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# Diverse labellar secretions in African *Bulbophyllum* (Orchidaceae: Bulbophyllinae) sections *Ptiloglossum*, *Oreonastes* and *Megaclinium*

MALGORZATA STPICZYŃSKA[1], KEVIN L. DAVIES[2]* and MAGDALENA KAMIŃSKA[3]

[1]*Faculty of Biology, University of Warsaw, Botanic Garden Al. Ujazdowskie 4, Warsaw 00-478, Poland*
[2]*School of Earth and Ocean Sciences, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, UK*
[3]*Department of Botany, University of Life Sciences, Akademicka 15, Lublin 20-950, Poland*

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Floral food-rewards of *Bulbophyllum* range from nectar to protein-rich mucilage and lipid-rich labellar secretions. For the first time, the structure of the labellum and the secretory process are investigated for four African *Bulbophyllum* species. The most specialized type of labellar organization occurred in *B. schinzianum*, the deep, narrow, median longitudinal groove consisting of palisade-like secretory cells flanked by trichomes containing lipid droplets, and the copious secretion containing sugar. This groove was absent or poorly defined, shallow and wide, in the remaining taxa, the scant secretion containing lipid. All taxa possessed a striate cuticle lacking cracks and pores, and micro-channels were present, cuticular blisters occurring only in *B. schinzianum*. The labellum contained storage parenchyma (*B. lupulinum*) or mesophyll-like parenchyma (*B. schinzianum*), but in section *Megaclinium* (*B. falcatum* and *B. maximum*), these were replaced by aerenchyma. In *B. schinzianum*, the form of the labellar groove, sweet fragrance and sugary secretion suggest pollination by Hymenoptera, the food-reward and fragrance indicating that pseudocopulation is unlikely. Conversely, the form of the labellum of taxa having smaller flowers, and the lipid-rich secretion, suggests pollination by small flies. The labellar aerenchyma may facilitate this process or even aid wind-assisted pollination. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, **179**, 266–287.

ADDITIONAL KEYWORDS: aerenchyma – anatomy – food-reward – histochemistry – lipid – micromorphology – sugar – ultrastructure.

## INTRODUCTION

It has long been known that many species of *Bulbophyllum* Thouars produce floral secretions that function as food-rewards and thus attract pollinators (van der Pijl & Dodson, 1969; van der Cingel, 2001). In most cases, this secretion is nectar, but there is evidence that certain representatives of the genus may also produce other food-rewards such as floral oils, often in conjunction with sugar (Pohl, 1935; van der Cingel, 2001; Nunes *et al*., 2014) or protein-rich mucilage (Davies & Stpiczyńska, 2014).

*Bulbophyllum* has a pantropical distribution, is one of the most diverse and largest of orchid genera containing an estimated 1200–2000 species and some 5% of all orchids, and occurs in Asia, Africa, Madagascar, the Neotropics and Australasia, although its distribution is not homogeneous throughout its entire range. It occurs mainly in the Palaeotropical region of Southeast Asia, but its centre of distribution is considered to be New Guinea. Asia contains the greatest number of species, followed by Africa and finally the Neotropics (Vermeulen, 1991; Dressler, 1993; Sieder, Rainer & Kiehn, 2007, cited in Smidt *et al*., 2011). Its

*Corresponding author. E-mail: kevinldavies@btinternet.com



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species often have complex pollination strategies (van der Cingel, 2001), with many species considered to display mimicry. For example, the flowers of the Asian species *B. makoyanum* (Rchb.f.) Ridl. [section *Recurvae* (Garay, Hamer & Siegerist) J.J.Verm.] are arranged in a circle on the inflorescence and collectively resemble a nectariferous, actinomorphic flower (Knerr, 1981). Furthermore, Koehler & Davenport (1983) showed that the similar inflorescence of *B. lepidum* (Blume) J.J.Sm. [synonym *B. flabellum veneris* (J. Koenig) Aver.], when examined using UV light (visible to many insects), resembles the capitulum of *Gazania uniflora* Sims (Asteraceae). The West African species *B. schinzianum* Kraenzl. (section *Ptiloglossum* Lindl.), one of the subjects of this paper, possesses a labellum that is thought to mimic the hairy body of an insect and may thus be pseudocopulatory (Jongejan, 1994; van der Cingel, 2001), whereas in the proposed pseudocarnivorous or pseudoparasitic *B. cimicinum* J.J.Verm. [section *Epicrianthes* (Blume) Hook.f.], the flower resembles a spider and is thought to attract spider-predatory or spider-parasitic flies or wasps (Christensen, 1994). Moreover, it has been proposed that pollination in some species is wind-assisted (Sazima, 1978; Borba & Semir, 1998).

Their flowers are often dull cream or yellow–green to purple–brown and frequently spotted and hirsute with mobile labella and appendages, and insect pollinators are attracted by a combination of fruity or malodorous and carrion-like scents (Tan & Nishida, 2000, 2005; Tan, Nishida & Toong, 2002) produced by osmophores. Some may be pollinated by foraging beetles and others, especially in West Africa, by bees and wasps, including stingless bees and ctenuchid wasps (Dressler, 1990, 1993; Johansson, 1974, cited in van der Cingel, 2001), but most are pollinated by flies (Diptera; Ong, 2011, 2012, 2013; Ong & Tan, 2011, 2012; Ong *et al*., 2011), often blow-flies (Calliphoridae), flesh-flies (Sarcophagidae) and signal-flies (Platystomatidae; Ong & Tan, 2011; Ong *et al*., 2011; Ong, 2012), but also Milichiidae flies (e.g. *Pholeomyia* spp., often females) that graze small, labellar hairs or lick fluid from the central labellar groove of the flower (Braga, 1977; Sazima, 1978), and fruit flies such as *Bactrocera* spp. (Tephritidae; Ong, 2011, 2013; Ong & Tan, 2011; Ong *et al*., 2011, and references therein) and *Drosophila* spp. (Drosophilidae; Ong & Tan, 2012) that also probe the median, longitudinal groove of the labellum. Furthermore, West African species of section *Megaclinium* G.A.Fischer & J.J.Verm., such as *B. magnibracteatum* Summerh., *B. imbricatum* Lindl., *B. maximum* (Lindl.) Rchb.f. (another subject of this paper), *B. purpureorhachis* (De Wild.) Schltr. and *B. resupinatum* Ridl. have flowers that, in terms of colour and form, would indicate sapromyophily.

Although labellar secretions and anatomy have been investigated for the Neotropical section *Didactyle* (Lindl.) Cogn. (Nunes *et al*., 2014) and the Asian section *Racemosae* Benth. & Hook.f. (Davies & Stpiczyńska, 2014) and *B. wendlandianum* (Kraenzl.) Dammer (section *Cirrhopetaloides* Garay, Hamer & Siegerist; Kowalkowska, Kozieradzka-Kiszkurno & Turzyński, 2014), detailed micro-morphological studies of *Bulbophyllum* flowers are scarce (e.g. de Pádua Teixeira, Borba & Semir, 2004; Nunes *et al*., 2014; Davies & Stpiczyńska, 2014) and, to date, have not been applied to West African *Bulbophyllum* spp.

Here we investigate the labellar structure and labellar secretory activity of four representative members of West African *Bulbophyllum* {one species each from sections *Ptiloglossum* and *Oreonastes* G.A. Fischer & J.J. Verm. (*B. schinzianum* Kraenzl. and *B. lupulinum* Lindl., respectively), and two species from section *Megaclinium* [*B. falcatum* (Lindl.) Rchb.f. and *B. maximum* (Lindl.) Rchb.f., of which the former is the type species]} and compare these characters with those recorded for Asian and Neotropical species of the genus.

## MATERIALS AND METHODS

Species used in this study include *Bulbophyllum schinzianum* (accession number KLD 201311; section *Ptiloglossum*), *B. lupulinum* (accession number KLD 201401; section *Oreonastes*), and *B. falcatum* and *B. maximum* (accession numbers KLD 201402 and KLD 201307, respectively; both assigned to section *Megaclinium*). Spirit-preserved, voucher material of each of these species was deposited at the herbarium of the Royal Botanic Gardens, Kew, under accession numbers *Davies 2014-5* (*B. schinzianum*), *Davies 2014-6* (*B. lupulinum*), *Davies 2014-7* (*B. falcatum*) and *Davies 2014-8* (*B. maximum*). The identity of all species was confirmed by J. J. Vermeulen (pers. comm., 2014). Abbreviations for authors of plant names follow Brummitt & Powell (1992) throughout.

The distribution of osmophore tissue was determined by immersing entire, living flowers for 20 min in a 0.01% (w/v) aqueous solution of neutral red, which stained this tissue red. Although insufficient material precluded the accurate determination of sugar concentration in presumed nectar, when possible, sugar concentration was estimated by means of an Eclipse hand-held refractometer (Bellingham & Stanley). Detection of free α-amino acids in secretions was accomplished by treating the latter with a drop of 0.2% (w/v) aqueous ninhydrin (2,2-dihydroxyindane-1,3-dione) solution on a microscope slide and gently warming the slide on a hotplate. The formation of a purple coloration indicates the presence of free α-amino acids. Intact flowers were immersed for

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5–20 min in a saturated ethanolic solution of Sudan III or a 0.3% (w/v) ethanolic solution of Sudan black B to determine localization of lipid-secreting tissues, and mucilage-secreting tissues were detected by immersing fixed labella (see below) in 0.05% (w/v) aqueous ruthenium red solution. These tissues were subsequently examined using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM), as follows.

Samples (*c.* 1 $mm^3$) from the distal, central and proximal parts of the labellum were excised and fixed in 2.5% (v/v) glutaraldehyde/4% (v/v) formaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4 °C, transferred and washed three times in 0.1 M sodium cacodylate buffer (pH 6.8) and post-fixed in 1.5% (w/v) osmium tetroxide solution for 1.5 h at 0 °C. Dehydration using a graded ethanol series and infiltration and embedding in Spurr resin (Spurr Low-Viscosity resin; Sigma) followed. Following polymerization at 60 °C, sections were cut at 70 nm for TEM using a Leica EM UC7 ultramicrotome and a diamond knife, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined using an FEI Tecnai Spirit G2 transmission electron microscope at an accelerating voltage of 90 kV.

Semi-thin sections (0.9–1.0 μm thick) were prepared for LM and stained for general histology using aqueous 1% (w/v) methylene blue/1% (w/v) azure II (1:1) for 5–7 min (MB/AII). The periodic acid-Schiff (PAS) reaction was also used to reveal the presence of insoluble polysaccharides (Jensen, 1962). Hand-cut sections of floral tissues (labellum, petals, sepals and column) were tested for lipids, starch and mucilage using a saturated ethanolic solution of Sudan III, aqueous IKI (iodine–potassium iodide) solution and ruthenium red solution, respectively. Staining for total proteins was accomplished using Coomassie brilliant blue R250 (CBB) (Fisher, 1968; Ruzin, 1999). A 10% (w/v) aqueous solution of $FeCl_3$ was used to test for catechol-type dihydroxyphenols (Gahan, 1984) and an ethanolic solution of phloroglucinol followed by concentrated HCl (Ruzin, 1999) was used to test for lignified cell walls. These two tests stained catechol-type dihydroxyphenols and lignin black and red, respectively.

Nikon Eclipse E200 (NIS-Elements AR) software, in conjunction with a Nikon DS-Fi2 and a Canon D500 camera, was used for LM micrometry and photomicrography, respectively. An FEI Tecnai Spirit G2 (TEM Imaging & Analysis computer program) was used for TEM.

For SEM, whole labella or representative parts of the flower were dehydrated and subjected to critical-point drying using liquid $CO_2$. They were then sputter-coated with gold and examined using a Tescan Vega II LS scanning electron microscope at an accelerating voltage of 30 kV.

## RESULTS

Many *Bulbophyllum* spp. are poorly delimited and may eventually be subject to realignment. Thus, the floral habit of the four species investigated here is shown for future reference (Fig. 1) and the results summarized in Table 1.

