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COMPARATIVE STRUCTURE OF THE OSMOPHORES IN THE FLOWER OF *Stanhopea graveolens* Lindley AND *Cycnoches chlorochilon* Klotzsch (ORCHIDACEAE)

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Abstract

The structure of the osmophores in *Stanhopea graveolens* and *Cycnoches chlorochilon* was studied by means of light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The scent glands are located in the basal part of the labellum. The surface of the osmophores is wrinkled or rugose, which increases the area of fragrance emission. On the surface of the epidermis, remnants of secretion are noticeable in *S. graveolens*, but these are absent in *C. chlorochilon*. The osmophore tissue is composed of secretory epidermal cells and several layers of subepidermal parenchyma, and it is supplied by vascular bundles that run in ground parenchyma. The secretory cells have large nuclei, a dense cytoplasm with numerous ER profiles, lipid droplets, and plastids with a substantial amount of starch, which are probably involved in the synthesis of volatile substances. In the cell walls of the osmophore cells, numerous pits with plasmodesmata occur that are likely to take part in symplastic transport of the scent compounds. The structure of the osmophores is similar in both investigated species. Both *S. graveolens* and *C. chlorochilon* are pollinated by euglossine bees, and such similarity results from adaptation to effective scent emission and attraction of pollinators.

Key words: micromorphology, ultrastructure, histochemistry, secretory tissue, scent glands, *Stanhopea*, *Cycnoches*, Epidendroideae

INTRODUCTION

Orchids expanded various adaptations to pollination and their flowers provide several kinds of plant-pollinator interactions (Dressler, 1993; van der Cingel, 2001). Since the pollen in Orchidaceae may be used as food only in primitive species, e.g. *Neuwiedia* (Kocyan and Endress, 2001), the most common and widespread reward for pollinators is nectar (van der Cingel, 2001). Moreover, some representatives of Oncidiinae, e.g. *Oncidium, Ornithophora, Gomesa,* reward pollinators with lipid-rich substances (Stpiczyńska and Davies, 2008; Alisciоni *et al*. 2009). Some species offer both nectar and fragrance reward, such as *Cyclopogon elatus* (Sw.) Schltr (Wiemer *et al*. 2009), or oil and fragrance, such as *Grobya amherstiae* Lindl. (Pansarin *et al*. 2009), whereas many other species offer exclusively floral fragrances as secondary floral attractants, e.g. *Gongora, Catasetum, Stanhopea, Cymbidium* (Williams & Whitten, 1983; Stpiczyńska, 1993; Martini et al. 2003).

Floral fragrances are produced by the osmophores (scent glands) occurring in a large group of plants (Vogel, 1990; Dressler, 1993). In euglossinophilous orchids scent is a combination of terpenes and aromatics (Williams & Whitten, 1983) and functions as an attractant for male euglossine bees (tribe Euglossini, Apidae). It is collected from the flower surface and stored in specialized hind tibiae of these bees (Dressler, 1982). Fragrance is used as a precursor for a sex pheromone (Stern et al. 1987; Vogel, 1990; van der Cingel, 2001). Flowers of orchids are not the exclusive source of floral fragrance for euglossine bees, and this mutualistic relationship is not symmetric, as the bees can collect fragrances from the flowers of other genera such as *Spathiphyllum, Anthurium* (Araceae), *Drymonia* and *Gloxinia* (Gesneriaceae), *Cyphomandra* (Solanaceae), *Dalechampia* (Euphorbiaceae) (Williams & Whitten, 1983 and references therein). Nevertheless, Pemberton and Wheeler (2006) reported that an equally valuable

source of essential oils for euglossine bees were leaves of *Ocimum basilicum* L., *Pimenta dioica* (L.) Merr or *Melaleuca quinquenervia* (Cav.) S.T. Blake. However, the reason why these bees collect fragrances is not entirely clear.

In orchids, osmophores may be located on the adaxial surface of sepals, petals, or part of the lip (Dressler, 1993). Such surface can be smooth or covered by papillae, unicellular trichomes, as in *Stanhopea saccata* Bateman (Curry et al. 1991), pear-shaped or spherical unicellular hairs with irregular cuticle in *Cymbidium tracyanum* Rolfe (Stpiczyńska, 1993), dome-shaped papillae in *Ophrys lutea* (Ascensăno et al. 2005), or papillose cells with smooth cuticle in *Acianthera* (de Melo et al. 2010).

Stanhopea graveolens Lindley (Stanhopeinae) and *Cycnoches chlorochilon* Klotzsch (Catasetinae) are fragrant orchids pollinated exclusively by male euglossine bees (Williams & Whitten, 1983). Duration of flowering and the period of fragrance secretion are significantly diverse in both species. In *Cycnoches*, anthesis lasts weeks, as long as the pollinia remain intact, whereas in *Stanhopea* flowers last only three days (Vogel, 1990). *Cycnoches* is well known for producing sexually dimorphic flowers and non-functional intermediate flowers are occasionally formed as well (Dressler, 1993). The lip in *Cycnoches* is upper due to the fact that these flowers are not resupinated (Bechtel et al. 1980). The hypochile is separated from the epichile by dark green waxy callus. Also, the labellum of *Cycnoches* has an enormous thickness (up to 9 mm thick) and represents the most voluminous osmophore in Orchidaceae (Vogel, 1990).

In Orchidaceae, the structure of osmophores has been studied with light microscopy (Vogel, 1990; Pansarin et al. 2009) and both scanning and transmission electron microscopy (Stern et al. 1987; Curry et al. 1991; Stpiczyńska, 1993; Stpiczyńska, 2001; Wiemer et al. 2009; de Melo et al. 2010). In the literature there are some detailed data on the structure of osmophores in several species of *Stanhopea* (Stern et al. 1987; Curry et al. 1991), but up to now the studies on the structure of the large and elaborated osmophore gland in *Stanhopea graveolens* has been neglected. Also, there have been no anatomical studies of the osmophores in *Cycnoches chlorochilon* at all.

The purpose of this study was to investigate and compare the micromorphology and ultrastructure of the osmophores in *Stanhopea graveolens* and *Cycnoches chlorochilon* using light microscopy, scanning and transmission electron microscopy. We selected these species because of significant differences in the flower life-span and, at the same time, the difference in the functioning of these secretory glands.

MATERIALS AND METHODS

The plants of *Stanhopea graveolens* Lindl. and *Cycnoches chlorochilon* Klotzsch used in this study are cultivated in the greenhouses of the Botanic Garden of the University of Warsaw.

