Nectar-secreting Floral Stomata in *Maxillaria anceps* Ames & C. Schweinf. (Orchidaceae)

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● *Background and Aims* Although it was generally assumed that *Maxillaria* spp. do not produce nectar, in recent years, nectar has been reported for a number of these orchids. Nevertheless, our current understanding of nectary structure and nectar secretion in *Maxillaria* is based solely on *M. coccinea* (Jacq.) L.O. Williams ex Hodge, which, since it shows many features characteristic of ornithophilous flowers, is atypical of this largely entomophilous genus. The aim of the present paper is to describe, for the first time, nectar secretion in a presumed entomophilous species of *Maxillaria*.
● *Methods* The structure of the nectary of *M. anceps* Ames & C. Schweinf., nectar composition and the process of nectar secretion were investigated using light microscopy, scanning electron microscopy, transmission electron microscopy, histochemistry, refractometry and high performance liquid chromatography.
● *Key Results and Conclusions* Nectar appears as droplets that are exuded by modified stomata borne upon the labellar callus and collects upon the labellum and at the base of the column-foot. Although such stomata are known to occur in a number of angiosperm families, this is the first time for them to be observed in orchids. The callus consists largely of parenchyma with raphides and is supplied by eight to ten collateral bundles. This tissue, together with the single-layered epidermis, seemingly contains terpenoids. During the bud stage, the callus cells contain an organelle complement consistent with secretory cells whereas by day 4 of anthesis, much of the cell is occupied by a vacuole. The nectar is sucrose-dominant but also contains low concentrations of glucose, fructose, free amino acids and possibly terpenoids. The high sugar concentration (approx. 66 %) is consistent with melittophily and may indicate that, like the majority of *Maxillaria* spp., *M. anceps* is visited by stingless bees (Meliponini).

Key words: Entomophily, HPLC, light microscopy, *Maxillaria*, melittophily, nectar, nectary, refractometry, SEM, stomata, TEM, terpenoids, ultrastructure.

INTRODUCTION

Maxillaria Ruiz & Pav. is a large, morphologically and anatomically diverse genus of Neotropical orchids (Senghas, 1993; Holtzmeier *et al.*, 1998; Davies, 1999). This diversity is reflected both in the pollination strategies employed by its members and in the type of floral reward offered to pollinators. This, perhaps, is hardly surprising since, on the basis of molecular evidence, *Maxillaria*, as it currently stands, is considered to be polyphyletic (Whitten *et al.*, 2000). These rewards include pseudopollen (Janse, 1886; Porsch, 1905; van der Pjil and Dodson, 1969; Davies and Winters, 1998; Davies *et al.*, 2000, 2003*a*; Davies and Turner, 2004; Matusiewicz *et al.*, 2004), wax or a viscid, resinous material secreted by the labellum (Porsch, 1905; van der Pjil and Dodson, 1969; Senghas, 1993; Davies *et al.*, 2003*a, b*; Flach *et al.*, 2004; Matusiewicz *et al.*, 2004; Singer and Koehler, 2004) and floral nectar (Davies *et al.*, 2003*a, b*; Singer and Koehler, 2003, 2004; Davies and Turner, 2004; Stpiczyńska *et al.*, 2004). However, a significant number of *Maxillaria* species seemingly offer no rewards whatsoever (Davies and Turner, 2004; Singer and Koehler, 2004).

In *Maxillaria*, pseudopollen is formed when multicellular, moniliform, labellar trichomes fragment forming short chains or individual cells containing rich reserves of protein and starch (Davies *et al.*, 2000, 2003*a*; Davies and Turner, 2004) and these are gathered by stingless bees (Meliponini) that pollinate the flowers (R. B. Singer, pers. comm., 2002; Singer and Koehler, 2004). However, to date, there is no unequivocal evidence that pseudopollen is ingested by these insects. Likewise, Meliponini are thought to gather labellar wax, possibly for nest-building (van der Pjil and Dodson, 1969; Flach *et al.*, 2004). The resinous, viscid material secreted by the labella of certain *Maxillaria* spp. contains lipoidal substances and aromatic amino acids and may thus be gathered for its nutritive value (Davies *et al.*, 2003*b*). Flach *et al.* (2004), using gas chromatography–mass spectrometry and nuclear magnetic resonance analysis have examined these secretions further and found that triterpenoids form the major component.