### SECTION *PTILOGLOSSUM*

#### Bulbophyllum schinzianum

The inflorescence is a simple raceme. Each flower has a sweet but faint fragrance and is subtended by a large, prominent bract. The flowers of this species remained open for only 1 day, the sepals shutting and the pedicel becoming reflexed on day 2. The broadly triangular sepals are pale green with purple–brown bands, whereas the narrow, forwardly pointing petals are similarly coloured with purple–brown towards the apices (Fig. 1A). The hinged mid-lobe of the labellum is dark brown and densely hirsute. The longest hairs, which measured up to 3 mm, were present on the abaxial surface of the labellum (Figs 1A, 2A). These hairs are unicellular, have cellulosic cell walls and their parietal cytoplasm contains lipid droplets (Fig. 2B). The distally located vacuoles contain dark-purple cell sap and this coloration is probably due to anthocyanins. Droplets of sweet-tasting liquid were present on the margins and abaxial surface of the sepals (Fig. 1A) and on the floral bracts and thoroughly wetted the entire labellum. However, despite rigorous examination of each of these structures, secretory tissue was found only in the median longitudinal groove of the labellum, and therefore it must be concluded that the occurrence of liquid elsewhere was largely due to the overflow of secretion onto unrelated structures during anthesis. Refractometry of droplets collected from the dorsal sepal gave a total sugar value of 61.7%. However, warming these droplets with ninhydrin solution indicated the absence of free α-amino acids. The glossy labellar callus remained unstained when treated with CBB (Fig. 2D), Sudan III (Fig. 2E) and ruthenium red solution. Only the column, in particular the alar appendages, stained with Sudan III. The floral bracts also remained unstained with this reagent.

The median longitudinal groove of the labellum contained secretory, palisade-like epidermal cells of mean dimensions 28.3 × 10.4 μm (Fig. 2F, G, J–L, N, O). Adjacent epidermal cells were larger (39.3 × 30.5 μm, on average), forming conical papillae or short trichomes (Fig. 2F, H, I). The subepidermal parenchyma comprised a single layer of small isodiametric cells (mean diameter = 21.7 μm) containing dense parietal cytoplasm and numerous plastids (Fig. 2G–I). Most of the labellum consisted of

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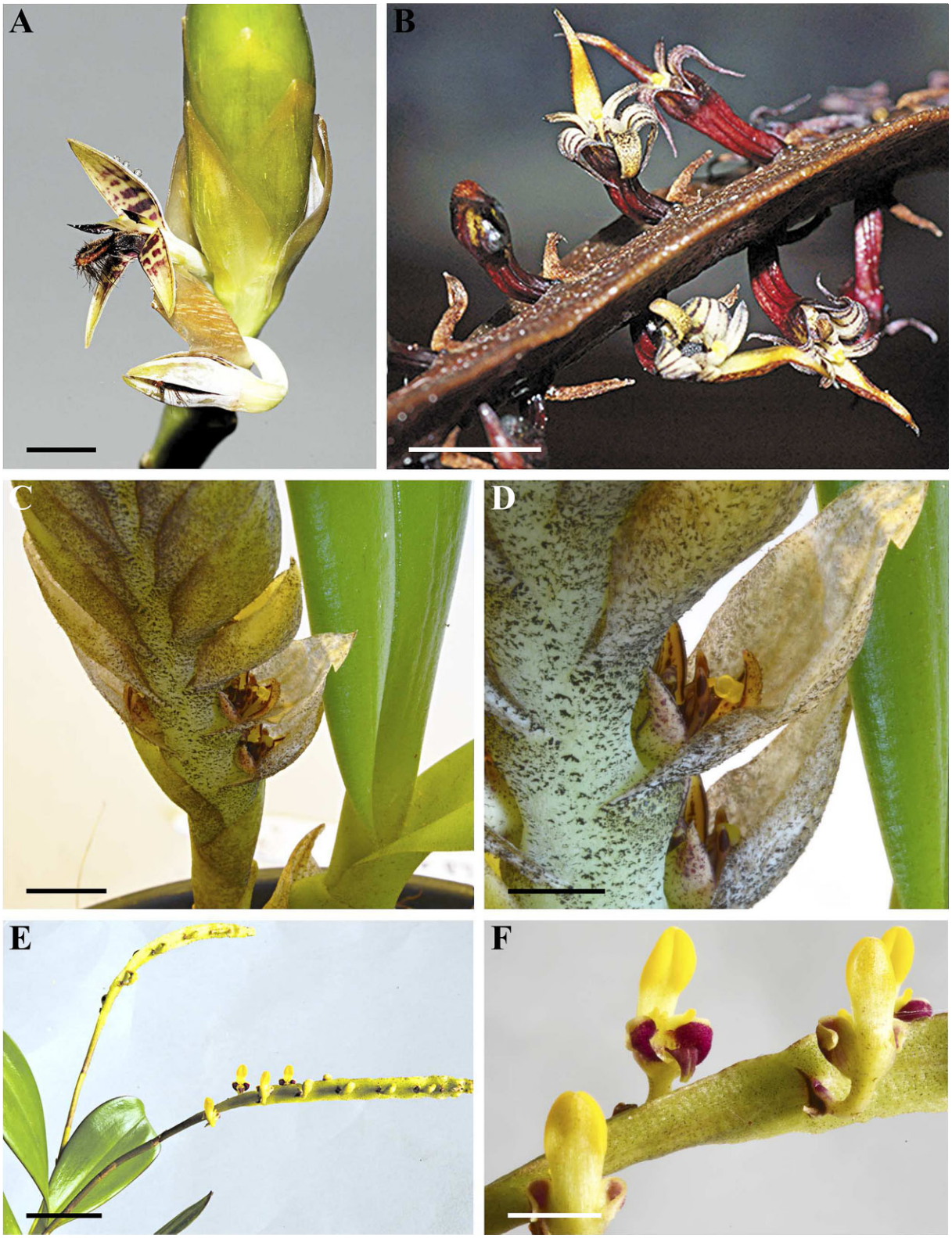


**Figure 1.** Habit of the four *Bulbophyllum* spp. investigated. (A) Habit of *B. schinzianum* showing fully opened flower, floral bracts and reflexed flower. (B) Inflorescence and flowers of *B. maximum*. (C) Basal part of inflorescence of *B. lupulinum* showing a single flower in the axil of each bract. (D) Flowers of *B. lupulinum* subtended by bracts. (E,F) Habit and inflorescence of *B. falcatum*. Scale bars = 8 mm, 5 mm, 1 cm, 6 mm, 2 cm and 6 mm, respectively.



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**Table 1.** Summary of anatomical and histochemical features of the labellum of four African species of *Bulbophyllum*

| Character | *Bulbophyllum schinzianum* Kraenzl. | *Bulbophyllum lupulinum* Lindl. | *Bulbophyllum falcatum* (Lindl.) Rchb.f. | *Bulbophyllum maximum* (Lindl.) Rchb.f. |
|---|---|---|---|---|
| Fragrance | + | – | – | – |
| Median longitudinal labellar groove | + | – | +/– | – |
| Secretion (entire flowers) | Copious | Scant | Very scant to absent | Scant |
| Sweet taste (sugars?) | + | – | – | – |
| Stains for proteins | – | – | – | – |
| Stains for α-amino acids | – | Not tested | Not tested | Not tested |
| Stains for lipids | – | – | – | – |
| Stains for mucilage | – | – | + (proximally on labellum) | – |
| Type of secretion (TEM) | Heterogeneous | Homogeneous | Not visible in section | Heterogeneous |
| Palisade-like secretory epidermal cells LM | + | – | – | + |
| Secretory tissue LM | | | | |
| Proteins | – | – | – | – |
| Lipids (also TEM) | +/– (+ in trichomes) | +++ | + | + |
| Starch (also TEM) | – | + | + | + |
| Mucilage | + (mainly outer cell wall) | – | + (mainly cell wall) | – |
| Phenolic compounds | – | – | – | – |
| Cuticle | | | | |
| Striate | + | + | + | + |
| Cuticular blisters | + | – | – | – |
| Cracks or pores | – | – | – | – |
| Micro-channels | + | + | + | + |
| Type of parenchyma | Mesophyll-like | Compact storage parenchyma | Aerenchyma | Aerenchyma |
| Idioblasts with raphides | + | + | + | + |
| Thickened idioblasts | – | – (+ sepals only) | – (+ sepals and petals) | – (+ sepals and petals) |

– = absent; + = present; +++ = abundant.

parenchyma that resembled the spongy mesophyll of leaves (Fig. 2F–O) and through which ran collateral vascular bundles enclosed in a parenchymatous bundle sheath, cells of which contained anthocyanins (Fig. 2I, L, M). Occasional idioblasts with raphides occurred scattered throughout the parenchyma (Fig. 2F, N).

The parietal cytoplasm of each palisade- like epidermal cell usually contained a centrally located nucleus, few plastids and, generally, one large vacuole (Fig. 2O). The cell walls were thin, cellulosic and had a relatively thick cuticle (Fig. 2G, H, O). Generally, secretion was not visible on the surface of the cells when viewed using LM. Neither the labellar groove nor its secretion stained for proteins with CBB, but the thick cuticle on the outer wall of epidermal cells and hairs stained for lipids with Sudan III (Fig. 2G, H). Furthermore, palisade-like cells, especially their outer tangential walls, stained for mucilage with ruthenium red solution (Fig. 2L). Histochemical tests with IKI and PAS showed that starch was generally absent from these cells, but present in subepidermal parenchyma and mesophyll-like parenchyma (Fig. 2I–K). Treatment of sections of the labellum with $FeCl_3$ and neutral red failed to reveal the presence of intracellular phenolic compounds and osmophore tissue, respectively.

SEM of the labellum demonstrated that secretion was present proximally and that the median longitudinal groove was lined with palisade-like cells and its surface verrucose and coated with secretion (Fig. 3A–D). Cuticular blisters resulting from the subcuticular accumulation of secretion were also present (Figs 2O,



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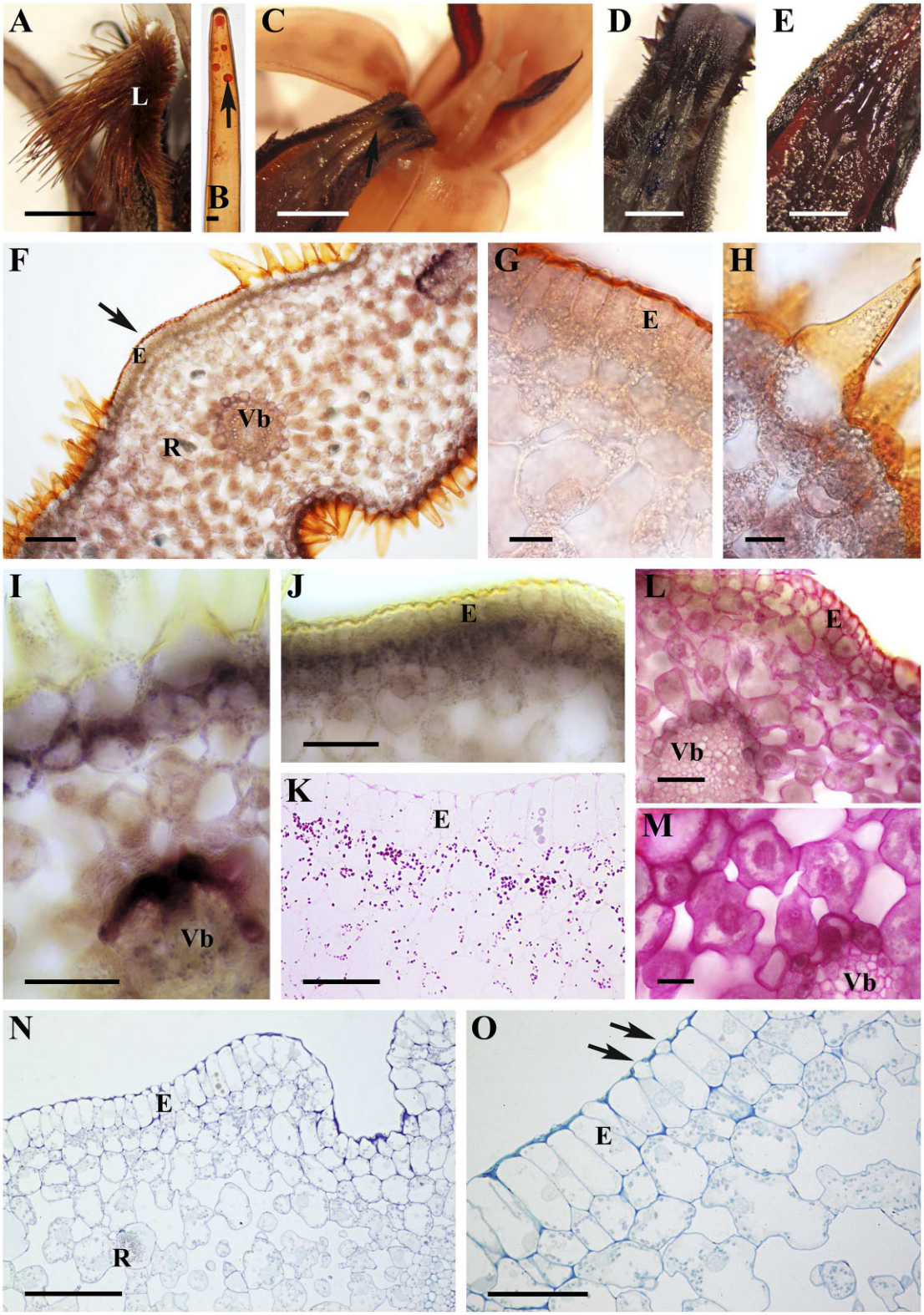