In the case of *S. graveolens,* osmophore tissue was sampled at the bud stage (buds 3-4 cm long, about one week before anthesis) and, additionally, on the second day of anthesis (Fig. 1 A-B), whereas from *C. chlorochilon* this tissue was sampled exclusively from male flowers on the 7^{th}-10^{th} day of anthesis. The osmophores were examined with light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Freshly cut pieces of secretory tissue (about 3×3 mm) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at a pH of 7.4 at room temperature for 2 h, and then washed in phosphate buffer. Afterwards, the material was post-fixed in 1.5% osmium tetroxide for 1.5 h and washed in distilled water. The fixed material was dehydrated in a graded ethanol series, infiltrated and embedded in LR White resin. The embedded material was cut at 60 nm for TEM using a Reichert Ultracut-S ultramicrotome and a glass knife, stained with uranyl acetate and post-stained in lead citrate (Reynolds, 1963). Then, the sections were examined with an FEI Technai G2 Spirit Bio TWIN transmission electron microscope, at an accelerating voltage of 120 kV. TEM images were taken using a Megaview G2 Olympus Soft Imaging Solution camera.

For light microscopy, pieces of osmophores were prepared as described for TEM, but resin blocks were cut into semi-thin sections (0.9-1.0 μm thick) and stained with 1% (w/v) aqueous methylene blue-Azur B solution for general histology. The presence of insoluble polysaccharides was tested with PAS staining (Jensen, 1962). Additionally, hand-cut sections were made using a razor blade and then tested for starch presence with Lugol's iodine solution (IKI). For lipids, the tissue was stained with alcoholic Sudan III (in hand-cut sections) and Auramine O (in semi-thin sections); in the latter, the staining reaction was examined by means of a Nikon Eclipse 90i microscope equipped with a fluorescein isothiocyanate filter. Also, the autofluorescence of chlorophyll in plastids was tested in *C. chlorochilon* using a Nikon Eclipse 90i fluorescence microscope with a UV-2B filter.

For the observations of the surface of the secretory epidermis, pieces of osmophore tissue were dehydrated in acetone, then subjected to critical-point drying using liquid CO_2, sputter-coated with gold and examined by means of a TESCAN/VEGA LMU scanning electron microscope, at an accelerating voltage of 30 kV.

RESULTS

Stanhopea graveolens

The labellum of *Stanhopea graveolens* is composed of three parts: basal hypochile, mesochile and distal epichile. The hypochile bears osmophore. The petals and sepals are faintly orange to yellow, while the hypochile is strongly orange (Fig. 1B).

In the flowers of *S. graveolens*, the surface of the osmophores is rugose (Fig. 1C-F) at the two stages investigated. In buds, the cuticle covering the epidermal cells is slightly wrinkled (Fig. 1C), whereas in open flowers it is highly sculptured, mainly at the tip parts of the rugae (Fig. 1D). On the surface of the epidermis, remnants of secretion and a distended cuticle are present (Fig. 1E-F).

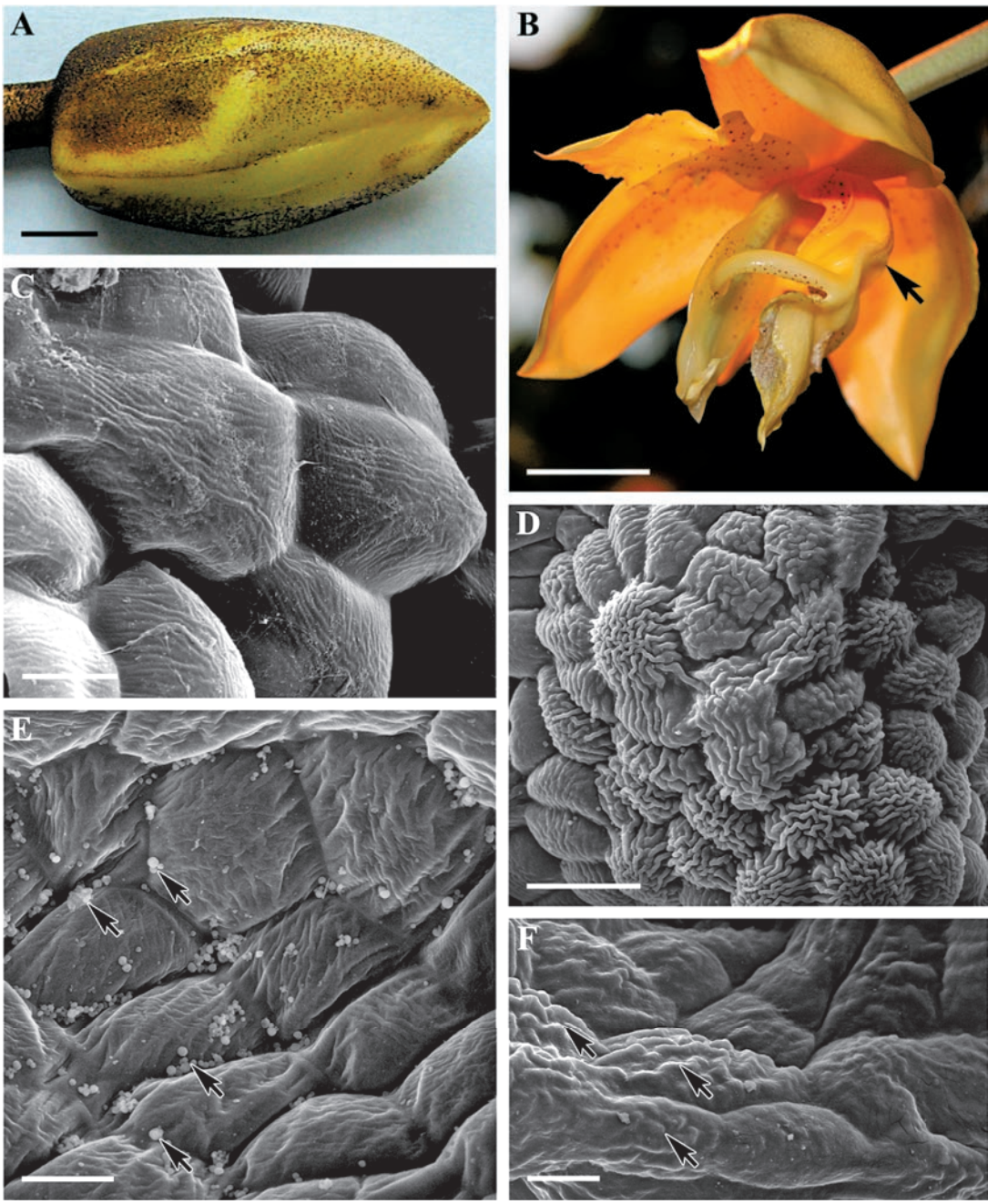

Fig. 1. A-F. Habit of the flower of *Stanhopea graveolens* in bud and at anthesis, macro images and SEM. (A) bud, one week before anthesis. Scale bar = 5 mm. (B) Open flower with a complex labellum and hypochile containing an osmophore (arrow). Scale bar = 20 mm. (C) Surface of the rugae with a slightly wrinkled cuticle at the bud stage. Scale bar = 10 µm. (D) Tip part of the rugae with highly sculptured cuticle in opened flower. Scale bar = 50 µm. (E) Details of the cavity formed between the rugae, remnants of secretion in the form of globular structures (arrows). Scale bar = 20 µm. (F) Blistered cuticle on the surface of the osmophore (arrows). Scale bar = 20 µm.

