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Osmophore and elaiophores of *Grobya amherstiae* (Catasetinae, Orchidaceae) and their relation to pollination

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Grobya amherstiae flowers release a honey-like scent produced by an osmophore, comprising a papillate epidermis. The scent attracts bee pollinators (*Paratetrapedia fervida*), which collect floral oils produced by elaiophores on the lip apex and column base. The secretory tissue of the elaiophore on the lip apex consists of both palisade-like epidermal cells and conspicuously elongated unicellular trichomes. From an anatomical point of view, this elaiophore differs in structure from those known in angiosperms to date. The elaiophore on the column base is exclusively composed of short unicellular trichomes. In addition, there is an elaiophore comprising a papillate epidermis on the internal surface of the lip. The elaiophores produce a heterogeneous secretion, composed of fatty acids and mucilage. The elaiophore on the internal surface of the lip produces oil in non-collectible amounts, but it is enough to maintain the interest of the bees, guiding them to the elaiophore on the column base, a necessary step in pollination. The former elaiophore is here identified as an oil guide and it plays an essential role in ensuring pollination. The presence of three types of elaiophores on the flowers of this species of Orchidaceae is peculiar and noteworthy.

ADDITIONAL KEYWORDS: anatomy – column – lip – floral glands – labellum – oil guide – secretion.

INTRODUCTION

In orchids, nectar is the most common and widespread floral reward. However, a significant number of species reward pollinators with lipid-rich substances (see van der Pijl & Dodson, 1966; Dressler, 1993; van der Cingel, 2001). These lipophilic substances are wax-like materials and oils, which are generally produced by surface areas of the lip. The oils are secreted by oil glands named elaiophores (van der Pijl & Dodson, 1966; Vogel, 1974).

In Orchidaceae, oil or viscid material has been recorded in four subtribes, namely Maxillariinae Benth. (van der Pijl & Dodson, 1966; Davies & Turner, 2004), Bifrenariinae Dressler (Davies & Stpiczyńska, 2006), Oncidiinae Benth. (Stpiczyńska, Davies & Gregg, 2007; Stpiczyńska & Davies, 2008) and Catasetinae Benth. (Davies, Stpiczyńska & Turner, 2006; Mickeliunas, Pansarin & Sazima, 2006). This lipophilic exudate has nutritional value and may be used as a source of food rather than merely as glue for building and repairing nests, as has been suggested (van der Pijl & Dodson, 1966; van der Cingel, 2001).

Some flowers emit a scent that may or may not be associated with pollinator attraction. The scent is produced by glands named osmophores and is mainly composed of volatile oils (Vogel, 1963, 1990). The osmophores are usually formed by a single layer of epidermal cells or they may have secretory papillae (Curry *et al.*, 1991; Endress, 1994; Ascensão *et al.*, 2005; Cseke, Kaufman & Kirakosyan, 2007).

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The genus *Grobya* (Cymbidieae: Catasetinae) is endemic to Brazil and includes five epiphytic species, of which *Grobya amherstiae* Lindl. is the most widespread (Barros & Lourenço, 2004). It occurs in mesophytic, semi-deciduous forests and the Atlantic Rainforest, mainly in eastern Brazil (Pridgeon, 1997).

Anatomical studies of structures that attract and reward pollinators are scarce in Orchidaceae (Teixeira, Borba & Semir, 2004; Pansarin & Amaral, 2006) and this is the first to deal with a species of *Grobya*. The aim of this work is to investigate the anatomy of floral glands of *G. amherstiae* and their relationships to the pollination mechanism.

MATERIAL AND METHODS

Fresh flowers were collected in the morning at anthesis from natural populations occurring at Serra do Japi, municipality of Jundiaí (State of São Paulo, Brazil). A voucher specimen (15/03/2004, L. Mickeliunas & E. R. Pansarin 47) was deposited in the herbarium UEC.

Flowers were fixed in formalin–acetic acid–alcohol (FAA) for 24 h (Johansen, 1940), in buffered neutral formalin (BNF) for 48 h (Lillie, 1965) and placed under low vacuum to ensure penetration of the fixative. Flowers were then stored in 70% ethanol. The material was dehydrated through a tertiary butanol series (Johansen, 1940), embedded in Paraplast and then sectioned. Longitudinal and transverse serial sections were cut at a thickness of 10–12 μm using a rotary microtome. For histological studies, the sections were stained with safranin O and Astra blue (Gerlach, 1969) and examined under polarized light for the occurrence of starch grains (Dickison, 2000).