**Figure 2.** See caption on next page.

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**Figure 2.** *Bulbophyllum schinzianum*, LM. (A) Labellum clothed abaxially with long hairs (trichomes). (B) Detail of trichome stained with Sudan III and containing lipid droplets (arrow). (C) Proximal part of labellum, the median longitudinal groove indicated by arrow. (D,E) Staining of the entire labellum with CBB and Sudan III failed to indicate the presence of proteins and lipids, respectively. (F) Section of proximal part of labellum indicating position of palisade-like cells (arrow). (G) Palisade-like cells and subepidermal parenchyma following treatment with Sudan III. Note intense staining of cuticle. (H) Cuticle of labellar hairs stained with Sudan III. (I) Treatment with IKI revealed that starch was present in non-secretory epidermal and subepidermal parenchyma cells. (J,K) Staining with IKI (J) and PAS (K) revealed that starch was largely absent from palisade-like cells, but occurred in subepidermal parenchyma cells. (L,M) Palisade-like cells, subepidermal parenchyma and mesophyll-like parenchyma stained with ruthenium red. Note that the outer tangential wall of palisade-like cells stains intensely with this reagent. (N) Palisade-like cells, subepidermal parenchyma and deeply located mesophyll-like parenchyma stained with MB/AII. (O) Detail of palisade-like cells. Cuticular blisters present on the outer tangential walls are indicated by arrows. Scale bars = 2 mm, 10 μm, 2 mm, 1 mm, 1 mm, 100 μm, 20 μm, 20 μm, 50 μm, 50 μm, 50 μm, 50 μm, 20 μm, 100 μm and 50 μm, respectively. E, palisade-like epidermis; L, labellum; R, raphides; Vb, vascular bundle.

3B, C). The cuticle of the palisade-like cells lining the labellar groove, although somewhat striate, was mainly smooth and no cracks or pores were observed. Secretion was absent from the labellar trichomes that occurred alongside the grove and on the distal part of the labellum (Fig. 3E, F).

TEM of the outer cellulosic periclinal wall and cuticle of the palisade-like cells revealed that the cuticle contained numerous micro-channels (Fig. 4A–D). Heterogeneous secretory material was present in depressions of the convoluted cuticle. This material consisted of two components, a homogeneous, electron-dense component (Fig. 4B) and a less electron-dense component that appeared almost fibrous in section (Fig. 4B–D). Secretory vesicles of various sizes collected in the parietal cytoplasm, alongside the plasmalemma, and fused with the latter (Fig. 4B, C). Invaginations of the plasmalemma and the vesicles, contents of which varied in electron-translucency, were observed. Therefore, it is likely that transport of material similar to that found on the surface of the cuticle is vesicle-mediated. The cytoplasm of palisade-like epidermal cells was granular and electron-dense and contained abundant profiles of smooth endoplasmic reticulum (SER) with dilated cisternae, numerous ribosomes, dictyosomes and numerous mitochondria. Elliptical plastids were present, each containing an electron-dense stroma, plastoglobuli and an array of internal tubules. Material present within these tubules appeared grey following conventional staining for TEM. The plastids, which usually lacked starch, were often associated with long profiles of SER (Fig. 4E). Vacuoles were usually electron-translucent or contained variously sized globular, electron-dense bodies. Occasionally, plasmodesmata were observed in primary pit-fields and these connected the cytoplasm of adjoining palisade-like epidermal cells.

Abundant mitochondria and rough endoplasmic reticulum (RER) profiles were present in subepidermal and parenchyma cells, but these cells possessed larger, starch-containing amyloplasts and their cytoplasm was less granular (Fig. 4F) than that of palisade-like epidermal cells. Plasmodesmata in the cell walls of subepidermal and parenchymal cells were much more numerous than in those of palisade-like cells.

### Section *Oreonastes*

#### Bulbophyllum lupulinum

The inflorescence is a laterally compressed spike, each small, sessile flower arising from a concave depression of the rachis and subtended by a prominent and wide, papery bract that greatly exceeds the length of the flower (Fig. 1C, D). The flower has no obvious scent. The sepals are flesh-coloured abaxially, suffused dark red or yellow, with dark red to purple margins, and bear brown trichomes. Adaxially, the sepals are bright yellow marked with dark red to purple. Each flesh-coloured petal is spathulate, marked dark red and bears a prominent dark red spot distally on its abaxial surface (Fig. 5A, B). The mobile labellum is yellow or flesh-coloured, marbled with dark red, and has two relatively indistinct longitudinal ridges separated by a dark red, flattened or slightly depressed area (Fig. 5A–D). Two glossy patches are often visible on the dark red, expanded base of the column-foot (Fig. 5A, B). Entire, fixed flowers and labellum stained uniformly for lipids following immersion in ethanolic solutions of Sudan Black B and Sudan III (Fig. 5C, D), whereas treatment with CBB and ruthenium red failed to detect the presence of proteins and mucilage, respectively. Likewise, treatment with neutral red failed to detect the presence of osmophore tissue.

The adaxial labellar epidermal cells were conical, globose or somewhat elongate in transverse section and of mean dimensions 22.4 × 24.4 μm (Figs 5E, F, I, K, 6A–C). Similarly, the abaxial labellar epidermal cells were also conical, but flattened or globose at the labellar margin and measured 20.5 × 22.1 μm, on average.

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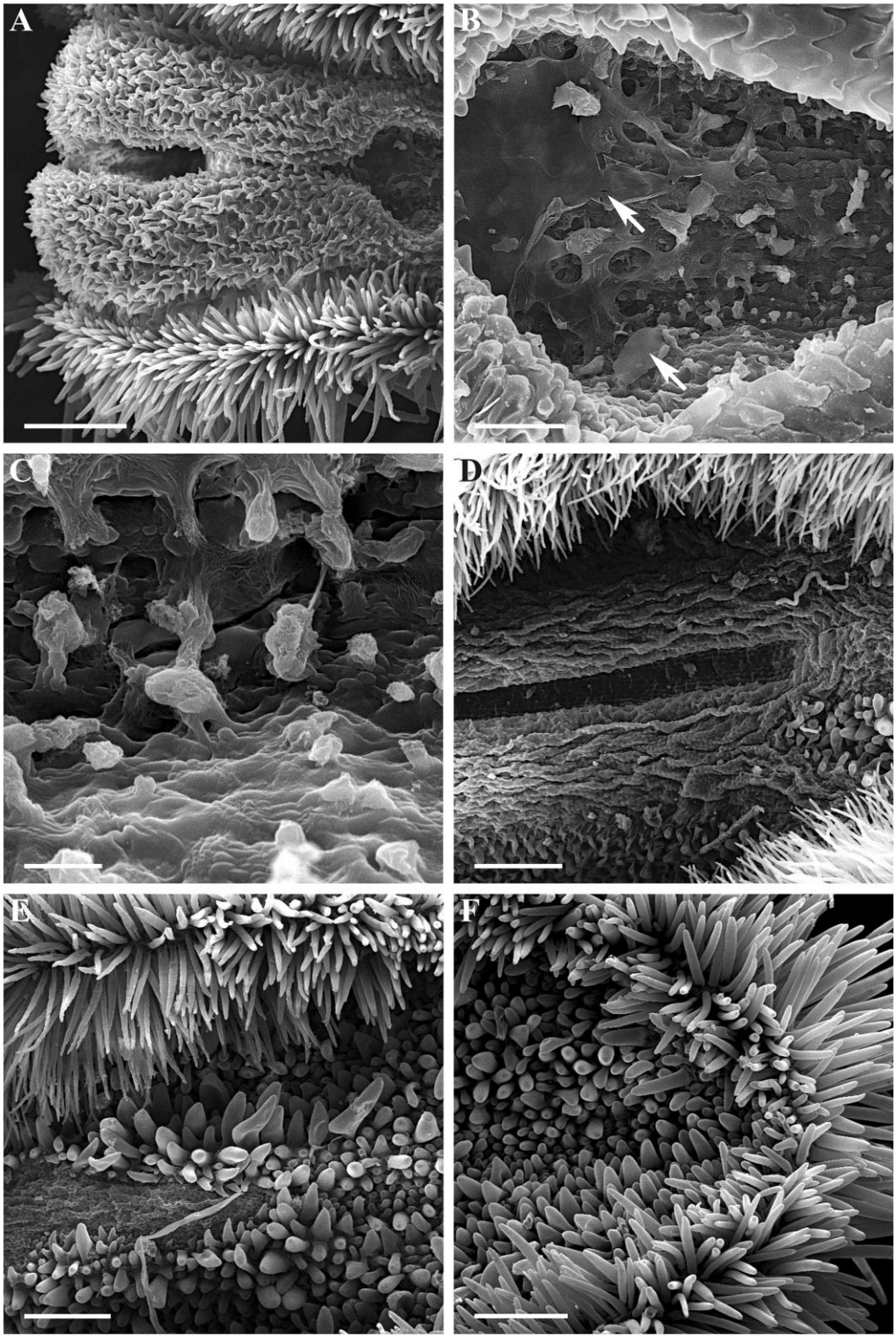


**Figure 3.** *Bulbophyllum schinzianum*, SEM. (A) Proximal part of labellum with median longitudinal groove. (B) Cells lining the groove are coated with secretory residues (arrows). (C) Cuticular blisters occur on the surface of the palisade-like cells lining the labellar groove. (D) Distally, non-secretory cells are found alongside the labellar groove. (E,F) Adaxial labellar hairs lack secretion on their surface. Scale bars = 200, 50, 10, 200, 200 and 200 μm, respectively.