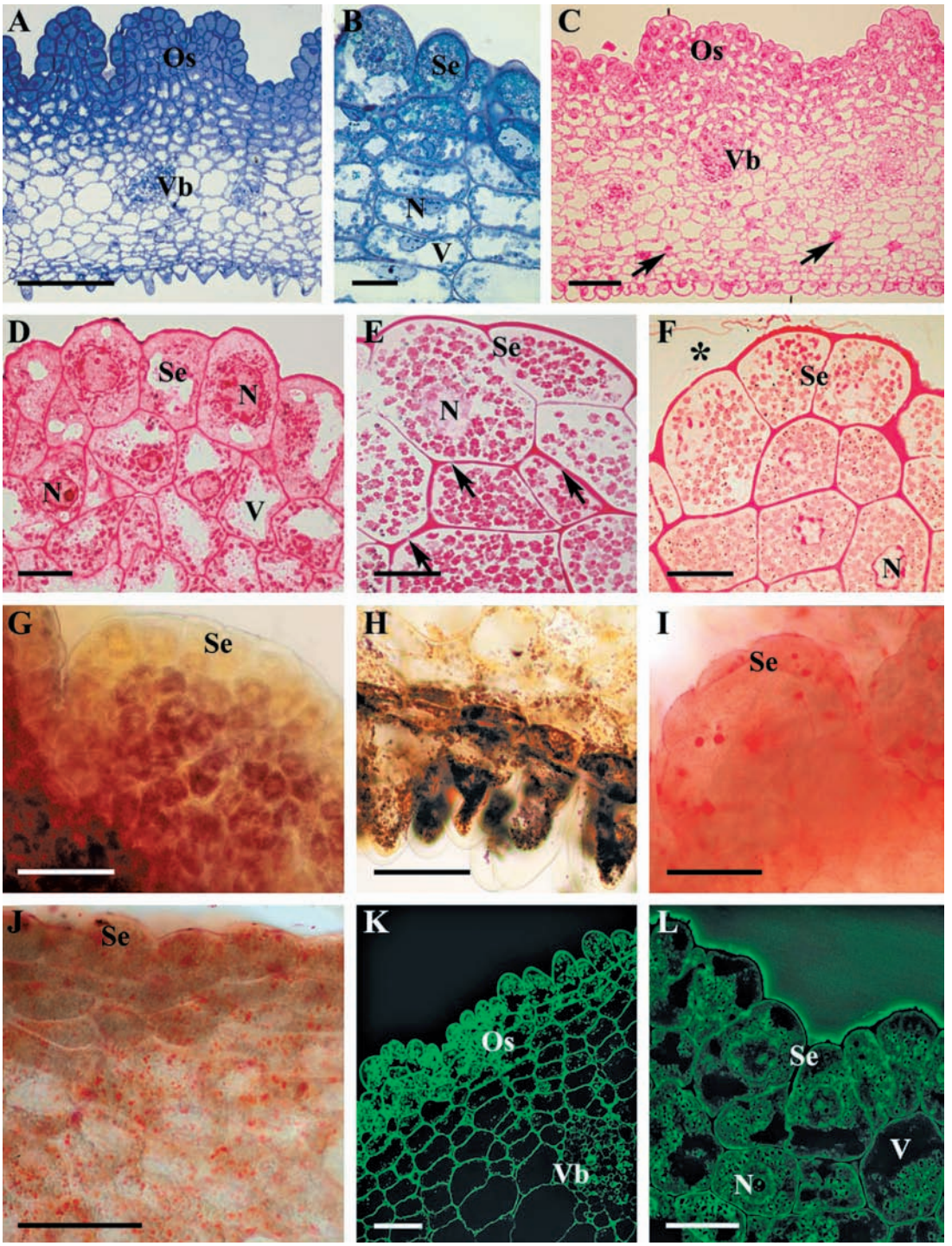

Fig. 2. A-L. Histology of the osmophores of *S. graveolens*, LM. (A) Transverse section through the labellum; note intense staining of the cytoplasm in the osmophore cells and vascular bundles in ground parenchyma. Scale bar = 500 µm. (B) Section showing a dense cytoplasm with lipid droplets and parietal cytoplasm in subepidermal parenchyma cells. Scale bar = 50 µm. (C) Section of the labellum showing vascular bundles and raphides in ground parenchyma. Scale bar = 100 µm. (D) Detail of osmophore cells at the bud stage with small vacuoles and perinuclear plastids. Scale bar = 20 µm. (E) Section showing secretory cells of the osmophore rugae in open flowers; note several pit fields in the subepidermal cells (arrows) and the presence of starch grains both in epidermal and secretory parenchyma cells. Scale bar = 25 µm. (F) Distended cuticle at the tip of the rugae (asterisk). Scale bar = 50 µm. (G) Hand-cut section of the osmophores at the bud stage; note numerous starch grains in the parenchyma. Scale bar = 100 µm. (H) Section showing the abaxial epidermis as well as underlying parenchyma cells with abundant starch grains. Scale bar = 100 µm. (I) Hand-cut section through the osmophore in bud stained with Sudan III; note few red-stained lipid droplets. Scale bar = 100 µm. (J) Sections of the osmophore from a flower at anthesis with numerous lipid droplets. Scale bar = 100 µm. (K) Section stained with auramine O showing light green fluorescent lipid droplets in the osmophore cells. Scale bar = 100 µm. (L) Fluorescence of the cuticle after staining with auramine O. Scale bar = 25 µm.