Some histochemical procedures were performed to detect the main classes of chemical compounds produced by floral glands: Sudan black B (Pearse, 1968) for total lipids; tannic acid–ferric chloride (Pizzolato, 1977) and Ruthenium red (Gregory & Baas, 1989) for mucilage. Fresh sections were tested for fatty acids with copper acetate–rubeanic acid (Ganter & Jolles, 1969) and with Nadi reagent for terpenoids (David & Carde, 1964). For all histochemical tests, appropriate controls were run simultaneously. Light microscopy observations were carried out using an Olympus BX51 microscope. Photomicrographs were captured on Kodak Pro Image (100 ASA) film. The terminology of trichomes follows Theobald, Krahulik & Rollins (1979).

RESULTS

Grobya amherstiae flowers (Fig. 1A) release a honey-like scent, mainly in the hottest hours of the day. The lip is yellow, trilobed, wet and covered at the apex by an abundant secretion (Fig. 1C). The flowers have two different kinds of glands related to the pollination mechanism: one osmophore and three types of elaiophores, which occur singly (Figs 1–4).

The osmophore occurs on the external surface of the lip (Figs 1A, 2A, arrow 'Os') and is of the epidermal type, composed of one layer of papillate epidermal cells (Fig. 2B, arrow 'Os'). These papillate cells stain positively for total lipids with Sudan black B (Fig. 2C, arrow 'Os') and for essential oils with Nadi reagent (Fig. 2D), confirming their secretory nature. No starch grains are detected in the secretory tissue or in the subepidermal parenchyma.

Elaiophores occur on the lip apex (Fig. 1A, B, arrow 'EL', 1C), on the column base (Fig. 1B arrow 'EC', 1D) and on the internal surface of the lip (Fig. 1B arrow 'OG'). The elaiophore secretory tissue on the lip apex consists of both trichomes (Figs 2A, 3A, arrow 'Tr', 3B) and palisade-like epidermal cells (Figs 2A, 3A; arrow 'PE', 3C). Trichomes are unicellular and conspicuously elongated, with a densely stained cytoplasm and a well-developed nucleus (Fig. 3B). The palisade-like epidermis also possesses a densely stained cytoplasm and a relatively large nucleus (Fig. 3C). The secretory epidermis has small projections and grooves (Fig. 3C, arrow), the latter of which and the elongated trichomes increase the secretory surface. On the column base (Fig. 3D), the elaiophore is composed of short, unicellular trichomes and, on the internal surface of the lip (Figs 2A, B arrow 'OG', 4E, F, arrows), of a papillate epidermis; both are secretory tissues where oil is produced. No starch grains are found in secretory tissues or in subepidermal parenchyma. The accumulated oil is released without cuticle detachment or disruption.

The secretion of the elaiophores stains positively for lipophilic and hydrophilic substances: staining with Sudan black B (Figs 2C, 4F, arrows) and tannic acid–ferric chloride (Fig. 4C) revealed total lipids and mucilage. These results are also confirmed using copper acetate–rubeanic acid (Fig. 4A, B) for fatty acids and Ruthenium red (Fig. 4D) for mucilage. Thus, besides secreting edible oil (fatty acids), the elaiophores also secrete mucilage, resulting in a heterogeneous secretion.

DISCUSSION

The flowers of *G. amherstiae* possess a complex system of floral glands that are crucial for the success of the pollination mechanism. The osmophore on the external surface of the lip produces the scent that attracts effective pollinators to the flowers (Mickeliunas *et al.*, 2006). Volatile oil is synthesized by the papillate epidermal cells and the absence of subsecretory parenchyma tissue in this species is a feature