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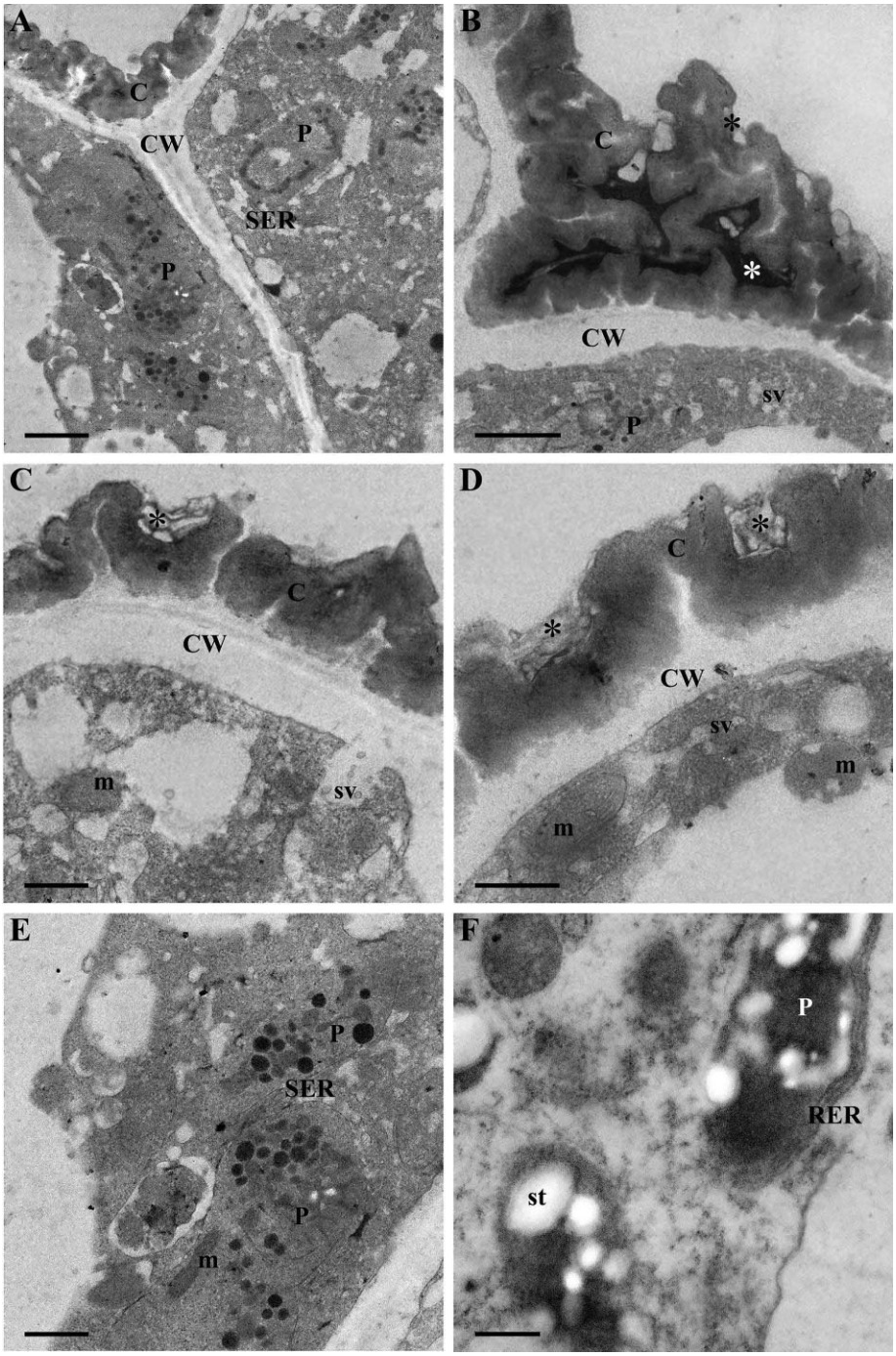


**Figure 4.** *Bulbophyllum schinzianum*, TEM. (A) Section through palisade-like epidermal cell showing electron-dense cytoplasm containing starchless plastids with plastoglobuli. (B) Secretory cell with parietal cytoplasm containing plastid and short SER profiles. A folded cuticle with micro-channels is present. Both an electron-dense, homogeneous, osmiophilic component of the secretion (white asterisk) and an electron-translucent, heterogeneous component (black asterisk) are present. (C) Heterogeneous secretion in depression of cuticle (asterisk). The plasmalemma is invaginated and secretory vesicles are visible in the periplasmic space. (D) Micro-channels are visible in the cuticle, and secretory material collects in cuticular depressions (asterisks). Mitochondria and secretory vesicles occur in the parietal cytoplasm. (E) Plastids with plastoglobuli, surrounded by SER and secretory vesicles, occur in palisade-like cells. (F) Plastids with small starch grains occur in parenchyma cells. Scale bars = 1, 1, 0.5, 0.5, 0.5 and 0.5 μm, respectively. C, cuticle; CW, cell wall; m, mitochondrion; P, plastid; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; st, starch; sv, secretory vesicle.



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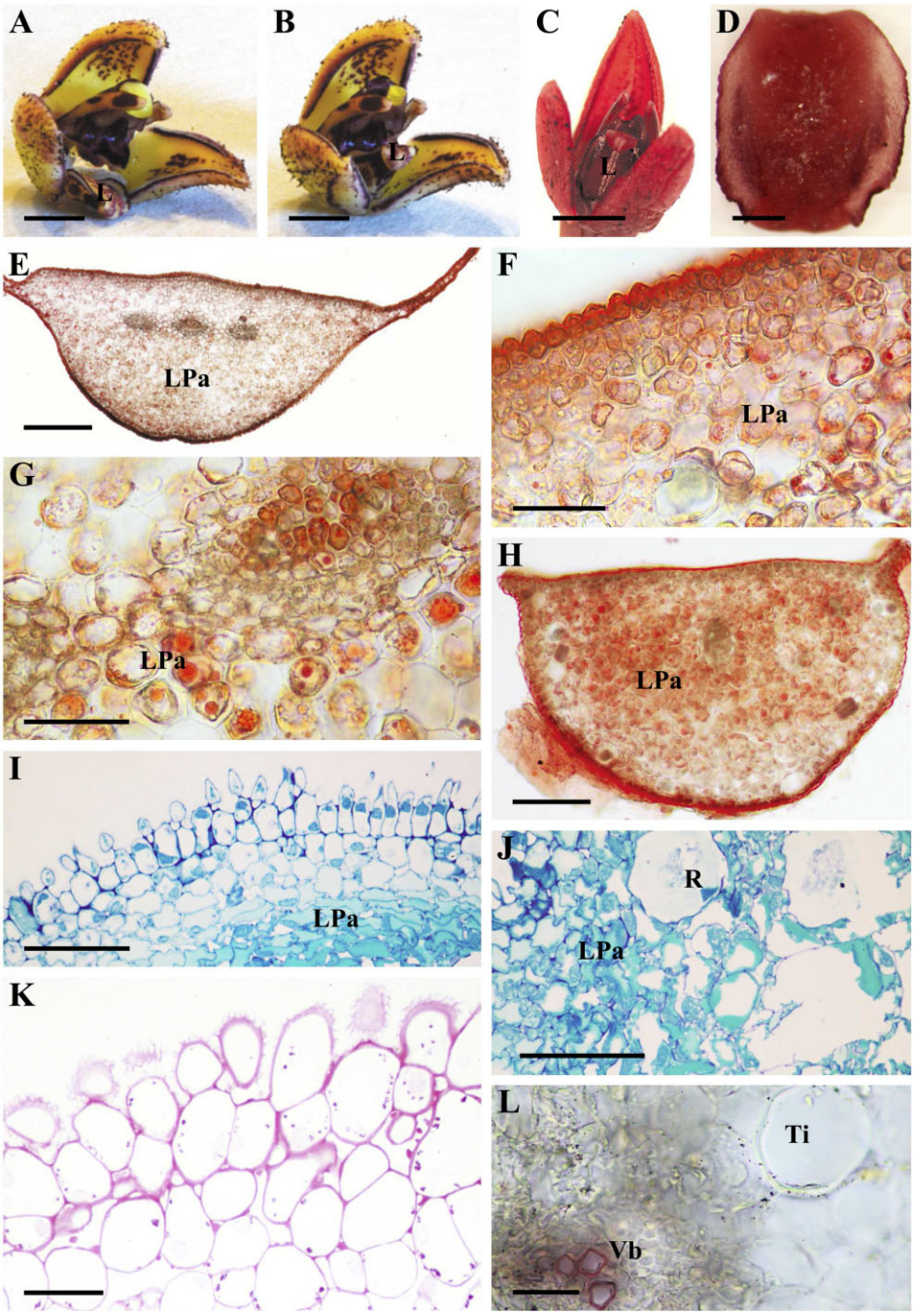


**Figure 5.** *Bulbophyllum lupulinum*, habit of flower, LM. (A,B) Flower showing both adaxial and abaxial surfaces of the labellum. (C) Flower uniformly stained red following immersion in ethanolic Sudan III solution. (D) Adaxial surface of labellum showing the absence of lipid-rich secretion following staining with Sudan III. (E) Transverse section of labellum stained with Sudan III. Note the three vascular bundles and lipid-storing parenchyma. (F) Adaxial epidermis and parenchyma stained with Sudan III. (G) Lipid droplets occur both in the storage parenchyma and in the vascular bundle. (H) Transverse section of petal showing epidermis with material that stains red following treatment with Sudan III, together with lipid-storing parenchyma. (I) Section of labellum stained with MB/AII showing epidermis, subepidermal parenchyma and lipid-storing parenchyma. (J) Lipid-storing parenchyma and idioblasts with raphides. (K) Epidermal cells and parenchyma stained with PAS to show the distribution of starch. (L) Transverse section of sepal following treatment with phloroglucinol + HCl showing vascular bundle and thickened idioblast. Scale bars = 2 mm, 2 mm, 2 mm, 0.5 mm, 250 μm, 50 μm, 50 μm, 250 μm, 50 μm, 50 μm, 25 μm and 25 μm, respectively. L, labellum; LPa, lipid-storing parenchyma; R, raphides; Ti, thickened idioblast; Vb, vascular bundle.



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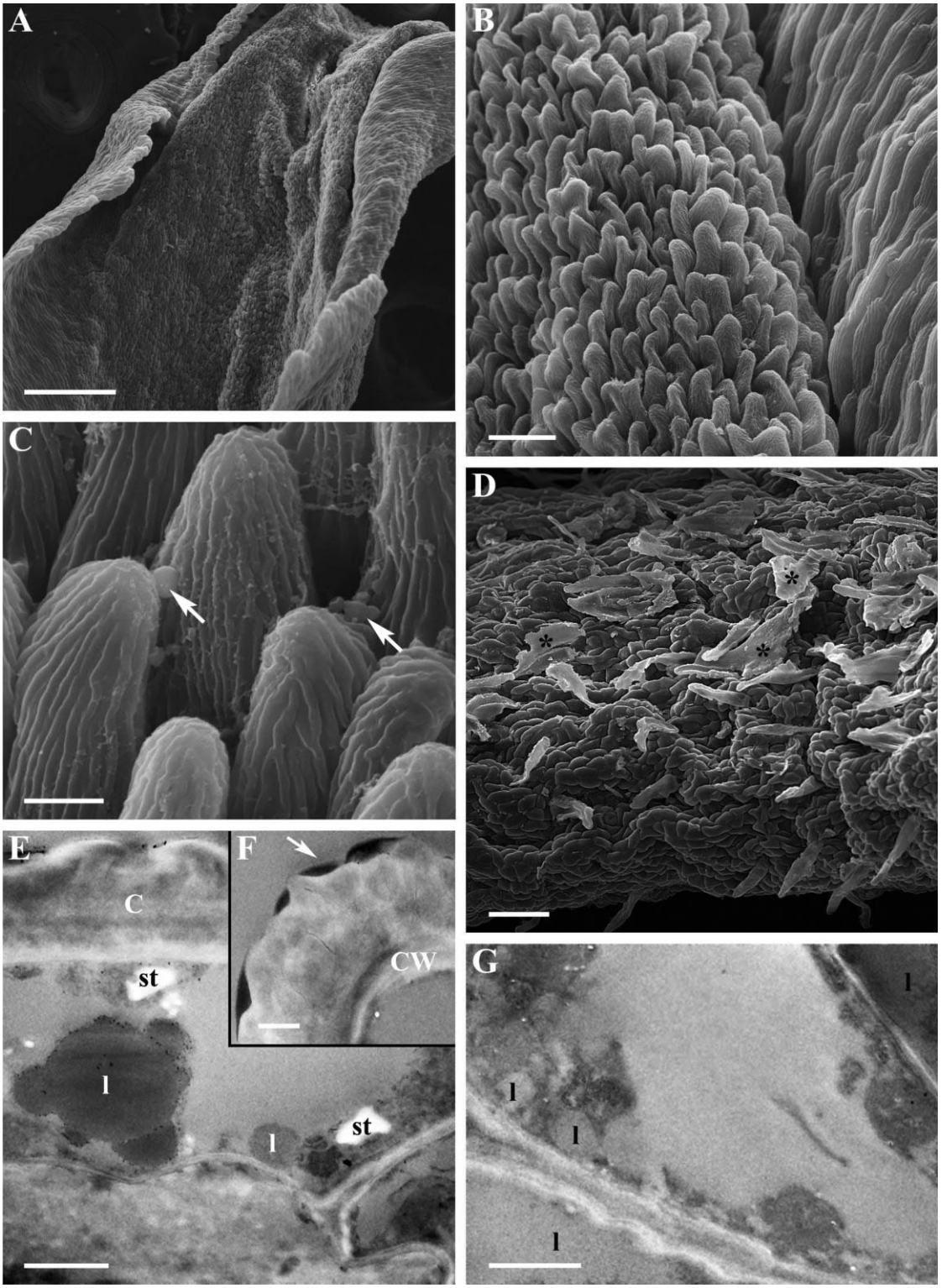


**Figure 6.** *Bulbophyllum lupulinum*, SEM and TEM. (A) Adaxial surface of labellum lacking median longitudinal groove. (B) Conical and somewhat elongate epidermal cells of central part of labellum. (C) Epidermal cells with finely striate cuticle and globular material (arrows) on their surface. (D) Wax deposits (asterisks) and trichomes present abaxially on the sepals. (E) Lipid droplets in epidermal cell. (F) Homogeneous material (arrow) on surface of cuticle with microchannels. (G) Lipids in cytoplasm of parenchyma cell. Scale bars, 200 μm, 20 μm, 5 μm, 100 μm, 2 μm, 500 nm and 1 μm, respectively. C, cuticle; CW, cell wall; l, lipid; st, starch.