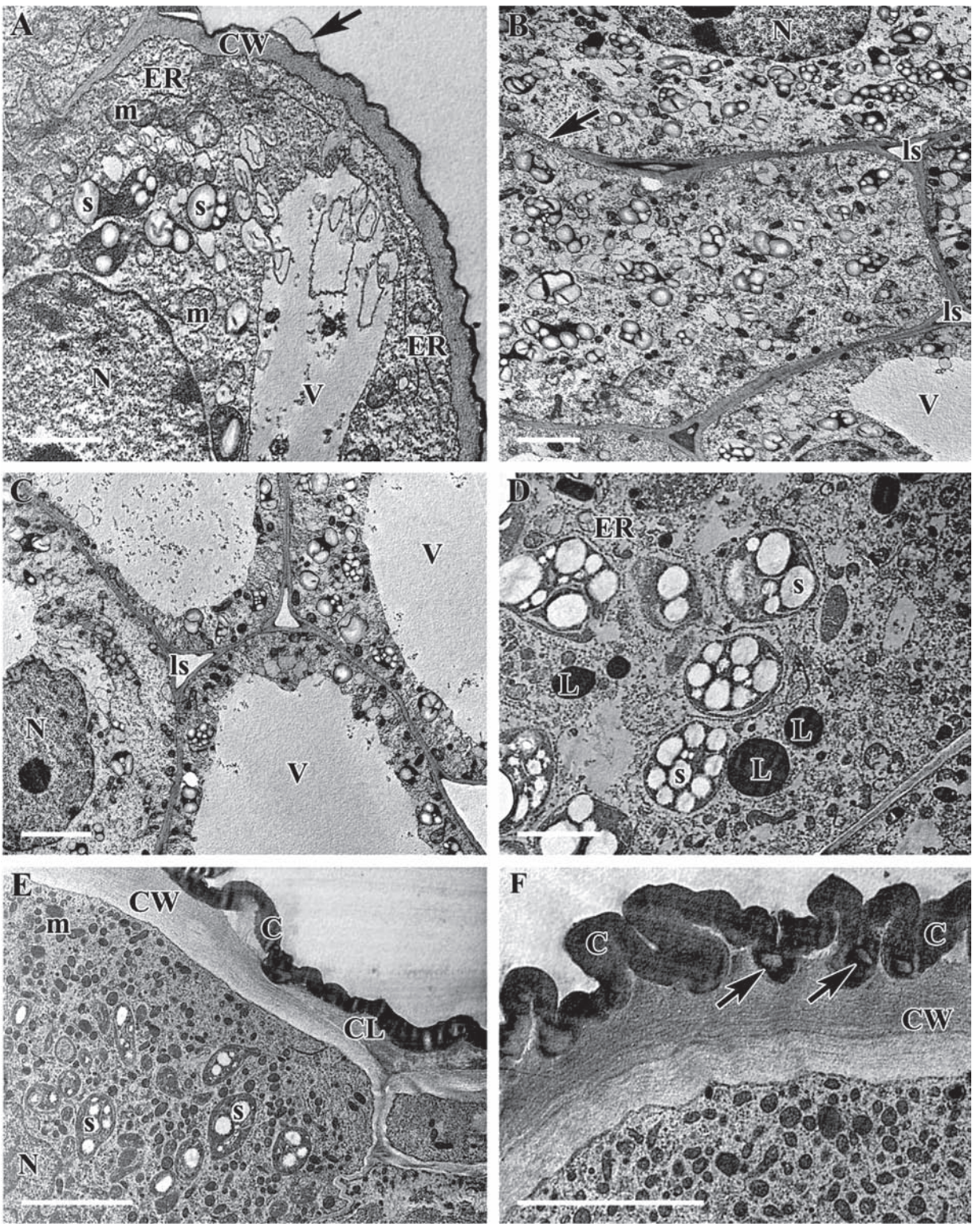

Fig. 3. A-F. Ultrastructure of the osmophores of *S. graveolens,* TEM. (A) Granular cytoplasm of the secretory epidermis containing mitochondria, amyloplasts, large nuclei and ER profiles that occur close to the external cell wall; note the blistered cuticle (arrow). Scale bar = 2 μm. (B) Subepidermal secretory cells showing numerous amyloplasts with starch grains, small intercellular spaces and plasmodesmata in the cell walls (arrow). Scale bar = 5 μm. (C) Detail of the subepidermal parenchyma with parietal cytoplasm. Scale bar = 5 μm. (D) Secretory epidermis at the open flower stage with lipid droplets, short ER profiles with an intimate connection to amyloplasts; note degraded starch grains. Scale bar = 2 μm. (E) Section showing the external cell wall in the epidermis and the bi-layered cuticle with an outer homogenous and inner reticulate layer. Scale bar = 5 μm. (F) Detail of a highly sculptured cuticle with spherical globules, probably derived from secreted terpenoids (arrows); note numerous mitochondria close to the outer cell wall. Scale bar = 5 μm.

The osmophore tissue is composed of secretory epidermis, several layers of subepidermal secretory parenchyma cells and underlying non-glandular parenchyma with vascular bundles (Fig. 2A-C). The cells of osmophore tissue in both investigated stages are compactly arranged (Fig. 2D-F) with only small intercellular spaces. In contrast to the ground parenchyma cells, their protoplasts intensely stain with methylene blue-azur B solution (Fig. 2 A-B).

The secretory cells (epidermal as well as subepidermal ones) have a large nucleus, with an average diameter of 16.18 μm at the bud stage and 19.55 μm at the anthesis stage. In the glandular epidermis, the nucleus is located in the central part of the cell, whereas in secretory parenchyma its position is parietal (Fig. 2B, D). The osmophore cells have one medium-sized vacuole or several smaller ones (Fig. 2A-D). The secretory cells contain numerous plastids that accumulate a substantial amount of starch in both investigated stages. In buds, numerous starch grains occur mainly in the parenchyma (Fig. 2D, G), whereas in the open flowers starch is present both in parenchyma and secretory epidermis (Fig. 2E-F). Starch is also abundant in the abaxial epidermis and in several layers of underlying parenchyma cells (Fig. 2H). In the secretory cells, lipid droplets occur both at the bud stage and in open flowers (Fig. 2I-K). Idioblasts with raphides are present in vacuoles of ground parenchyma, predominantly near the abaxial epidermis (Fig. 2A, C).

TEM observations indicate that in the cytoplasm of the secretory cells there are numerous mitochondria and relatively short profiles of endoplasmic reticulum (ER) that occur predominantly close to the external cell wall of the epidermis at the bud stage, whereas in open flowers ER forms short profiles with an intimate connection to amyloplasts (Fig. 3A-D). Numerous mitochondria are arranged close to the external wall of the epidermal cells (Fig. 3E-F). Dictyosomes are observed neither at the bud stage nor at the open flower stage. Amyloplasts are the most frequent form of plastids in the osmophore cells and they have scantily developed internal tubules, a dark stroma and several starch grains (3-9, in both investigated stages) (Fig. 3A-E). Starch grains with an irregular corroded surface are noticeable in the osmophore cells at the anthesis stage (Fig. 3D-E).

The outer cell walls of the secretory epidermal cells are relatively thick, in the range of 1.45-3.28, and they are covered with a cuticle that stains intensely with Sudan III and auramine O, both at the bud and open flower stages (Fig. 2I, K-L). The cuticle is single-layered and homogenous (Fig. 3A) or bi-layered with an outer homogenous and inner reticulate layer (Fig. 3E-F). The latter structure of cuticle is observed particularly in the cells located at the tip part of the rugae, where it is deeply wrinkled (Fig. 1D). The cuticle is intact, without pores, but distensions are visible in SEM, LM and TEM (Figs. 1F, 2F, 3A, respectively). In the cuticle, spherical globules are noticeable, putatively derived from secreted trepenoids (Fig. 3F). Also, remnants of secreted material in the form of globular structures are present on the surface of the epidermal cells, especially of those situated in the cavities that are formed between the rugae (Fig. 1E).