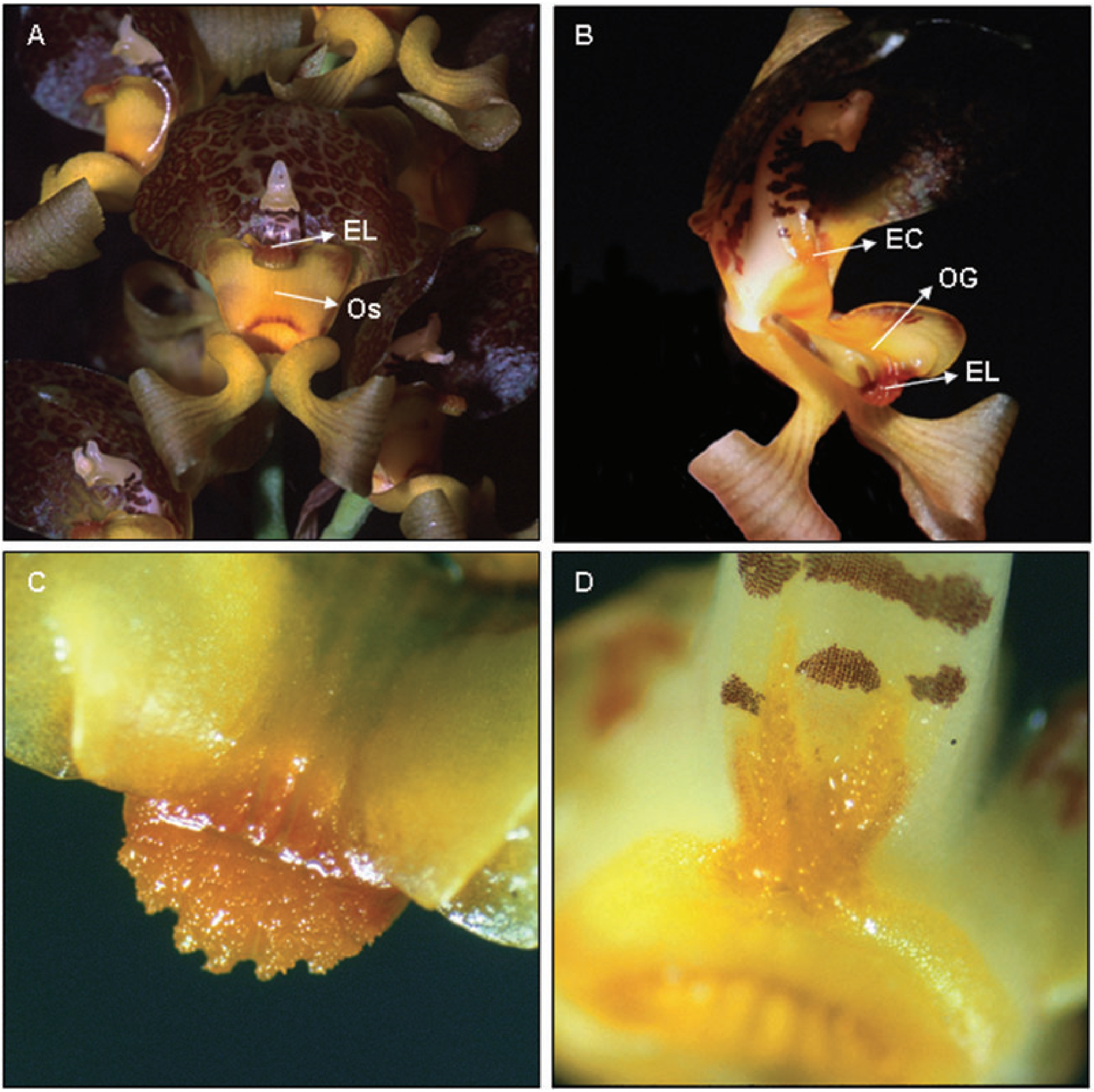

Figure 1. A–D, *Grobya amherstiae*. A, flower in frontal view, showing the elaiophore on the lip apex (EL) and the osmophore (Os) on the external surface of the lip. B, flower with the lip down, showing the elaiophores (EL, elaiophore on the lip apex; EC, elaiophore on the column base; OG, oil guide). C, detail of the elaiophore on the lip apex. D, detail of the elaiophore on the column base.

that contrasts with other orchid species (Vogel, 1990; Curry & Stern, 1991; Curry *et al.*, 1991; Stpiczyńska, 1993; Ascensão *et al.*, 2005; Pansarin, Pansarin & Sazima, 2008).