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The subepidermal parenchyma usually comprised two layers of small isodiametric cells (mean diameter = 18.6 μm) with dense parietal cytoplasm. Most of the labellum consisted of parenchyma through which ran three collateral vascular bundles (Fig. 5E, G). Occasional large idioblasts with raphides were present. Anatomically, the structure of the petal, except for the single vascular bundle (Fig. 5H), was similar to that of the labellum, whereas adaxially, the sepals possessed globose, thick-walled epidermal cells that abaxially were modified to form short unicellular trichomes (Fig. 6D). Thickened idioblasts were scattered through the subepidermal parenchyma, but were present exclusively in sepals (Fig. 5L).

Secretion was not visible on the adaxial surface of labellar cells under LM (but see SEM and TEM below). Neither the epidermal nor the parenchyma cells stained for proteins with CBB. However, the thick cuticle on the outer wall of epidermal cells stained for lipids with Sudan III, indicating that it was the staining of the cuticle rather than that of the scant secretion that resulted in the uniform staining of entire flowers treated with this reagent. Staining with Sudan III also revealed numerous lipid droplets within cells of the epidermis and ground parenchyma, together with those of the xylem and phloem parenchyma (Fig. 5E–G). Similar staining results were obtained for sepals and petals, but here waxy deposits were also present (Figs 5H, 6D). Histochemical tests with IKI and PAS revealed the presence of small quantities of starch in epidermal and parenchyma cells (Fig. 5K). Treatment of sections of the labellum, petals and sepals with $FeCl_3$ revealed the absence of phenolic compounds, whereas testing with phloroglucinol +HCl showed the presence of lignin in the walls of xylem elements, but not in those of thickened idioblasts. Here, the walls remained cellulosic (Fig. 5L).

Investigations using SEM failed to reveal the presence of secretion, other than an occasional globule on the surface of the adaxial epidermis of the labellum (Fig. 6C). The cuticle was striate and no cracks, pores or cuticular blisters were observed (Fig. 6B, C). Wax deposits and trichomes (Fig. 6D) were present on the abaxial epidermis of sepals, but were absent adaxially.

Observations by TEM of the outer periclinal wall and cuticle of adaxial epidermal cells of the labellum revealed traces of osmiophilic homogeneous material in the depressions of the striate cuticle (Fig. 6E, F). Numerous micro-channels were present in the epidermal cuticle (Fig. 6F) and conspicuous lipid droplets were observed within the cytosol and vacuoles of epidermal and parenchyma cells (Fig. 6E, G). The parietal cytoplasm of parenchyma was particularly electron-dense and consequently observation of ultrastructural details was often difficult. Nevertheless it consisted mainly of SER and abundant free ribosomes. Mitochondria and plastids, the latter containing minute starch grains, were also abundant (Fig. 6E), but dictyosomes were not observed.

### Section *Megaclinium*

Bulbophyllum falcatum

The inflorescence is a modified, simple raceme, each of the small flowers arising on a short pedicel inserted onto the laterally compressed rachis and subtended by a small, narrow, papery bract. The dorsal sepal and petals are yellow and the lateral sepals and mobile labellum are predominantly red (Figs 1E, F, 7A), the labellar mid-lobe lacking or having a poorly defined median longitudinal groove (Fig. 7A–E). The flowers, which are long-lasting, lacked fragrance and, to the naked eye, no secretion appeared to be present, although on occasion, silvery patches, possibly dried secretion residues, were seen in the throat of the flower and along the centre of the labellum.

Immersing whole flowers in Sudan III, CBB and ruthenium red did not generally indicate selective staining for lipids, protein or mucilage (Fig. 7B–D), but trichomes present on the surface of sepals and petals stained strongly with these reagents. The proximal part of the labellar lamina also stained with ruthenium red (Fig. 7D).

The epidermal cells of the labellum were globose or conical and of mean dimensions 14.5 × 12.3 μm. They had thin, cellulosic walls and a relatively thick cuticle, but secretion was not visible on their surface, when viewed using LM.

Aerenchyma occurred beneath the epidermis (Fig. 7E–I). This tissue consisted of cells of variable shape and size, containing dense parietal cytoplasm and numerous plastids. Vascular bundles running through the aerenchyma were enclosed by parenchymatous bundle sheaths (Fig. 7E–G), but the strands of phloem and xylem lacked fibre support. Occasional idioblasts with raphides were located hypodermally.

Neither epidermal nor aerenchyma cells stained selectively for proteins with CBB. However, the epidermal cuticle stained for lipids with Sudan III and lipid droplets occurred in cells of the epidermis and aerenchyma (Fig. 7I). Furthermore, the cell walls of epidermal cells also stained slightly for mucilage with ruthenium red solution. Histochemical testing with IKI and PAS demonstrated the presence of numerous starch grains in the epidermal and aerenchyma cells (Fig. 7G, H), but no phenolic compounds were present. Starch was also present in the parenchyma cells of petals and sepals, but in contrast to the labellum, parenchyma here was of the typical, more compact type referred to as ground parenchyma

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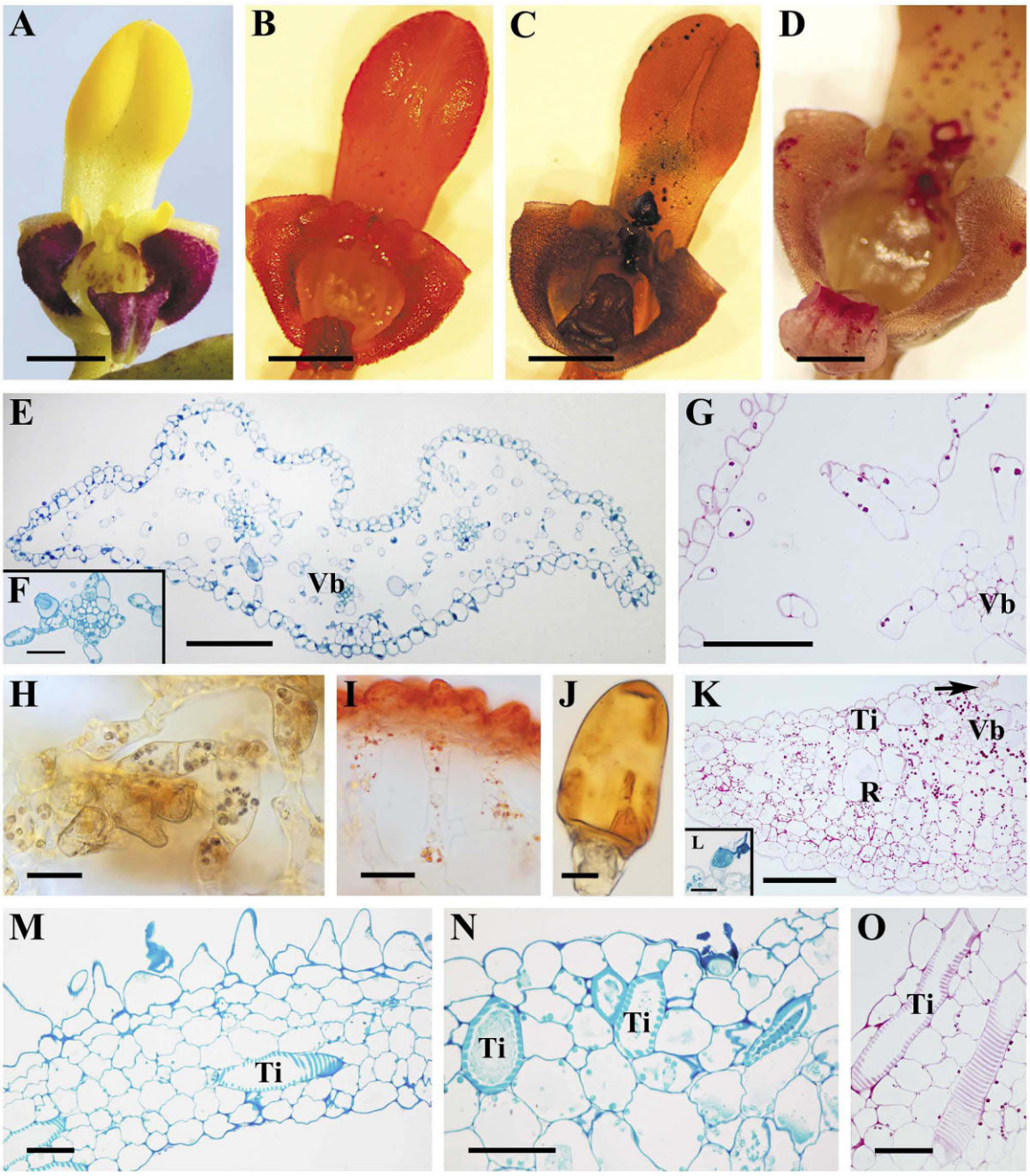


**Figure 7.** *Bulbophyllum falcatum*, habit of flower and LM. (A) Flower showing articulated labellum. (B–D) Whole flower stained with Sudan III, CBB and ruthenium red, respectively. (E) Transverse section of labellum stained with MB/AII showing epidermis and underlying aerenchyma with vascular bundles. (F) Detail of vascular bundle lacking mechanical strengthening tissue. (G,H) Starch in epidermal and aerenchyma cells, as revealed using the PAS reaction (G) and IKI (H). (I) Staining of cuticle and small lipid droplets in aerenchyma cells following treatment with Sudan III. (J) Secretory trichome composed of a head and basal cell. (K) Section of petal showing compact ground parenchyma containing numerous starch grains, as detected using the PAS reaction. Idioblasts with raphides and subepidermally located thickened idioblasts are also present. Secretory hair is indicated by arrow. (L) Detail of hair, the head and secretion stained strongly with MB/AII. (M) Longitudinal section of lateral sepal showing thickened idioblasts. (N) Section of petal showing thickened idioblasts. Note the granular content of thickened idioblasts following staining with MB/AII. (O) Thickened idioblasts in lateral sepal showing annular thickening of cell wall (PAS reaction). Scale bars = 2 mm, 2 mm, 2 mm, 1 mm, 300 μm, 30 μm, 50 μm, 20 μm, 20 μm, 10 μm, 100 μm, 20 μm, 50 μm, 50 μm and 50 μm, respectively. R, raphides; Ti, thickened idioblast; Vb, vascular bundle.