Cycnoches chlorochilon

The flowers of *Cycnoches chlorochilon* are greenish-yellow and fleshy. They are dimorphic and vary from each other only by the structure of the column. The osmophore is located in the egg-shaped cavity of the labellum (Fig. 4A-B).

The surface of the gland is wrinkled or slightly rugose (Fig. 4C-D). The epidermal cells are covered with a predominantly smooth cuticle without visible pores, but cuticular blisters are present (Fig. 4E-F). In fresh flowers, on the surface of the osmophore small droplets of liquid substance are visible by naked eye (Fig. 4B), but in SEM no residues of secretion are noticeable (Fig. 4E).

The secretory tissue is composed of epidermal cells and several layers of small subepidermal glandular parenchyma (Fig. 5A-F). Collateral vascular bundles run in the ground parenchyma located underneath. In the secretory and ground parenchyma, many large idioblasts are noted with phenolic content and/or raphides (Fig. 5A).

The outer walls of the secretory epidermal cells are thick, 3.91 μm on average, and composed of cellulose. The cuticle covering the walls is also thick, 2.37 μm on average, and stains intensely with Sudan III and auramine O (Fig. 5D, G). Both the epidermal and secretory parenchyma cells contain relatively large vacuoles (Fig. 5A-B, E-F). The secretory parenchyma cells are of irregular shape and have large intercellular spaces (Fig. 5A-B, F). Plastids accumulate abundant amounts of starch (Fig. 5C, E) and, additionally, chloroplasts are present in the parenchyma cells, but they are almost completely lacking in the epidermis (Fig. 5H). Either the epidermal or glandular parenchyma cells are filled with large quantities of lipid droplets (Fig. 5B-D). In some parts of the osmophore tissue, lipids almost completely fill the cells (Fig. 5F). TEM observations indicate the presence of large lipid droplets, predominantly close to the outer wall of the epidermal cells (Fig. 6A-C).

The cytoplasm of the secretory cells is highly granular with abundant ER profiles and leucoplasts containing starch grains (Fig. 6A-F), though few plastids without starch may be also observed (Fig. 6E). Dictyosomes are observed neither in the epidermal nor parenchymal cells. In the vacuoles, osmophilic precipitates and small vesicles occur (Fig. 6D, F). The presence of plasmodesmata is frequent in the walls of

the epidermis and the adjacent parenchyma (Fig. 6E). The cuticle which covers the outer cell wall of the epidermal cells is predominantly homogenous and single-layered (Fig. 6B-C). Under the cuticular distensions, material of fibrillar or globular structure could be observed (Fig. 6A, D).

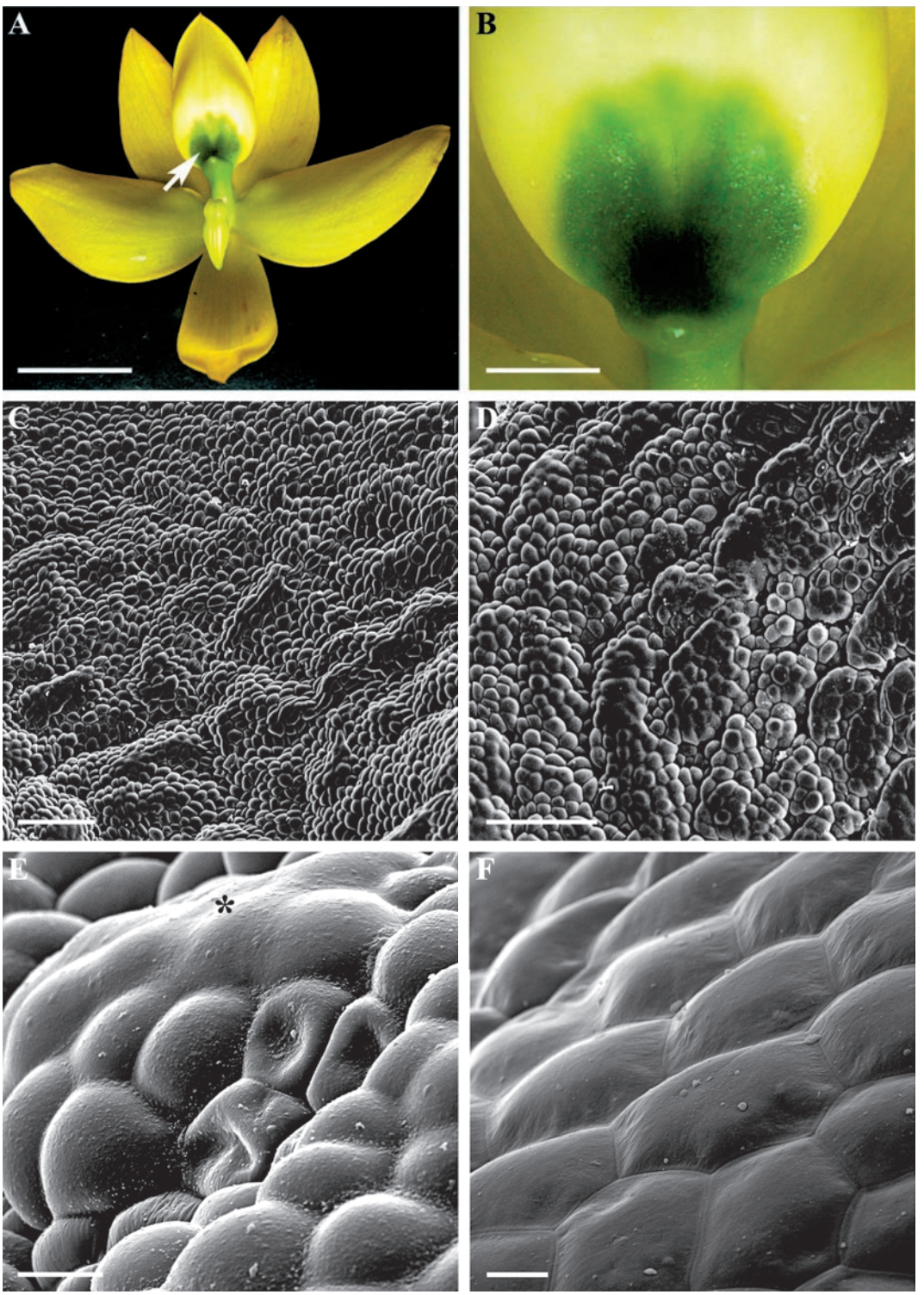

Fig. 4. A-F. Habit of the flower and osmophores of *Cycnoches chlorochilon,* macro images and SEM; (A) Greenish-yellow male flower in the middle of anthesis with an osmophore (arrow). Scale bar = 25 mm. (B) Osmophore in the basal part of the labellum; note small droplets of liquid substance. Scale bar = 6 mm. (C) Wrinkled surface of the osmophore. Scale bar = 200 µm. (D) Detail of the wrinkled and slightly rugose surface. Scale bar = 200 µm. (E) Cuticular blister (asterisk) on the epidermal cells. Scale bar = 20 µm. (F) Smooth cuticle of the secretory epidermis. Scale bar = 20 µm.

