of *G. varicosa*, are positioned at a close, sharp angle to the column. The tabula infrastigmatica is very obvious in *G. flexuosa* and *G. varicosa* but less so in *G. riograndensis*. In this last species, the basal, transverse, crinkled platform of the callus may also function as a tabula infrastigmatica.

All these characters, along with the presence of elaiophores, support the hypothesis that these species are pollinated by oil-gathering bees. For many Oncidiinae, floral oils are collected by female bees of the genera *Centris* Fabricius, *Tetrapedia* Klug, and *Paratetrapedia* Moure (Apidae) (Buchmann, 1987; Singer & Cocucci, 1999; Torretta et al., 2011), and it has been proposed that oil may be mixed with pollen to provide food for larval stages of these bees or to line their nests (Neff and Simpson, 1981; Alves-dos-Santos et al., 2007).

Many Oncidiinae attract pollinators by mimicking reward-offering flowers that share their native habitats (Williams et al., 2001), although little is currently known about in situ pollination. Recently, a study of *G. bifolia* showed a female *Centris trigonoides* Lepeletier (Apidae: Centridini) foraging for floral oil on the callus of this orchid (Torretta et al., 2011). The bee alighted on the callus, grasped the tabula infrastigmatica with its mandibles, and collected oil by scraping the callus with its front and middle legs. While in this position, the insect came into

Table. List of oil-rewarding species of *Gomesa* with anatomical descriptions of their elaiophores. Species in bold are included in the phylogeny of subtribe Oncidiinae (Neubig et al., 2012).

Taxa	Anatomy of elaiophore	Reference
Gomesa amicta	No	Singer et al., 2006
G. bifolia	Yes	Aliscioni et al., 2009
G. cornigera	No	Singer et al., 2006
G. cuneata	No	Singer et al., 2006
G. echinata	No	Singer et al., 2006
G. flexuosa	Yes	This study
G. hookeri	No	Alcántara et al., 2006
G. kautskyi	No	Singer et al., 2006
G. loefgrenii	Yes	Stpiczynska et al., 2007
G. longicornu	No	Singer et al., 2006
G. paranaensis	Yes	Singer & Cocucci, 1999
G. pubes	No	Singer et al., 2006
G. radicans	Yes	Stpiczynska & Davies, 2008
G. recurva	Yes	Stpiczynska et al., 2007
G. riograndensis	Yes	This study
G. varicosa	Yes	This study
G. venusta	Yes	Stpiczynska & Davies, 2008
G. welteri	No	Singer et al., 2006

contact with the viscidium, which adhered to the clypeal region.

The presence of oil as a pollinator reward in diverse clades of subtribe Oncidiinae (Renner & Schaefer, 2010; Neubig et al., 2012) indicates that elaiophores have arisen on several occasions in response to pollinator pressures. Floral oil is also present in other phylogenetically unrelated families (or some of their members), such as Malpighiaceae and Iridaceae. Therefore, in a wider context, pollination by oil-collecting bees may play an important role in the convergence of this character.

The morphology of Oncidiinae flowers is probably the result of a complex mixture of Batesian and Müllerian mimicry (Roy & Widmer, 1999). Based on spectral reflectance analyses, Powell (2008) established that many Oncidiinae with yellow flowers closely match those of yellow Malpighiaceae species, thus satisfying one of the criteria for Batesian mimicry. Nevertheless, in recent years the number of species known to possess elaiophores has increased significantly, with some authors proposing that the deceit/pollination syndrome may be more restricted in Oncidiinae than originally thought (Reis et al., 2006; Torretta et al., 2011).

Neubig et al. (2012) mentioned the likelihood of complex mimicry relationships between Malpighiaceae species, oil-producing Oncidiinae, and oil-deceit Oncidiinae. Whether Müllerian mimicry followed by Batesian mimicry can explain most cases involving the appearance and evolution of new oil hosts, however, depends on obtaining further field data on oil-bee behaviour (Renner & Schaefer, 2010).

Machaka-Houri et al. (2012) reported that the reproductive success of the deceptive Orquidaceae is lower than in species with reward. Although the species studied in the current work present elaiophores, future analyses of reproductive success, to quantify the oil production, would be important to determining whether oil is valuable as a real reward.

Important advances have been made in our understanding of the phylogeny of Oncidiinae. The most recent phylogenetic study of this subtribe was based on 46 species of *Gomesa*; these represent about one-third of the entire genus (Neubig et al. 2012), of which 12 species possess oil-reward flowers, and of these, elaiophore anatomy has been studied in 9. Consequently, although our understanding of oil-secreting glands in *Gomesa* has perhaps been over-simplified, such studies have nonetheless provided us with our first insight into the general trends that operate in this genus.