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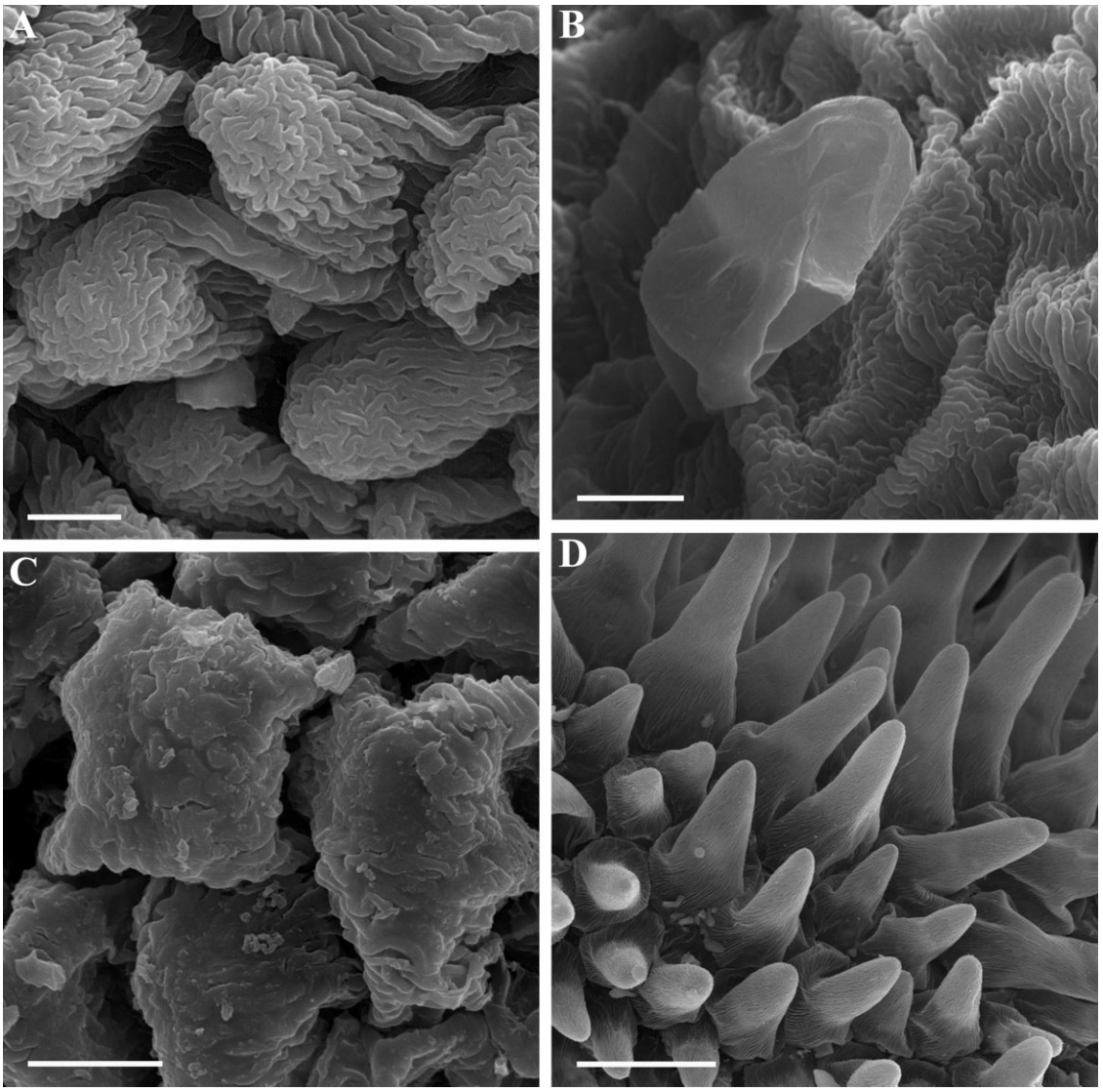


**Figure 8.** *Bulbophyllum falcatum*, SEM. (A) Striate cuticle of adaxial surface of labellum. (B) Secretory trichome. (C) Wax deposits on surface of epidermal cells of petals. (D) Conical papillae and trichomes are present adaxially on lateral sepals. Scale bars = 5, 10, 10 and 50 μm, respectively.

(Fig. 7K, M, O). The adaxial epidermis of the lateral sepals consisted of trichomal cells, whereas that of the dorsal, median sepal and petals was formed of globose cells (Figs 7K–O, 8A–D). Bicellular hairs were present on the abaxial and adaxial epidermis of both median dorsal sepals and petals and the abaxial epidermis of the lateral sepals. Each bicellular hair comprised a small basal cell sunken into the epidermis and a larger, more elongate head (Figs 7J, 8B). These hairs were coated with secretion that stained strongly with Sudan III and MB/AII, but did not stain with the PAS reaction (Fig. 7J–L). Thickened idioblasts, of mean diameter 38.4 μm, were also present hypodermally in the sepals and petals. These had cellulosic cell walls and helical thickening (Fig. 7K, M, O). Staining with MB/AII revealed that the lumina of thickened idioblasts contained granular material (Fig. 7N).

The cuticle, as revealed by SEM, was striate and lacked cracks, pores and cuticular blisters (Fig. 8A–D). Furthermore, except for that produced by bicellular hairs, SEM did not indicate the presence of secretion on the surface of the petals, sepals or labellum (Fig. 8A–D). Occasionally, epidermal wax deposits were observed on the petals and median dorsal sepal (Fig. 8C), and TEM revealed the presence of microchannels in the cuticle of epidermal cells of the labellum (Fig. 9A–C).

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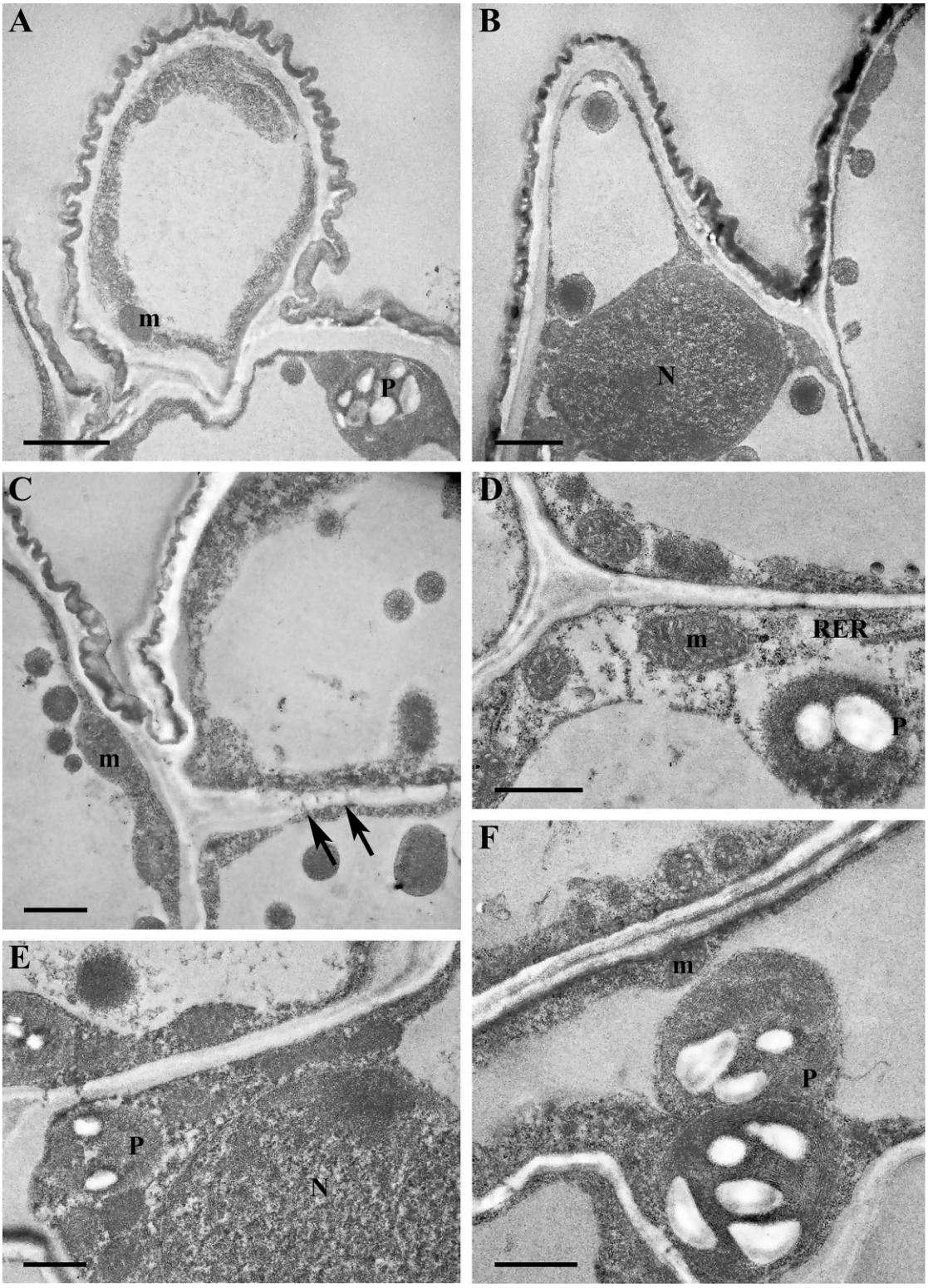


**Figure 9.** *Bulbophyllum falcatum*, labellum TEM. (A) Section through epidermal cell showing parietal cytoplasm and striate cuticle on cell wall. (B,C) Intravacuolar globular material occurs in both epidermal and subepidermal cells. In (C), plasmodesmata connecting adjoining epidermal and subepidermal cells are indicated by arrows. (D–F) Plastids containing starch grains but lacking plastoglobuli occur in epidermal (E) and subepidermal (D,F) cells. Scale bars = 2, 2, 1, 1, 1 and 1 μm, respectively. m, mitochondrion; N, nucleus; P, plastid; RER, rough endoplasmic reticulum.



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As with SEM, secreted material was not visible on the surface of the cuticle of labellar epidermal cells when viewed using TEM (Fig. 9A–C). The thin, parietal cytoplasm of epidermal and parenchyma cells was granular and electron-dense and mostly contained RER bearing numerous ribosomes (Fig. 9A–F). Mitochondria were also abundant, but dictyosomes were not observed. The plastids contained an electron-dense stroma with starch grains, but plastoglobuli were absent (Fig. 9D–F). Vacuoles contained relatively large electron-dense globular bodies (Fig. 9A–C, E). Plasmodesmata in the walls of aerenchyma cells were much more numerous than those observed for epidermal cells.

Bulbophyllum maximum

Again, the inflorescence is a modified, simple raceme, the small flowers arising from the laterally compressed rachis and subtended by a narrow, papery bract. The dorsal sepal is white, distally yellow, the lateral sepals and petals are white, striped with purple–black, and the mobile labellum is pale yellow, spotted black and seemingly lacking a median longitudinal groove (Fig. 1B). Proximally, the labellar margins are serrate (Figs 10C, 11A). The ventral surface of the column base is black and the peduncule ovary is red. The flowers, which open one or two at a time and last about 1 day, lack any obvious fragrance. Opalescent droplets of secretion were seen in stark contrast against the black ventral surface of the column base, but were absent from the labellum. Testing with Sudan III, CBB and ruthenium red solution did not demonstrate the presence of lipids, proteins or mucilage, respectively, on the adaxial surface of the labellum (Fig. 10A–C).

The adaxial labellar surface comprised globose epidermal cells marginally and elongate, epidermal cells centrally (Figs 10D, G–L, 11A–C). In transverse section, these central cells, of mean dimensions 21.7 × 8.0 μm, were seen to have a more or less palisade-like arrangement (Fig. 10J–L) and contained parietal cytoplasm with a centrally located nucleus. Each cell usually contained large vacuoles. The thick, outermost cellulosic walls of adaxial and abaxial epidermal cells possessed a thick cuticle that stained intensely with Sudan III (Fig. 10D).

The subepidermal parenchyma consisted of a single layer of smaller isodiametric cells, of mean diameter 16.5 μm. Most of the labellum consisted of aerenchyma through which ran three collateral vascular bundles; the vascular elements, however, were not supported by fibres. Large idioblasts containing raphides were scattered throughout the aerenchyma (Fig. 10G, J, K). Aerenchyma, however, was absent from the other parts of the flower, and here it was replaced by ground parenchyma and, especially in the hypodermal region of the sepals and petals, by large thickened idioblasts with helical or annular-type thickenings (Fig. 10E). Bicellular epidermal, secretory trichomes (Fig. 10E, F) were present on all parts of the flower, with the sole exception of the labellum.

Histochemical tests revealed the presence of numerous intracellular lipid droplets in the epidermal and aerenchyma cells (Fig. 10D), but no surface protein or mucilage (Fig. 10H, I). Treatment with PAS also revealed the presence of small quantities of starch in aerenchyma and epidermal cells, but these were almost undetectable when IKI was used to test for this polysaccharide (Fig. 10G, L). Treatment with $FeCl_3$ did not reveal the presence of phenolic compounds in labellar cells and no secretion was detected on the surface of the labellum using LM. Furthermore, SEM observations of the striate cuticle of epidermal cells failed to detect the presence of cracks, pores, blisters or secreted material (Fig. 11A–D). In fact, under SEM, secretion appeared to accumulate only on the surface of secretory hairs (Figs 10F, 11E).