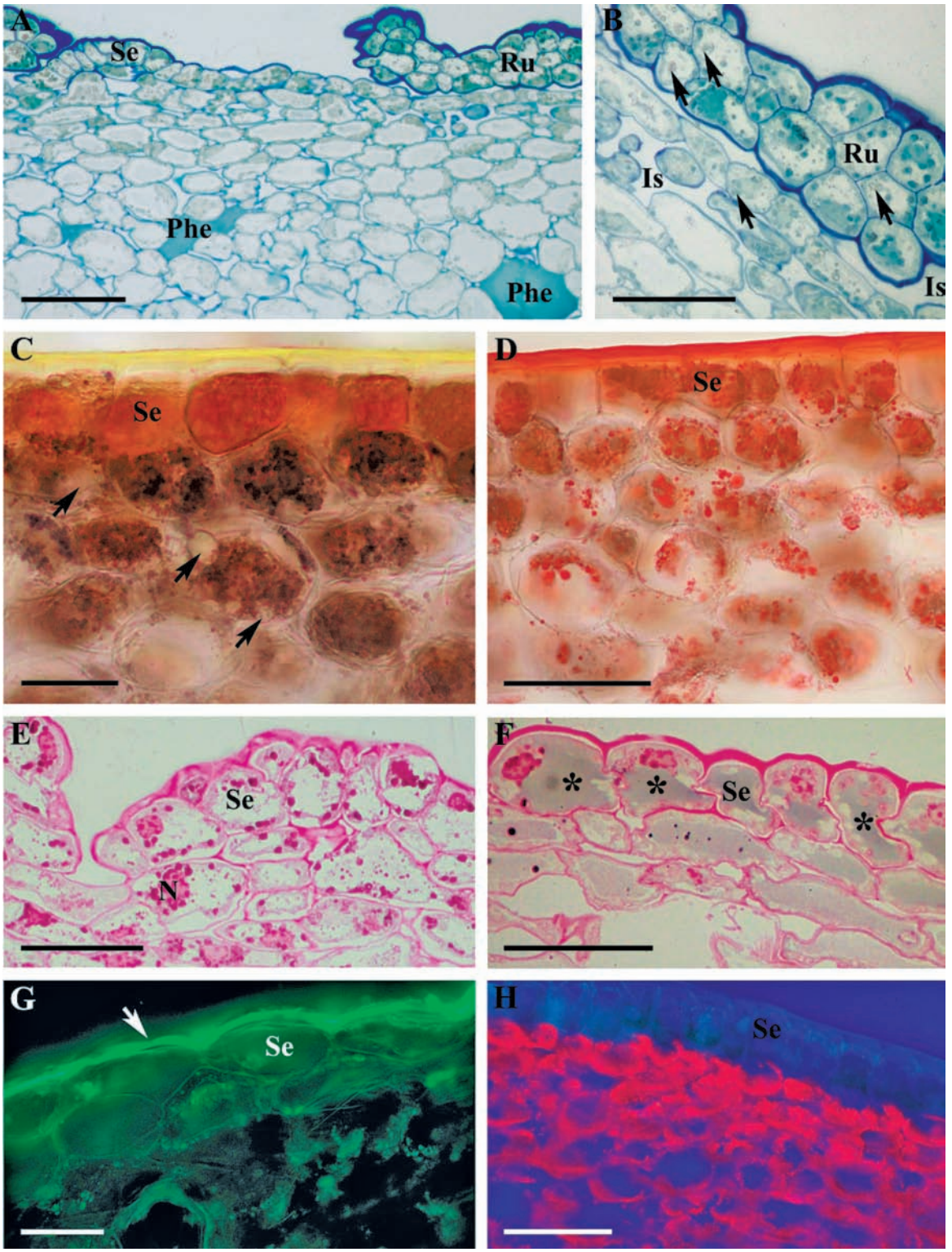

Fig. 5. A-H. Histology of the osmophore of *C. chlorochilon*, LM. (A) Section through the osmophore; note the rugae and phenolic content in the idioblasts. Scale bar = 100 µm. (B) Details of the rugae with numerous lipid droplets (arrows) and intercellular spaces in secretory parenchyma. Scale bar = 50 µm. (C) Hand-cut section stained with IKI, showing starch grains in secretory parenchyma cells; note numerous lipid droplets (arrows). Scale bar = 50 µm. (D) Hand-cut sections after treatment with Sudan III; note intensely staining cuticle and lipid droplets in the osmophore cells. Scale bar = 100 µm. (E) Details of the secretory cells with relatively large vacuoles and plastids with several starch grains. Scale bar = 50 µm. (F) Section showing the osmophore cells almost completely filled with lipids (asterisks). Scale bar = 50 µm. (G) Hand-cut section following treatment with auramine O with an intensely fluorescent cuticle (arrow). Scale bar = 50 µm. (H) Autofluorescence of the chlorophyll in the subepidermal parenchyma cells. Scale bar = 100 µm.