Unfortunately, in the absence of adequate elaiophore anatomy and pollination system data for all species currently assigned to *Gomesa*, mapping these characters on phylogeny is not yet possible. Even so, it is still possible to postulate some likely hypotheses. Currently, there is sufficient evidence to consider *Gomesa* a monophyletic genus, and, in this context, one of the most parsimonious explanations for the distribution of oil glands in the genus is that they evolved only once in the common ancestor, but that this capacity to secrete oil has subsequently been lost on several occasions. Determining the exact number of elaiophore gains/losses in *Gomesa* requires much more anatomical data than are currently available for floral secreting tissues. Nevertheless, based on the currently available information, epithelial elaiophores are the most commonly encountered secretory structure and probably represent the ancestral condition for the genus. These elaiophores could subsequently diversify within different subclades, finally resulting in diverse anatomical organisation and oil-secreting mechanisms.

Based on the phylogeny of Neubig et al. (2012), the presence of elaiophores appears in both ancestral and derived branches. For example, *Gomesa venusta* (Drapiez) M.W.Chase & N.H.Williams (cited as *Oncidium trulliferum* Lindl.), whose elaiophores were described by Stpiczyńska and Davies (2008), is placed near the base of the tree, in contrast to *G. flexuosa* (studied here) which belongs to one of more recent subclades.

Owing to the presence of elaiophores in representatives of different subclades of *Gomesa*, it is likely that in the ancestor of this genus this structure arose only once and thus represents a synapomorphy. Once present, the elaiophore may have evolved in several ways (epithelial or trichomatous) and developed diverse secretory mechanisms (cuticular distension or trans-mural passage). Furthermore, in some species of *Gomesa*, the elaiophores were lost, as in several other angiosperm genera and families where the oil-reward was lost more often than it was gained (Renner & Schaefer, 2010). However, the elaiophores of many more species of *Gomesa* have still not been investigated, and until this is done such hypotheses remain tentative.

Clearly, to determine whether *Gomesa* species are predominantly Batesian or Müllerian mimics, further phylogenetic studies that incorporate additional species are needed, and more morphological and anatomical studies are required in the genus. However, the current results are promising to help understand the way this particular pollination system has evolved in the large genus of Oncidiinae.

Acknowledgements

We are grateful to the anonymous referees, E Kellogg and R Saurral, for comments on the manuscript and the English revision and to the staff of the Lucien Hauman Botanical Garden (Facultad de Agronomía, Universidad de Buenos Aires) and S. Rosenfeldt (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires) for making plant material available. Funding was provided by Buenos Aires University (UBACYT-20020090100068). NG is supported by a UBACYT fellowship, and JPT and SSA by CONICET, Argentina.

References

Alcántara S, Semir J & Solferin VN (2006). Low genetic structure in an epiphytic Orchidaceae (*Oncidium hookeri*) in the Atlantic rainforest of south-eastern Brazil. *Annals of Botany* 98: 1207–1213.

Aliscioni SS, Torretta JP, Bello ME & Galati BG (2009). Elaiophores in *Gomesa bifolia* (Sims) M.W. Chase & N.H. Williams (Oncidiinae: Cymbidieae: Orchidaceae): structure and oil secretion. *Annals of Botany* 104: 1141–1149.

Alves-dos-Santos I, Machado IC & Gaglianone MC (2007). História natural das abelhas coletoras de óleo. *Oecologia Brasiliensis* 11: 544–557.

Buchmann SL (1987). The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics* 18: 343–369.

Chase MW (2009). A new name for the single species of *Nohawilliamsia* and corrections in *Gomesa* (Orchidaceae). *Phytotaxa* 1: 57–59.

Chase MW, Williams NH, Faria AD, Neubig KM, Amaral MCE & Whitten WM (2009). Floral convergence in Oncidiinae (Cymbidieae; Orchidaceae): an expanded concept of *Gomesa* and a new genus *Nohawilliamsia. Annals of Botany* 104: 387–402.

Coté GG & Gibernau M (2012). Distribution of calcium oxalate crystals in floral organs of Araceae in relation to pollination strategy. *American Journal of Botany* 99: 1231–1242.

van der Cingel NA (2001). *An Atlas of Orchid Pollination: America, Africa, Asia, and Australia*. Rotterdam: A.A. Balkema.

D'Ambrogio de Argüeso AC (1986). *Manual de Técnicas en Histología Vegetal*. Buenos Aires: Hemisferio Sur.

Davies KL & Stpiczyńska M (2008). The anatomical basis of floral, food-reward production in Orchidaceae. In: Teixeira da Silva J (ed.) *Floriculture, Ornamental and Biotechnology: Advances and Topical Issues*, Vol. V. Isleworth, Middlesex: Global Science Books, pp. 392–407.