Investigations using TEM revealed the presence of micro-channels in the striate cuticle of epidermal cells (Fig. 12A) and, contrary to SEM, the presence of electron-dense, osmiophilic secreted material on the surface of the cuticle. Moreover, similar material, often present in relatively large quantities, occurred in epidermal and subepidermal parenchyma cells (Fig. 12A–C). The cytoplasm of epidermal and subepidermal cells contained abundant RER, small vesicles and mitochondria (Fig. 12B, D). The ovoid plastids contained minute starch grains and several small plastoglobuli, whereas plastids present in parenchyma cells accumulated larger starch grains (Fig. 12B, D). The vacuoles contained osmiophilic globules, electron-translucent material or small, flocculent deposits, but no myelin-like figures were recorded.

## DISCUSSION

Three types of labellar organization were recognized. Of these, the most specialized occurred in *B. schinzianum*.

Flowers of *B. schinzianum* differed from those of the other species investigated in being larger, sweetly fragrant, densely hirsute and possessing a well-defined, relatively deep and narrow, median longitudinal labellar groove consisting of palisade-like secretory cells, flanked by unicellular trichomes that contained abundant lipid droplets. Furthermore, the labellum produced a copious, heterogeneous, sweet-tasting secretion. Similar, heterogeneous (sugary, yet oil- or lipid-rich) secretion was also reported by Pohl (1935) for the Asian species *B. lobbii* Lindl. [section *Sestochilos* (Breda) Benth. & Hook. f.] and *B. macranthum*

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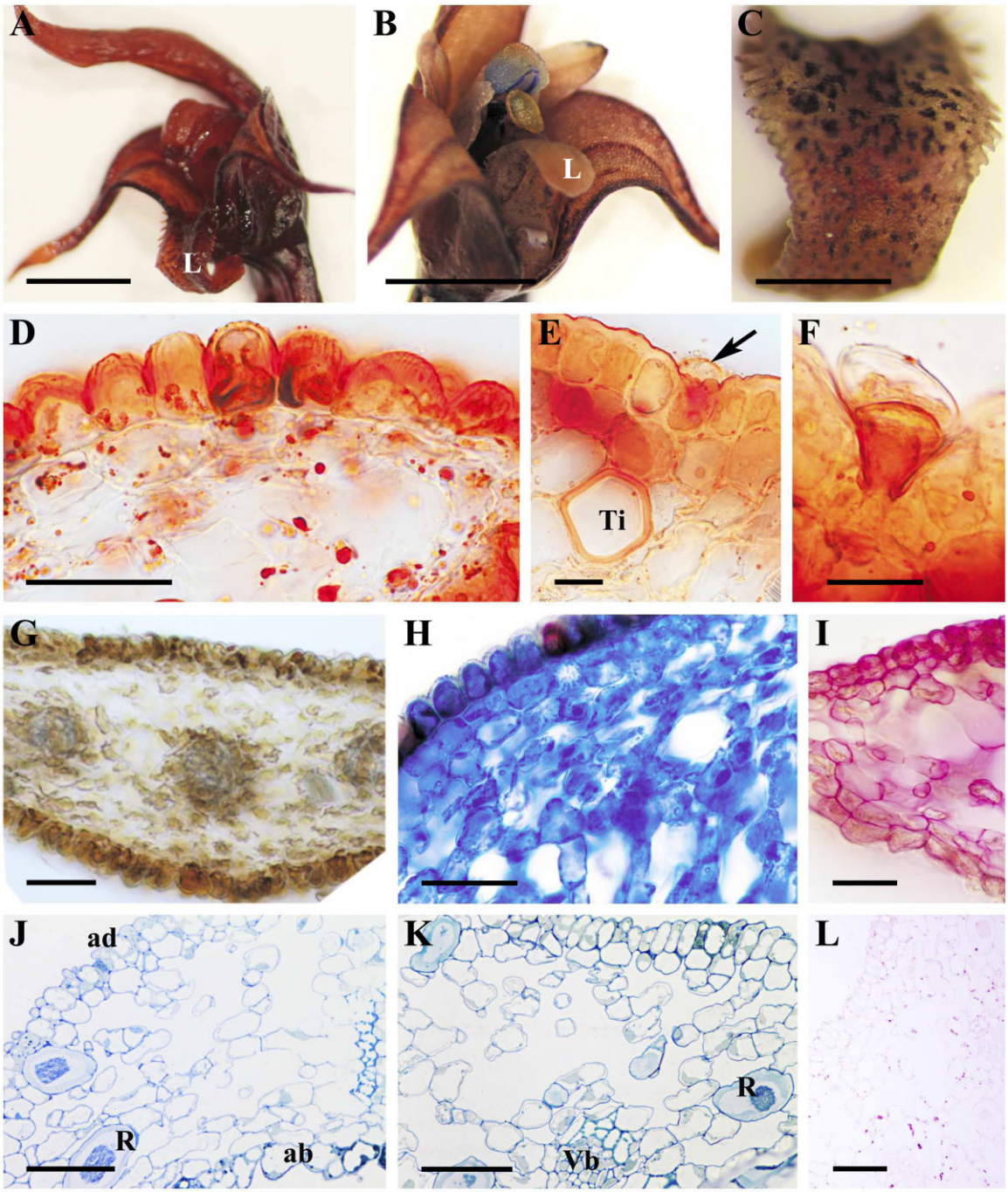


**Figure 10.** *Bulbophyllum maximum*, LM. (A,B) Entire flower stained with Sudan III and CBB, respectively. (C) Labellum stained with ruthenium red. (D) Longitudinal section of labellum showing adaxial epidermis and parenchyma stained with Sudan III. (E) Section of petal with thickened idioblast and secretory trichome (arrow). (F) Detail of trichome coated with secretion that stained with Sudan III. (G) Transverse section of labellum following treatment with IKI. (H) Section of labellum stained with CBB indicating the absence of protein-containing surface secretion. (I) Staining with ruthenium red failed to reveal the presence of surface mucilage. (J) Longitudinal section of labellum stained with MB/AII showing the adaxial and abaxial epidermis, aerenchyma and idioblasts containing raphides. (K) Adaxial epidermal cells more or less in palisade-like arrangement and aerenchyma with vascular bundle. (L) Treatment with PAS revealed the presence of inconspicuous starch grains in both epidermal and aerenchyma cells. Scale bars = 2 mm, 2 mm, 0.5 mm, 50 μm, 20 μm, 20 μm, 100 μm, 50 μm, 50 μm, 100 μm, 50 μm and 50 μm, respectively. ab, abaxial epidermis; ad, adaxial epidermis; L, labellum; R, raphides; Ti, thickened idioblast; Vb, vascular bundle.

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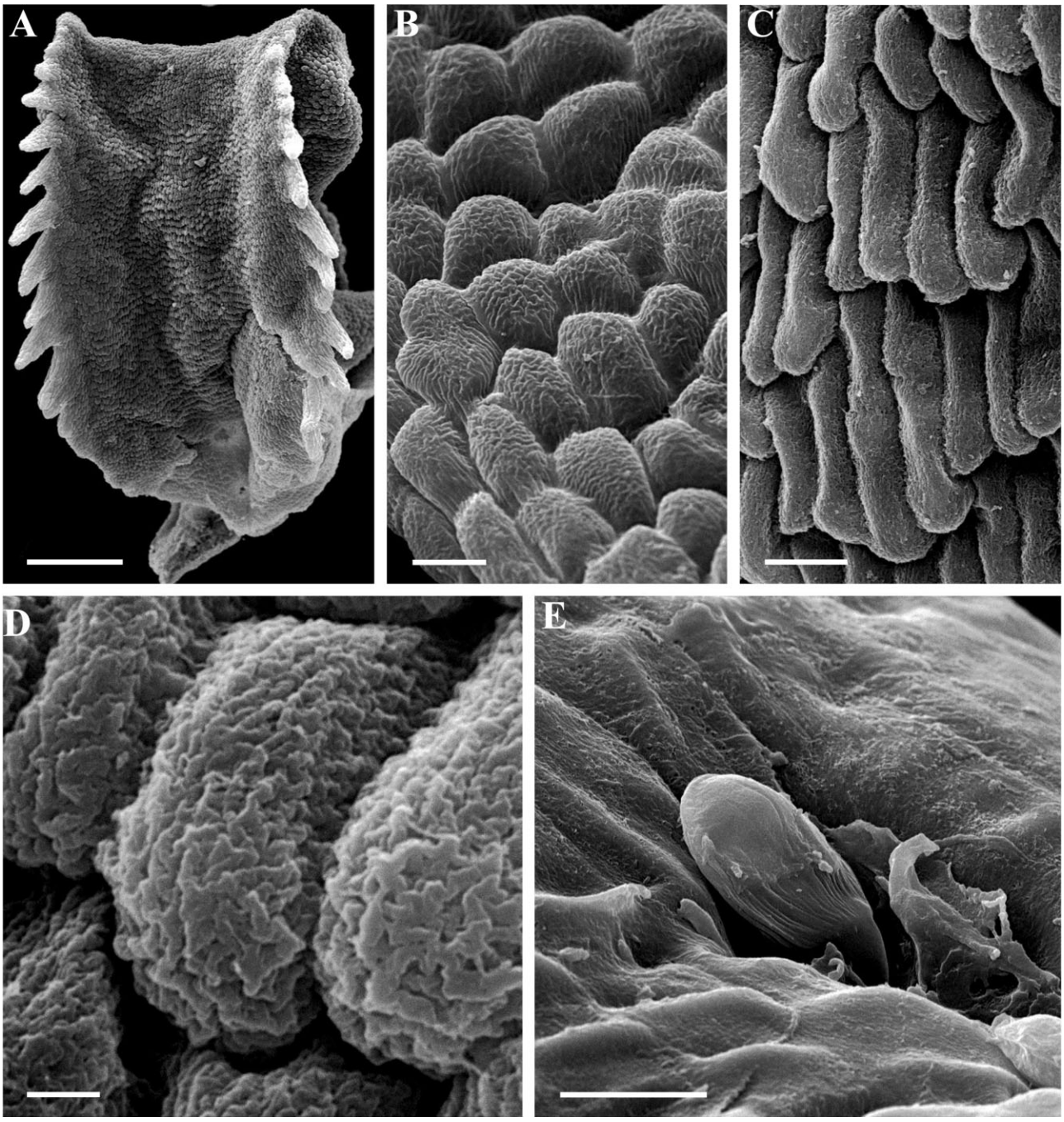


**Figure 11.** *Bulbophyllum maximum*, SEM. (A) Adaxial surface of labellum, the central region containing verrucae. (B) Globose, epidermal cells in the median part of labellum. (C) Elongate epidermal cells along margin of labellum. (D) Epidermal cells with striate cuticle. (E) Secretory trichome on adaxial surface of lateral sepal, the trichome surrounded by secretory residues. Scale bars = 200, 10, 10, 2 and 20 μm, respectively.