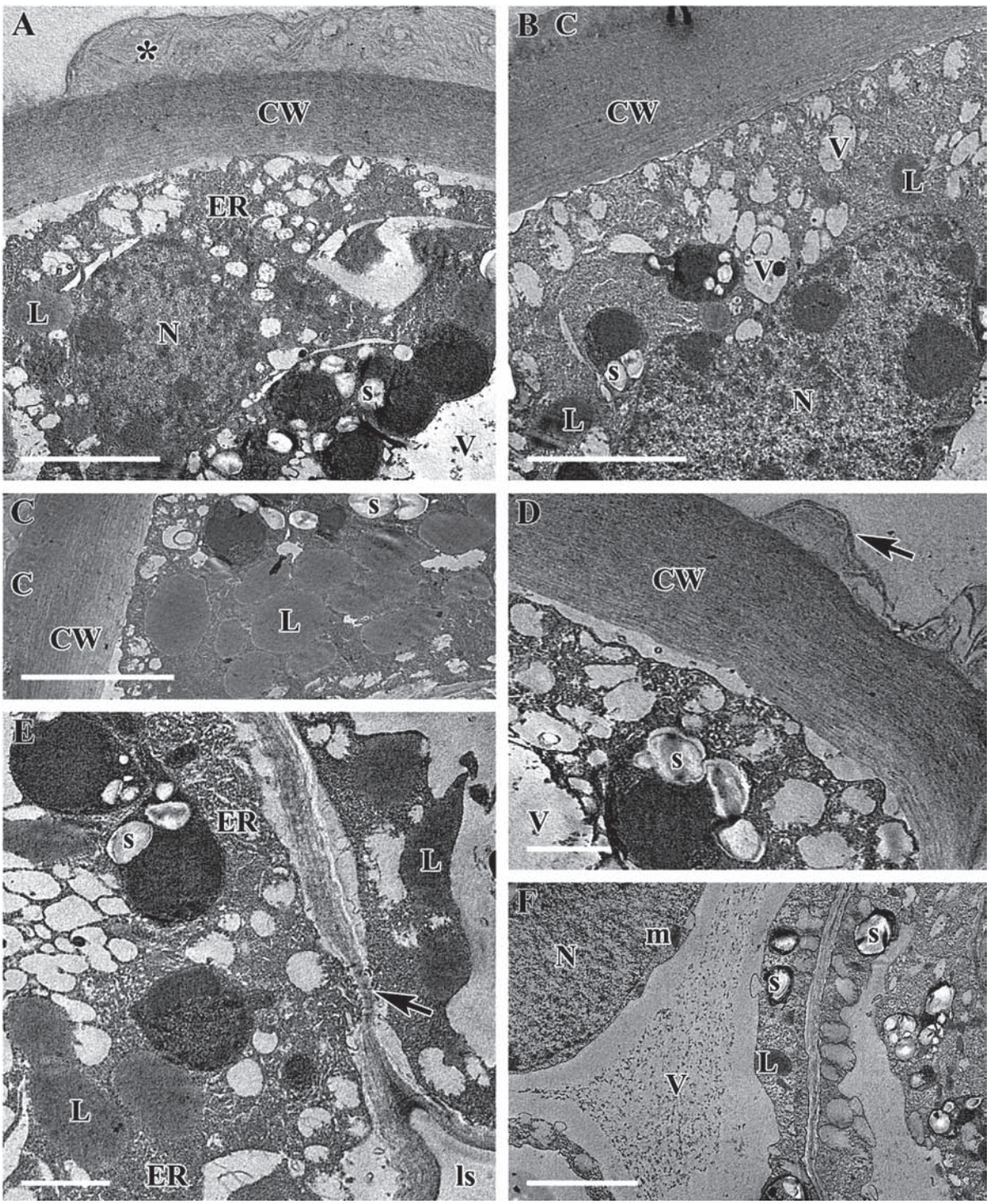

Fig. 6. A-F. Ultrastructure of the osmophore cells of *C. chlorochilon,* TEM. (A) Detail of thick-walled epidermal cells with lipid droplets, ER profiles and plastids with starch grains and small vacuoles; note the globular structure under the distended cuticle (asterisk). Scale bar = 5 μm. (B) Cell wall of epidermis with a homogenous, single-layered cuticle. Scale bar = 5 μm. (C) Section showing plastids with starch and numerous lipid droplets close to the outer cell wall. Scale bar = 5 μm. (D) Details of epidermal cells with small vacuoles; note fibrillar material under the distended cuticle (arrow). Scale bar = 2 μm. (E) Cytoplasm of secretory cells with abundant ER profiles, lipid droplets, intercellular space, plasmodesmata (arrow) and plastids with starch grains. Scale bar = 2 μm. (F) Parietal cytoplasm in subepidermal parenchyma with lipid droplets and vacuoles. Scale bar = 5 μm.

Abbreviations:
C, cuticle; CL, cuticular layer; CW, cell wall; ER, endoplasmic reticulum; Is, intercellular space; L, lipid droplet; m, mitochondrion; N, nucleus; Os, osmophore tissue; Phe, phenolic content; Ru, rugae; Se, secretory epidermis; s, starch; V, vacuole; Vb, vascular bundle.

DISCUSSION

Stanhopea graveolens and *Cycnoches chlorochilon* both have well-defined secretory glands, similarly as many other fragrant orchids (Curry et al. 1991; Stpiczyńska, 2001; de Melo et al. 2009). The flowers of *S. graveolens* and *C. chlorochilon* lack nectar and other food rewards, which is a common feature among euglossinophilous taxa (Williams and Whitten, 1983; van der Cingel, 2001). Therefore, osmophores are parts of the flower directly associated with attraction of pollinators.

In Orchidaceae, the osmophores may be located in different parts of the perianth. Likewise, in orchids pollinated by euglossine bees, including the species studied here, the scent glands seem to be associated only with the basal part of the labellum. Besides, osmophores are commonly found on the adaxial surface of the labellum, what is thus far the most widespread location of scent glands among Orchidaceae (Stern et al. 1987; Vogel, 1990). On the other hand, Wiemer et al. (2009) reported the location of the osmophores on the abaxial side of the lip in *Cyclopogon elatus*. However, in many orchids the lip is not the exclusive position for fragrance production and the osmophores can be situated in sepals in *Acianthera* species (de Melo et al. 2009) or both in sepals and the labellum in *Cymbidium tracyanum* (Stpiczyńska, 1993). The morphology of the osmophores in the investigated taxa and in other euglossinophilous orchids varies considerably. In *S. graveolens*, the surface of the gland is rugose with a highly sculptured cuticle, while in *C. chlorochilon* the osmophore has a predominantly wrinkled surface and a smooth cuticle. Besides, Curry et al. (1991) reported different characters of the osmophore in several species of *Stanhopea* and *Sievekingia,* suggesting that a more convoluted surface characterized by papillae, rugae or trichomes offers a larger area for fragrance volatilization. Therefore, the elaborated osmophore surface of *S. graveolens* seems to be more derived and adapted to scent emission than a rather smooth surface of the osmophore in *C. chlorochilon*.

The high cytoplasmic density, relatively large nuclei (probably polyploid), the abundance of starch in the epidermis and subepidermal layers in both investigated species are typical for scent-producing cells (Vogel, 1990). In contrast to *Grobya amherstiae* with the osmophore of epidermal type (Pansarin et al. 2009), in *S. graveolens* and *C. chlorochilon* the secretory tissue is composed of epidermal and underlying small parenchyma cells. The differences between epidermal and subepidermal cells in each taxa examined were only slightly noticeable, which coincides with the study conducted by Stern et al. (1987) suggesting that their structure was essentially the same.

The presence of starch in osmophore tissue, as observed in *S. graveolens* and *C. chlorochilon*, is a common feature of scent glands (Stern et al. 1987; Curry et al. 1991; Weryszko-Chmielewska et al. 2007; de Melo et al. 2009; Pansarin et al. 2009), indicating that starch is utilized as a source of energy for fragrance production (Vogel, 1990). Starch grains are abundantly stored in the pre-secretory stage and, following the increase in glandular activity, its degradation and hydrolization begin initially in the epidermis and progresses in the secretory parenchyma. Consequently, starch grains with a corroded and irregular surface, as a result of enzymatic decomposition, are observed in plastids at the anthesis stage in both investigated species, but they were also noticed in the nectary cells of *Cucurbita pepo* L. in later anthesis (Nepi et al. 1996). However, the absence of starch in plastids in the osmophore was observed in *Gymnadenia conopsea* (Stpiczyńska, 2001) as well as in *Cyclopogon elatus* (Wiemer et al. 2009). Besides, Stern et al. (1987) reported the presence of lipid droplets in osmophore cells at the bud and open flower stages in *S. wardii and S. oculata,* which was also found in *S. graveolens*. However, in some parts of the osmophore in *C. chlorochilon* lipids almost completely filled the cells and stained red with Sudan III and grey with Schiff's reagent. A similar result of lipid staining was noted on the surface of the glandular papillae in *Maxillaria elatior* and *Mormolyca tenuibulba* – the species that secrete resinaceous material composed of terpenoids (Davies and Stpiczyńska, 2012 in press). However, terpenoids occur also as a component of resin in many other plants. Therefore, monoterpenes predominate in the volatile compounds of conifer resins, such as Pinaceae, and play a major defensive role against insects and pathogens, but also occur in essential oils in *Mentha piperita* L. However, sesquiterpenes dominate in the resin of numerous angiosperms, e.g. in several tropical plants from the families Fabaceae, Dipterocarpaceae or Burseraceae (Langenheim, 2003).