Elaiophores, such as those recorded here for *G. amherstiae*, producing oil to reward their pollinators, have been documented for some orchid groups (e.g. van der Pijl & Dodson, 1966; Davies & Turner, 2004; Davies & Stpiczyńska, 2006; Stpiczyńska *et al.*, 2007; Stpiczyńska & Davies, 2008), but it is important to investigate the structure of these glands to assess their actual nature and function, because some orchid species have elaiophore-like structures that do not produce a reward and attract their pollinators by deception (Ackerman, 1986; Pansarin *et al.*, 2008).

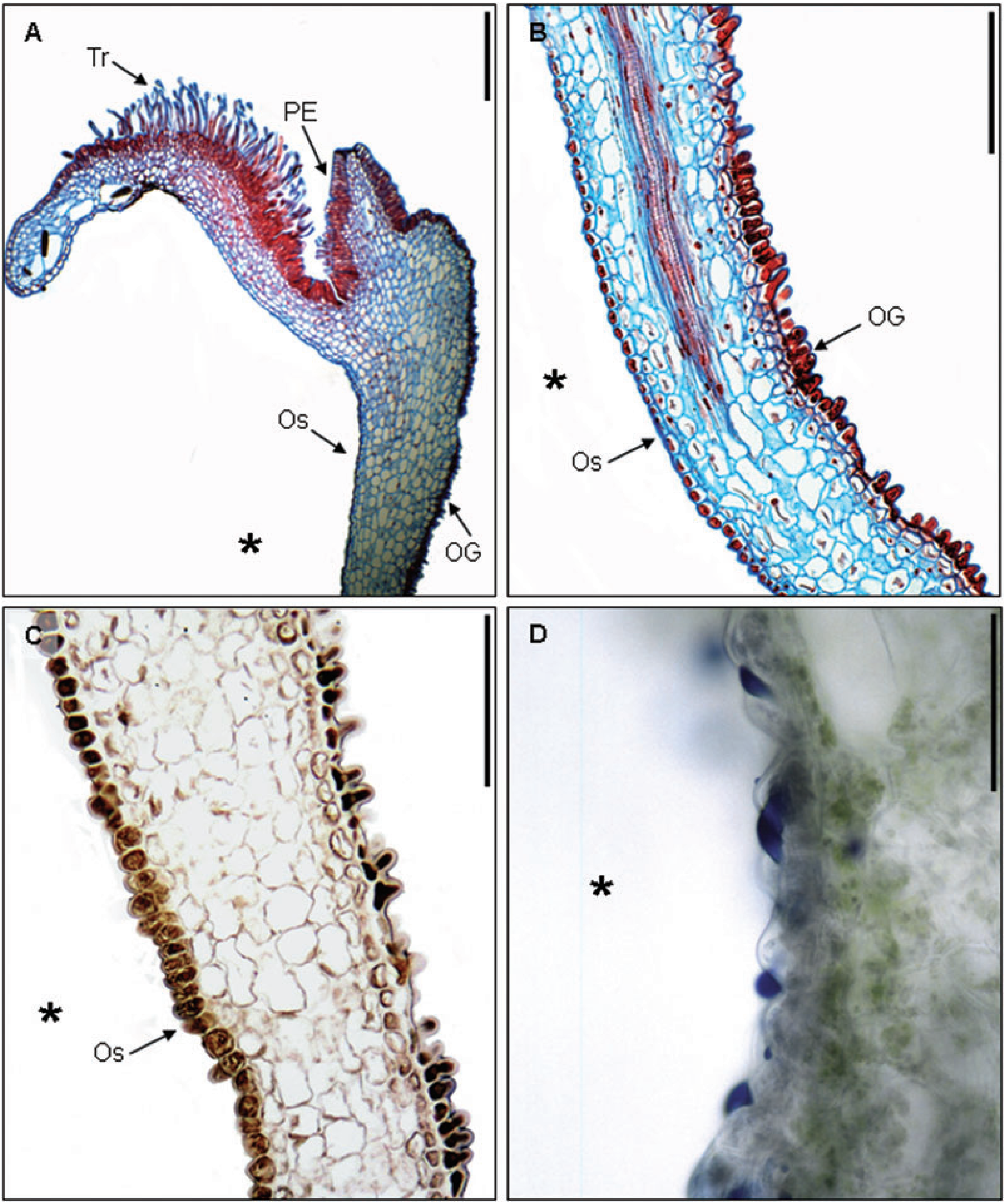

Figure 2. A–D, *Grobya amherstiae*. A, longitudinal section of the lip apex, showing the elaiophore (Tr, trichomes; PE, palisade-like epidermis; OG, oil guide) and osmophore (Os). B–C, osmophore on the external surface of the lip (arrow 'Os') and oil guide on the internal surface of the lip (arrow 'OG'). D, detail of the osmophore. A–B, safranin O and Astra blue; C, Sudan black B; D, Nadi reagent. Scale bars: A–C, 100 μm; D, 50 μm (*external surface).