Lindl. (section *Stenochilus* J.J.Sm.) and this is often referred to in the literature as 'nectar'. Recently, the presence of lipids and sugar in the floral secretion of *B. lobbii*, a fragrant species considered to have retained several features characteristic of bee-pollinated species (Christensen, 1994), was confirmed by the present authors (K. L. Davies, unpubl. results, 2013). In *B. schinzianum*, the depth of the labellar groove, with its narrow aperture, suggests that it may be accessed only by insects with a moderately long, narrow proboscis, and this, with the sweet fragrance and sugary secretion, contributes to pollinator



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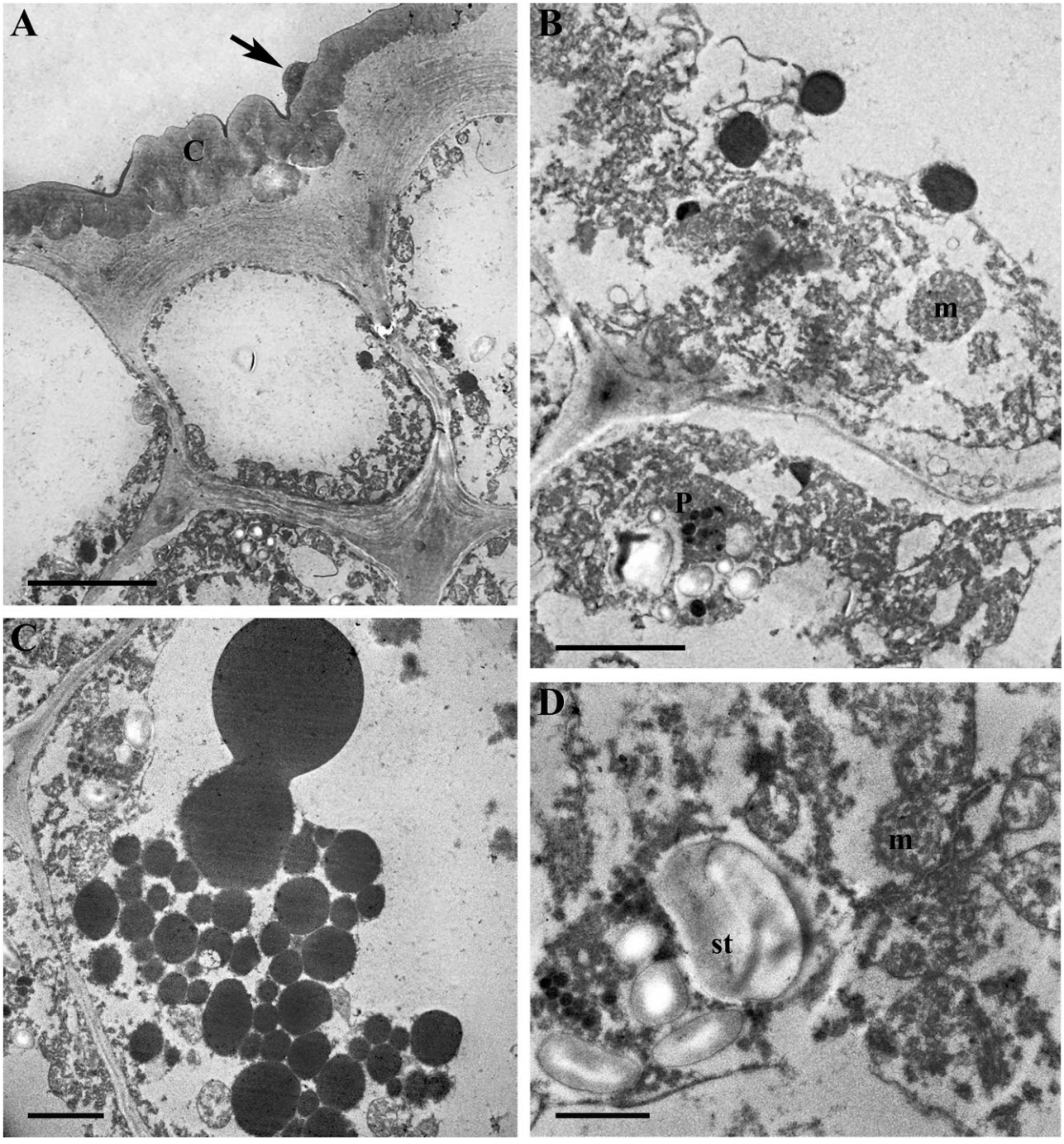


**Figure 12.** *Bulbophyllum maximum*, TEM. (A) Epidermal cells containing electron-dense globules. Similar material is present on the surface of the thick cuticle (arrow). (B) Electron-dense globules, together with plastids containing starch and plastoglobuli, also occur in subepidermal parenchyma cells. (C) Numerous intravacuolar, electron-dense globules are present in subepidermal parenchyma cells. (D) Detail of cytoplasm of subepidermal parenchyma showing mitochondria, RER profiles and plastid containing both starch grains and plastoglobuli. Scale bars = 5, 2, 2 and 1 μm, respectively. C, cuticle; m, mitochondrion; P, plastid; st, starch.

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selection and suggests that the pollinators may in fact be foraging Hymenoptera (bees, or perhaps even wasps). This is contrary to previous claims (Jongejan, 1994; van der Cingel, 2001) that this species is pseudocopulatory and attracts pollinators solely by mimicry, as its flowers produce both fragrance and a food-reward. The secretion is so copiously produced that it eventually overflows onto much of the flower and the expanding floral bracts, where it also becomes available to opportunistic insect visitors.

In the remaining taxa, the flowers were considerably smaller and lacked perceptible fragrance and the median longitudinal labellar groove was absent, or at best poorly defined, relatively shallow and wide, and the flat or slightly depressed secretory area produced a scant, homogeneous or heterogeneous secretion. A combination of histochemistry and TEM revealed that the secretory cells contained lipid droplets and/or an osmiophilic surface secretion. This combination of characters would suggest that these taxa are probably myophilous. Indeed, it is known that myophilous Neotropical representatives of *Bulbophyllum*, mainly pollinated by milichiid flies, also possess lipid-secreting cells along the median, longitudinal groove of the labellum (de Pádua Teixeira *et al*., 2004; Nunes *et al*., 2014 and references therein). However, unlike our specimens, these Neotropical species are said to produce an acid- or carrion-like fragrance, although this usually disappeared within hours, and certainly by the end of the first day of anthesis, making it difficult to detect (Borba & Semir, 1998; Silva *et al*., 1999).

A third type of labellar organization occurs in the often malodorous, fly-pollinated members of sect. Racemosae, and here, the median longitudinal groove is narrow, well-defined and furrow-like (Davies & Stpiczyńska, 2014).

The Latin term *sulcus* (furrow) has generally been applied to the labellar groove. However, for the purpose of distinction between the various types of organization, it is proposed that the Latin term *euripus* (channel, conduit or strait, usually with strong currents) be adopted for the type of well-defined, relatively deep, median labellar groove found in *B. schinzianum*, and that the term *stagnum* (pool, standing water) be used when there is no obvious median labellar groove and the secretion merely accumulates directly on the labellar surface or in a shallow and relatively wide depression (that may, nonetheless, extend for much of the length of the labellum).

A striate cuticle was present in all species, but only in *B. schinzianum* were cuticular blisters observed. This could perhaps be related to differences in the pollination strategies of the investigated taxa, *B. schinzianum* having larger flowers capable of supporting larger pollinators that, in turn, have greater capacity to rupture the cuticular blisters when foraging for food. Despite this, no tearing of the cuticle, cracks or pores were observed in any of the species. Nevertheless, cuticular micro-channels, similar to those observed for Asian *B. wangkaense* Seidenf. (Davies & Stpiczyńska, 2014) and *B. wendlandianum* (Kowalkowska *et al*., 2014) were present in every species, indicating that they all probably produce fragrances, even though these may not always be perceptible to humans.

In all cases, the epidermal cells contained a complement of organelles characteristic of secretory cells. These included, prominent nuclei, vacuoles and mitochondria. Plastids containing starch grains and plastoglobuli were also present, but those of *B. schinzianum* generally lacked starch (possibly due to hydrolysis during the secretory process), and here, unlike the plastids of subepidermal cells, tubules replaced typical plastid lamellae, indicating a degree of specialization. Similarly, plastids of *B. falcatum* lacked plastoglobuli. RER predominated in *B. falcatum* and *B. maximum*, whereas in *B. schinzianum* and *B. lupulinum*, it was largely replaced by SER. In some species, there was evidence of vesicle-mediated transport of secretory material. Unlike members of the Asian section *Racemosae*, dictyosomes were generally not observed, and mucilage and protein were not detected in the secretion or in the secretory cells (Davies & Stpiczyńska, 2014). However, occasional dictyosomes were seen in the palisade-like secretory cells of *B. schinzianum* and mucilage was present in this species, particularly in the outer tangential walls of secretory cells. Similarly, mucilage was detected in the walls of the epidermal cells of *B. falcatum*. Instead of protein, the secretory cells of all the investigated taxa (and the flanking trichomes in *B. schinzianum*) contained lipid droplets, as reported for certain Neotropical *Bulbophyllum* spp. (de Pádua Teixeira *et al*., 2004; Nunes *et al*., 2014).

The subepidermal parenchyma cells generally contained starch and it is thought that this polysaccharide is hydrolysed and the products mobilized to form components of the secretion (e.g. *B. schinzianum*) and used in energy production for the secretory process. This, in conjunction with numerous mitochondria and evidence of vesicle-mediated transport, indicates that secretion here is of the granulocrine type.

The labellum of each taxon consisted predominantly of parenchyma, but the type of parenchyma present varied from species to species, indicating further evidence of specialization. Thus, whereas the parenchyma of *B. lupulinum* was of the typical compact, storage type (ground parenchyma), that of *B. schinzianum* more closely resembled the spongy mesophyll of leaves, whereas that of members of

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section *Megaclinium* (*B. falcatum* and *B. maximum*) was represented by aerenchyma. It was remarkable to find this tissue here, as it is generally considered an adaptation for gaseous longitudinal transport and buoyancy in partly or entirely submerged hydrophytes, where it is usually associated with lacunae supported by lignified sclereids, such as astrosclereids. Aerenchyma is characterized by irregularly shaped cells with cellulosic walls, these cells often forming chains that transverse an extensive intercellular space system. Idioblasts with non-lignified helical or annular thickenings were often present (in sepals and petals), with more typical idioblasts containing raphides (in all parts of the perianth). It is speculated that the possession of a labellum consisting largely of intercellular space would be necessary for so small an insect to activate such a sensitive pollination mechanism and that the thickened idioblasts would offer a degree of mechanical support to the remaining parts of the perianth. Furthermore, de Pádua Teixeira *et al*. (2004), in reference to *B. weddellii* (Lindl.) Rchb.f., stated that the parenchyma of the lip and its limb was spongy, and that the latter 'appears to be involved in pollination, since any movement of the narrow lip by the wind will force the fly towards the column (Borba & Semir, 1998)'. Thus, from an anatomical perspective (yet to be observed in the field), it is possible that the presence of labellar aerenchyma may be an adaptation for wind-assisted pollination, as reported for certain species of Neotropical *Bulbophyllum* by Sazima (1978) and Borba & Semir (1998). Thickened idioblasts are known to occur in the leaf mesophyll and in the cortex of roots and pseudobulbs of many epiphytic members of Epidendroideae, and may have arisen independently in several lineages (Stern, 2014). They also function as water-storage cells and prevent cell collapse during periods of drought (Olatunji & Nengim, 1980) and, given the small size of these flowers, and the extremes of the African climate, this is an important consideration. Thickened idioblasts were formerly referred to as 'tracheoidal idioblasts' or 'tracheoidal elements' (Foster, 1956; Olatunji & Nengim, 1980). However, as Stern (2014) correctly asserted, this terminology is ill-advised because, unlike xylem tracheids, the thickenings may not be lignified (as here) and articulations (absent in tracheids) may be present. Furthermore, they have no physical, ontogenetic or functional similarity with tracheary elements. Although their occurrence in vegetative organs has been well documented (Stern, 2014 and references therein), reports of their presence in floral tissues are scarce. Nevertheless, Nunes *et al*. (2014) have reported idioblasts with raphides and cellulosic helical thickenings in the parenchyma of the dorsal sepal, petal, labellum and ovary of representatives of *Bulbophyllum* sect. *Didactyle*. By contrast, although thickened idioblasts were absent from the labella of sect. *Racemosae*, idioblasts containing raphides were common (Davies & Stpiczyńska, 2014).

Given the enormity of *Bulbophyllum* (as many as 2000 species on three continents), we recognize that a much greater concerted effort to characterize the anatomical characteristics of floral secretory tissue is required before arriving at any generalizations. A small number of researchers (e.g. de Pádua Teixeira *et al*., 2004; Davies & Stpiczyńska, 2014; Kowalkowska *et al*., 2014; Nunes *et al*., 2014) have already commenced on this colossal task. However, as far as we are aware, to date, the present study is the first to investigate the anatomy of floral secretory tissues in African species of the genus. With so many diverse taxa distributed over so large an area and pollinated by such a variety of insects, *Bulbophyllum* will provide a fascinating field of study for the orchid anatomist for many years to come.

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