Until recently, it was generally accepted that *Maxillaria* spp. do not produce nectar. However, nectar secretion is now known to occur in a number of species including *M. imbricata* Barb. Rodr., *M. rigida* Barb. Rodr., *M. coccinea* (Jacq.) L.O. Williams ex Hodge, *M. pendens* Pabst, *M. parviflora* (Poepp & Endl.) Garay and *M. sophronitis* (Rchb.f.) Garay (Roubik, 2000; Davies *et al.*, 2003*a, b*; Singer and Koehler, 2003; Stpiczyńska *et al.*, 2004).

Based upon data obtained from those *Maxillaria* spp. generally available in cultivation in the UK (Davies and Winters,

1998; Davies *et al.*, 2000, 2003*a*, *b*; Davies and Turner, 2004) and supplemented by field data obtained by Singer and Koehler (2004), it is estimated that some 56 % of species do not reward pollinators, whereas 13 % produce wax or viscid material, 16 % produce pseudopollen and 8 % produce nectar. A further 7 % produce trichomes that do not closely resemble typical pseudopollen-producing hairs but may, nonetheless, function as pseudopollen ($n = 100$ species).

To date, the only species of *Maxillaria* to be studied in detail in terms of nectary structure and nectar secretion is *M. coccinea* (Stpiczyńska *et al.*, 2004). The flowers of this species exhibit a number of features consistent with ornithophily and are presumed to be pollinated by hummingbirds. As such, this species is atypical for the genus. For example, the weakly zygomorphic flower is scarlet, shows diurnal anthesis and has a backwardly curved labellum. The floral tissues are tough and can withstand contact with a hard beak, and a strong fold in the labellum partly closes the floral tube at the level of the anther and stigma, thus forcing the visiting hummingbird to push its beak against the column so as to gain entry. A 'faucet and sink' arrangement occurs in this species and the nectary is represented by a small protuberance on the ventral surface of the column. Nectar collects in a semi-saccate reservoir formed by the fusion of the labellum and base of the column-foot. The nectary comprises a single-layered epidermis and three to four layers of small, subepidermal cells below which occur large parenchyma cells. The epidermal cells lack ectodesmata and it is thought that nectar passes along the apoplast and symplast and accumulates beneath the permeable, reticulate cuticle, which becomes distended prior to the discharge of nectar. The secretory cells are collenchymatous and have an organelle complement typical of cells involved in secretion, comprising nuclei, mitochondria, rough endoplasmic reticulum (ER) and plastids containing many plastoglobuli but few lamellae. Their thickened, cellulose walls contain numerous pits and plasmodesmata. Moreover, nectary cells often contain large, intravacuolar, protein bodies.

In previous papers (Davies *et al.*, 2003*a*, *b*; Davies and Turner, 2004), it has been stated that *M. jenischiana* (Rchb.f.) C. Schweinf. also produces nectar and that this collects upon the labellum and at the base of the column-foot. However, it seems that this species is seldom seen in cultivation in the UK and, since no monograph has been published for the genus and some of the features present in the specimen examined were inconsistent with those described for *M. jenischiana*, it was not possible to identify the specimen with certainty. Consequently, further advice was sought and as a result, the specimen of *M. jenischiana*, and the subject of this present paper, has been redetermined as *M. anceps* Ames & C. Schweinf. (G. Carnevali, pers. comm., 2004). This plant shows diurnal anthesis and has strongly zygomorphic, relatively open, greenish-white flowers that last approx. 1 month in cultivation. The labellum is modified to form a landing platform and bears copious nectar upon its surface. However, no nectar guides were visible to the human eye but whether fragrance is present is less certain, although one of the authors could detect a sweet but unpleasant scent. Consequently, it is speculated that *M. anceps* is melittophilous and is perhaps more typical of nectariferous *Maxillaria* spp. than the presumed ornithophilous *M. coccinea*. Thus, the aim of this paper is to describe, for the first time, the secretion and chemical composition of nectar in a presumed entomophilous species of *Maxillaria* and to compare this with data previously obtained for *M. coccinea* (Stpiczyńska *et al.*, 2004).