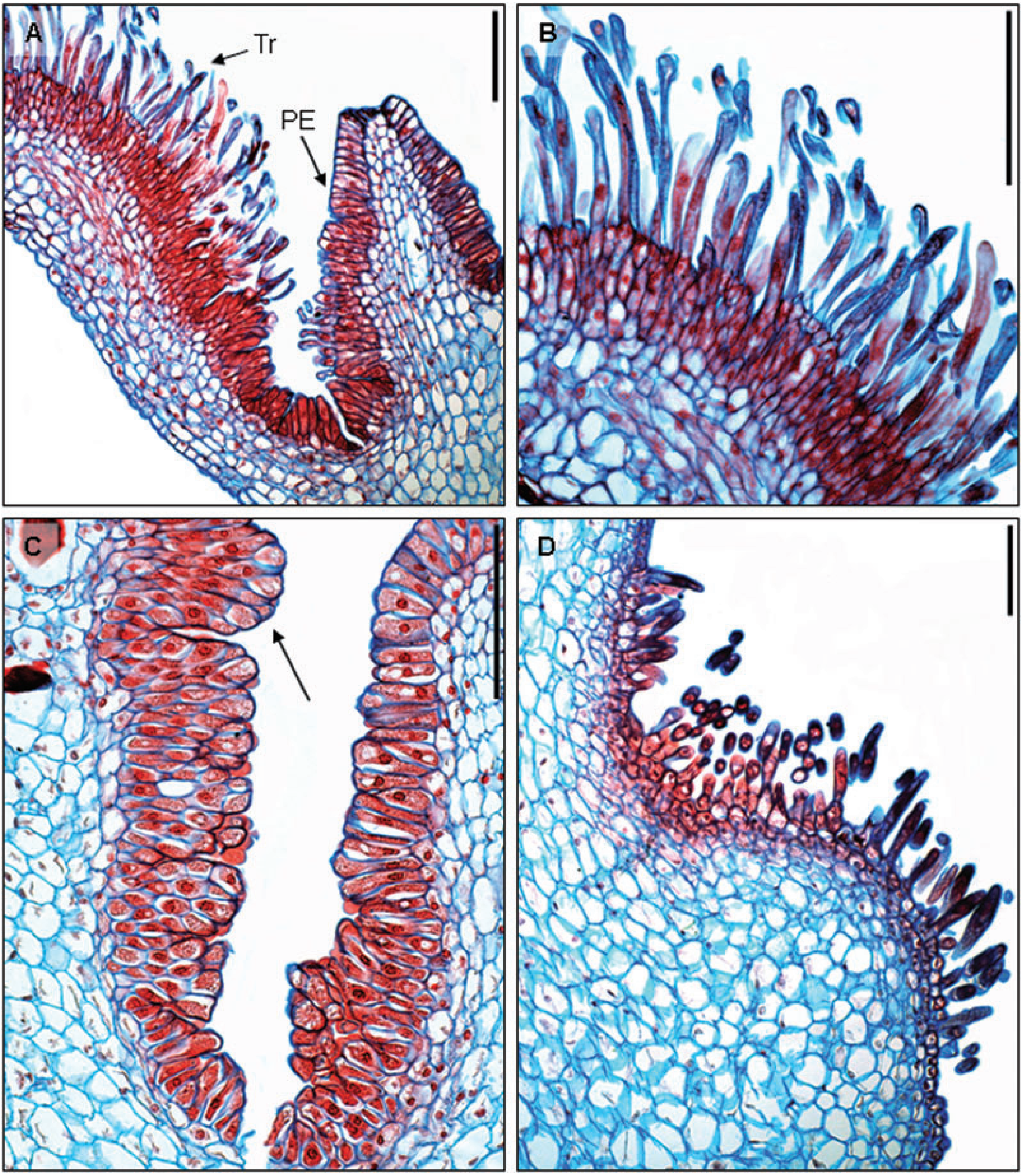

Figure 3. A–D, *Grobya amherstiae*. A–C, elaiophore on the lip apex, showing the trichomes and palisade-like epidermis. B, detail of the elongated unicellular trichomes. C, detail of the palisade-like epidermis, showing the grooves (arrow). D, elaiophore on the column base, with short unicellular trichomes. A–D, safranin O and Astra blue. Scale bars: A–D, 100 μm.