Davies KL & Stpiczyńska M (2009). Comparative histology of floral elaiophores in the orchids *Rudolfiella picta* (Schltr.) Hoehne (Maxillariinae *sensu lato*) and *Oncidium ornithorhynchum* HBK (Oncidiinae *sensu lato*). *Annals of Botany* 104: 221–234.

Dressler RL (1993). *Phylogeny and Classification of the Orchid Familiy*. Oregon: Dioscorides Press.

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. *Journal of Chemical Ecology* 30: 1045–1056.

Franceschi V & Nakata P (2005). Calcium oxalate in plants: formation and function. *Annual Review of Plant Biology* 56: 41–71.

Machaka-Houri N, Al-Zein MS, Westbury DB, Talhouk SN (2012). Reproductive success of the rare endemic *Orchis galilaea* (Orchidaceae) in Lebanon. *Turkish Journal of Botany* 36: 677–682.

Neff JL & Simpson BB (1981). Oil-collecting structures in the Anthophoridae (Hymenoptera): morphology, function, and use in systematics. *Journal of the Kansas Entomological Society* 54: 95–123.

Neubig KM, Whitten WM, Williams NH, Blanco MA, Endara L, Burleigh JG, Silveira K, Cushman JC & Chase MW (2012). Generic recircumscriptions of Oncidiinae (Orchidaceae: Cymbidieae) based on maximum likelihood analysis of combined DNA datasets. *Botanical Journal of the Linnean Society* 168: 117–146.

Pabst GFJ & Dungs F (1977). *Orchidaceae Brasilienses*. Hildesheim: Brücke Verlag.

Pacek A & Stpiczyńska M (2007). The structure of elaiophores in *Oncidium cheirophorum* Rchb. f. and *Ornithocephalus kruegeri* Rchb. f. (Orchidaceae). *Acta Agrobotanica* 60: 9–14.

Pacek A, Stpiczyńska M, Davies KL, Szymczak G (2012). Floral elaiophore structure in four representatives of the *Ornithocephalus* clade (Orchidaceae: Oncidiinae). *Annals of Botany* 110: 809–820.

Pansarin EM & Pansarin LM (2011). Reproductive biology of *Trichocentrum pumilum*: an orchid pollinated by oil-collecting bees. *Plant Biology* 13: 576–581.

Reis MG, Faria AD, Bittrich V, Amaral MCE & Marsaioli AJ (2000). The chemistry of flower rewards—*Oncidium* (Orchidaceae). *Journal of the Brazilian Chemical Society* 11: 600–608.

Reis MG, Singer RB, Goncalves R & Marsaioli AJ (2006). The chemical composition of *Phymatidium delicatulum* and *P. tillandsioides* (Orchidaceae) floral oils. *Natural Product Communications* 1: 757–761.

Renner SS & Schaefer H (2010). The evolution and loss of oil-offering flowers: new insights from dated phylogenies for angiosperms and bees. *Philosophical Transactions of the Royal Society B* 365: 423–435.

Roy BA & Widmer A (1999). Floral mimicry: a fascinating yet poorly understood phenomenon. *Trends in Plant Science* 4: 325–330.

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 J (ed.) *Floriculture, Ornamental and Plant Biotechnology*, Vol. 4, Isleworth, Middlesex: Global Science Books, pp. 569–582.

Stpiczyńska M, Davies KL & Gregg A (2007). Elaiophore diversity in three contrasting members of Oncidiinae (Orchidaceae). *Botanical Journal of the Linnean Society* 155: 135–148.

Stpiczyńska M & Davies KL (2008). Elaiophore structure and oil secretion in flowers of *Oncidium trulliferum* Lindl. and *Ornithophora radicans* (Rchb.f.) Garay & Pabst (Oncidiinae: Orchidaceae). *Annals of Botany* 101: 375–384.

Torretta JP, Gomiz NE, Aliscioni SS & Bello ME (2011). Biología reproductiva de *Gomesa bifolia* (Orchidaceae, Cymbidieae, Oncidiinae). *Darwiniana* 49: 16–24

Vogel S (1974). Ölblumen und ölsammelnde Bienen. *Tropische und Subtropische Pflanzenwelt* 7: 285–547.

Williams NH, Chase MW & Whitten WM (2001). Phylogenetic positions of *Miltoniopsis*, *Caucaea*, a new genus *Cyrtochiloides*, and *Oncidum phymatochilum* (Orchidaceae: Oncidiinae) based on nuclear and plastid DNA data. *Lindleyana* 16: 272–285.