MATERIALS AND METHODS

Fresh flowers of *M. anceps* were examined by means of a hand lens, an Olympus SZX12 stereo-microscope and a TESLA BS-300 series scanning electron microscope (SEM) in order to locate the position of the nectary and the site of nectar secretion. Flowers were also examined microscopically when in bud (1 d before opening), at the commencement of anthesis (1–2 d after opening) and 3–4 d after opening. Hand-cut sections through nectar-secreting tissue were tested for starch and lipids using IKI and a saturated alcoholic solution of Sudan III, respectively. The tissue was fixed, cut and stained for both light microscopy and transmission electron microscopy (TEM) as previously described (Stpiczyńska *et al.*, 2004). A Nikon Eclipse 600 microscope with screen measurement version 4·21 software was used for micrometry and photomicrography.

Preliminary tests for reducing sugars in the nectar of *M. anceps* using Clinistix (Beyer PLC) showed that the nectar of flowers prior to, or at the point of anthesis, whilst still tasting sweet, contained no glucose whereas that of older flowers gave a positive reaction for this substance. This test works on the principle that glucose (but not fructose) is oxidized in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide. The latter, in the presence of peroxidase, oxidizes the chromogen system through purple to blue. On the basis of these results, it was suspected that initially the nectar of *M. anceps* mainly contains sucrose but that this, as anthesis progresses, is converted to glucose.

Flowers were observed for the first 4 d of anthesis and nectar samples were collected by means of calibrated, glass microcapillary tubes. The nectar was weighed using an analytical balance and its sugar concentration measured using a PZ0 RL-3 refractometer and expressed as a percentage (w/w solution). The concentration of amino acids was determined using the method described by Dafni (1992) and a saturated ethanolic solution of Sudan III was used to test for lipids. Terpenoids were detected by examining spots of fresh nectar using a Wood's Famed–1 L6/58 UV lamp, and these, together with other secretory products, display autofluorescence following exposure to UV or violet light (Roshchina, 2003). Finally, the sugar composition of nectar was determined by subjecting two pooled samples of nectar, weighing 13 mg and 27 mg, respectively, to high performance liquid chromatography (HPLC) (Rossomando, 1998).

RESULTS

Nectary structure

Maxillaria anceps has strongly zygomorphic, relatively open, greenish-white flowers with a well-developed

labellum (Fig. 1). The nectary is represented by a simple, yellowish, labellar callus (Fig. 2). Nectar is secreted onto the labellar surface (Fig. 2) and may collect at the base of the column-foot. The callus consists largely of parenchyma (Fig. 3A and B). The secretory epidermal cells and sub-secretory parenchyma cells measure 20·73 μm (range 15·32–27·07 μm) and 24·88 μm (range 13·92–33·34 μm) in diameter, respectively whereas those of the ground parenchyma are larger and have a mean diameter of 49·12 μm (range 34·68–61·43 μm). Some of the ground parenchyma cells contain raphides, whereas others contain flocculent, intravacuolar precipitates (Fig. 3C–E). Eight to ten collateral vascular bundles supply the callus (Fig. 3B and E). Staining with toluidine blue indicated that the walls of secretory cells consist of cellulose. Vacuoles of epidermal and subepidermal cells, as seen in semi-thin sections, stain an intense blue-green with this reagent and thus, probably contain terpenoids. These cells, in unstained sections, also contain yellow-grey, spherical bodies, some 7·42 μm (range 5·02–16·23 μm) in diameter and these, too, stain blue-green with toluidine blue (Fig. 3C).

The adaxial, secretory epidermis bears relatively few stomata and these are absent from the abaxial surface. The circular, stomatal aperture is almost entirely covered by a thick cuticle (Fig. 4A) and transverse sections through stomata show that the thickened, outer, tangential walls of guard cells, adjacent to the stomatal aperture, have well-developed, cuticular projections (Fig. 4B) that form a peri-apertural ridge (Fig. 4A). Moreover, stomata of fresh material stain selectively with very dilute aqueous solutions of neutral red (0·03 %) and toluidine blue (0·005 % in 0·005 % sodium tetraborate solution). The callus epidermis is glabrous (Fig. 4C–E), whereas conical papillae are present (Fig. 4F) on other parts of the labellum, as previously described for this (as *M. jenischiana*) and a great number of other *Maxillaria* species (Davies and Turner, 2004). Peculiar, stalked glandular trichomes occur occasionally upon the adaxial surface of the callus and these appear to exude a colourless secretion (Fig. 5A).