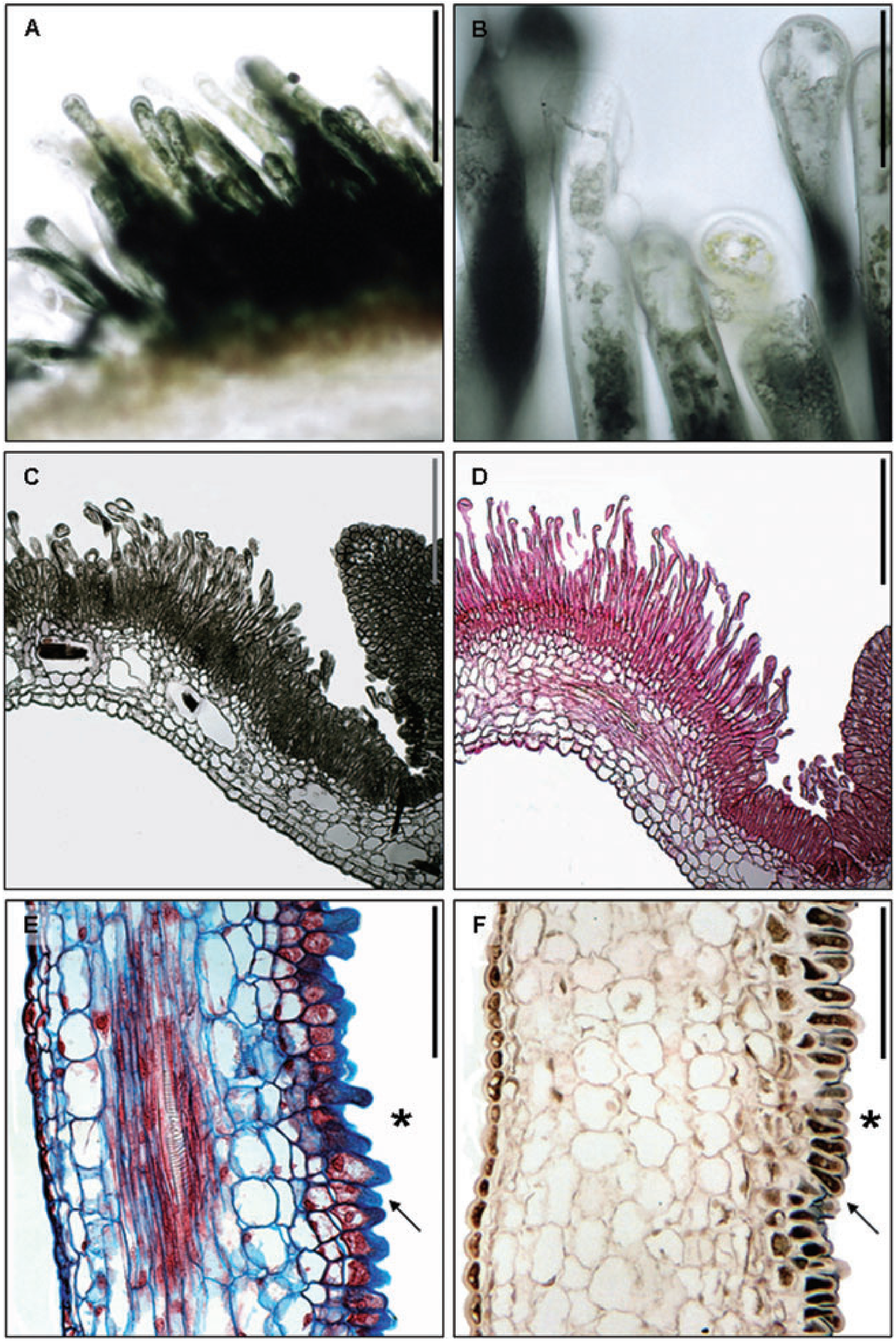

Figure 4. A–F, *Grobya amherstiae*. A–D, elaiophore on the lip apex, showing the trichomes and palisade-like epidermis. E–F, elaiophore (oil guide) on the internal surface of the lip (arrow) composed of papillate epidermis. A–B, copper acetate–rubeanic acid; C, tannic acid–ferric chloride; D, Ruthenium red; E, safranin O and Astra blue; F, Sudan black B. Scale bars: A–C, E–F, 100 µm; D, 50 µm (*internal surface).

The anatomy of the elaiophore on the lip apex of *G. amherstiae* is novel in Orchidaceae as the secreted material is produced by two types of structures (trichomes and palisade-like epidermis) that occur together in the same region, increasing and optimizing the secretory surface. This elaiophore structure differs from the types described by Vogel (1969, 1974), Singer & Cocucci (1999), Stpiczyńska *et al.* (2007) and Stpiczyńska & Davies (2008). The trichome type of elaiophore on the column base is similar to others that occur in some orchids (Vogel, 1974) and a papillate epidermis of the elaiophore on the internal surface of the lip is also found in other orchids (Singer & Cocucci, 1999; Davies & Stpiczyńska, 2006; Stpiczyńska *et al.*, 2007; Stpiczyńska & Davies, 2008).

Grobya amherstiae is pollinated by females of *Paratetrapedia fervida* Smith (Anthophoridae) bees that visit the flowers to collect edible oil from the elaiophores on the lip apex and on the column base (Mickeliunas *et al.*, 2006). The elaiophores produce not only fatty acids but also acid mucilage which, added to the oil, makes the secretion more fluid and therefore facilitates its collection by bees. Other floral glands, such as wet stigma and nectaries, might be related to biosynthesis of complex mixtures (heterogeneous secretions) of different chemical compounds (Castro & Demarco, 2008). Lipids and mucilage have been reported in stigmatic exudates (Cresti *et al.*, 1986; Endress, 1994) and in the nectar (Endress, 1994; Fahn, 2000) of some angiosperm species. However, as far as is known, there are no records of heterogeneous secretions for flowers of Orchidaceae.