Relatively large intercellular spaces in the parenchyma cells are observed in *C. chlorochilon*, which is a common feature of scent glands indicating their glandular character and engagement in intense metabolism. However, the chlorophyll content and intercellular spaces in subepidermal parenchyma cells in *C. chlorochilon* are associated with gas exchange and photosynthesis, which may extend the activity of the osmophore by providing assimilates, since the scent glands in *C. chlorochilon* stay active for weeks. Autofluorescence of chlorophyll in the osmophore cells of *Galanthus nivalis* L. was also noted (Weryszko-Chmielewska and Chwil, 2010). However, anthesis in *S. graveolens* lasts only three days as well as the chlorophyll is lacking and the cells are more compactly arranged, which shows that fragrance production is related only to consumption of reserve materials but not to assimilation (Vogel, 1990). In both investigated species,

idioblasts containing rahpides or phenolic content were frequently present close to the secretory tissues. A similar location of idioblasts with raphides accompanied various kinds of secretory tissues in orchids, namely nectaries, elaiophores and resin glands (Stpiczyńska et al. 2007; Stpiczyńska et al. 2011; Davies and Stpiczyńska, 2012, in press).

Numerous mitochondria, well-developed ER profiles and lipid droplets are common features of the osmophore cells in the investigated species and in other representatives of Orchidaceae (Curry et al. 1991). Terpenoids that are volatile components of fragrant oils are synthesized in plastids or in plastids and ER via the mevalonic acid pathway (Curry, 1987; Stern et al. 1987). Afterwards, they are modified in the ER. Numerous lipid droplets, not connected with the ER, were present in the cytoplasm of both species, while larger globules occurred in *C. chlorochilon*. The lipid droplets may be transformed into very small vesicles, then being accumulated in the periplasmic space (Stern et al. 1987) or both periplasmic and intercellular spaces (de Melo et al. 2009). However, these kinds of accumulation were not noticed in our study.

In the investigated species, symplastic transport of the volatile compounds probably exists, since the secretory cells are interconnected by numerous plasmodesmata. Unlike the osmophore cells in other orchids (Stern et al. 1987; Stpiczyńska, 1993), we did not observe dictyosomes in the secretory cells in both examined species at all. On the other hand, a low frequency of dictyosomes was reported in a few species from the *Acianthera* genus, showing compatibility with the nitrogen-rich nature of their secretions (de Melo et al. 2009).

The cuticle in *S. graveolens* may be composed of two different layers and such structure of the cuticle has already been observed in glandular cells of Orchidaceae (Stpiczyńska et al. 2011). However, the thickness of the cuticle in osmophores is related to the type of secretion (Vogel, 1990). In *S. graveolens* the presence of secretion on the surface is noticeable, while in *C. chlorochilon* it is lacking. Moreover, the cuticle may reduce the loss or uptake of gasses, which is true for water, carbon dioxide, oxygen or volatile organic compounds such as, e.g., terpenes (Riederer, 2006). Though, the volatilization of fragrance compounds by cuticular diffusion has already been described in many orchids (Stern et al. 1987; Stpiczyńska, 2001), whereas in a few *Acianthera* species it seems to be associated with stomata occurrence (de Melo et al. 2009). But for highly lipophilic organic compounds, such as terpenoids, the cuticle is the preferred pathway of volatilization, even when the stomata are open (Riederer, 2006). On the other hand, the cuticle with microchannles which are involved in the transit of fragrance substances to the cell exterior has been observed in the osmophores of other plants, e.g. *Orbea variegata* L. (Płachno et al. 2010) or *Passiflora suberosa* L. (Amela Garcia et al. 2007).

S. graveolens and *C. chlorochilon* are representatives of two different tribes and subtribes of Epidendroideae (Maxillarieae, Stanhopeinae and Cymbidieae, Catasetinae, respectively) and, moreover, they distinctly differ in the lifespan of their flowers and longevity of osmophore activity. However, both species show similar micromorphology and histological characters of the glands. Such similarities in the structure of the osmophore result from the adaptation of euglossine--bee-pollinated taxa to effective scent emission and attraction of pollinators.

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Porównanie budowy osmoforów w kwiatach *Stanhopea graveolens* Lindley i *Cycnoches chlorochilon* Klotzsch (Orchidaceae)

Streszczenie

W niniejszej pracy badano strukturę osmoforów w kwiatach *Stanhopea graveolens* Lindley oraz *Cycnoches chlorochilon* Klotzsch wykorzystując mikroskopię świetlną (LM), skaningową elektronową (SEM) oraz transmisyjną elektronową (TEM). Gruczoły wydzielające zapach zlokalizowane są u podstawy warżki. Powierzchnia osmoforów jest pomarszczona lub brodawkowata, co ułatwia emisję substancji zapachowych. W SEM na powierzchni epidermy u *S. graveolens* widoczne są pozostałości wydzieliny, natomiast u *C. chlorochilon* pomimo tego, że wydzielina pokrywa warżkę świeżych kwiatów, to w SEM nie jest widoczna. Tkanka osmoforowa jest utworzona z komórek wydzielniczych epidermy oraz kilku warstw subepidermalnych komórek miękiszowych i jest zaopatrywana przez wiązki przewodzące zlokalizowane w miękiszu zasadniczym. Komórki wydzielnicze mają duże jądro, gęstą cytoplazmę, ER, krople lipidowe oraz plastydy z ziarnami skrobi. W ścianach komórek osmoforów znajdują się liczne jamki z plazmodesmami, które przypuszczalnie biorą udział w symplastycznym transporcie związków zapachowych. Osmofory badanych gatunków mają bardzo podobną budowę anatomiczną. Zarówno *S. graveolens* jak i *C. chlorochilon* są zapylane przez pszczoły *Euglossine,* natomiast podobieństwa w strukturze ich osmoforów wynikają z przystosowania do efektywnego wydzielania zapachu, a tym samym skutecznego zwabiania zapylaczy.