Whereas elaiophores on the lip apex and on the column base produce rewards and are situated in important regions of the flower that lead the bees to perform pollination, the elaiophore on the internal surface of the lip secretes small and non-collectible amounts of oil and mucilage, but it exerts an important function in the pollination mechanism. According to Mickeliunas *et al.* (2006), female bees land on the lower surface of the lip or directly on the elaiophore of the lip apex. Under the weight of the bees, the lip tips down. Afterwards, oriented by the elaiophore on the internal surface of the lip, the bee moves to the elaiophore on the column base. In the process, it passes the equilibrium position and the lip recovers its original position, throwing the bee lengthwise onto the column. The dorsal thorax (scutellum) of the bee is positioned just below the viscidium. To pull itself out, the bee has to struggle and, as it does so, the pollinarium sticks to its scutellum (Mickeliunas *et al.*, 2006). Thus, the elaiophore on the internal surface of the lip is here identified as an oil guide, an essential structure in ensuring removal and deposition of a pollinium on the stigmatic surface (Mickeliunas *et al.*, 2006).

Osmophores and elaiophores are important structures in the pollination process of many flowering plants (e.g. Vogel, 1969, 1974, 1990), including orchid species (e.g. Teixeira *et al.*, 2004; Pansarin & Amaral, 2006). Thus, studies of the anatomy and histochemistry of these glands, along with accurate studies on pollinators and floral biology, are important in highlighting the role of such structures in pollination mechanisms.

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