The cellulose cell walls of the secretory epidermis are thin (0·6–1·52 μm; mean 1·07 μm) but the outer, tangential walls (Fig. 5B and C) are much thicker (1·43–4·06 μm; mean 2·58 μm) and are covered by an uninterrupted cuticle (Fig. 4C) that becomes distended while the flower is in bud (Fig. 4C and E) but appears to collapse as anthesis progresses (Fig. 4D). Numerous plasmodesmata maintain cytoplasmic continuity between contiguous cells. During the bud stage, secretory cells contain dense cytoplasm with abundant mitochondria, dilated ER profiles, dictyosomes (Golgi bodies) and coated vesicles (Fig. 5D). Generally, smooth ER predominates although rough ER is also common. Abaxial, subepidermal cells contain amyloplasts with abundant starch (Fig. 6A and B) whereas their adaxial
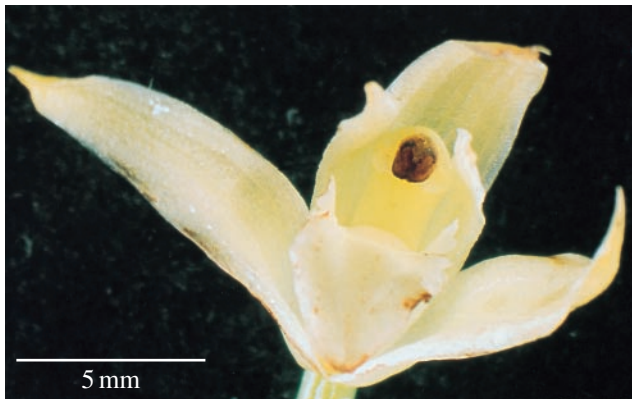

FIG. 1. Zygomorphic flower of *Maxillaria anceps* showing well-developed labellum. Scale bar = 5 mm.
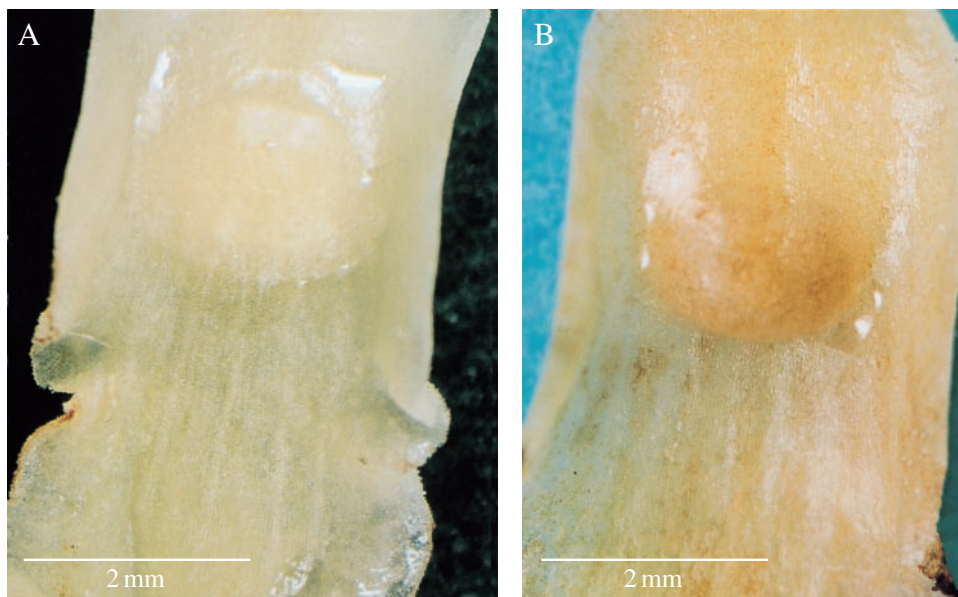

FIG. 2. (A) Labellar surface during early anthesis showing simple callus and associated drops of nectar. Scale bar = 2 mm. (B). Labellar surface at day 4 with nectar accumulating at distal end of callus. Scale bar = 2 mm.
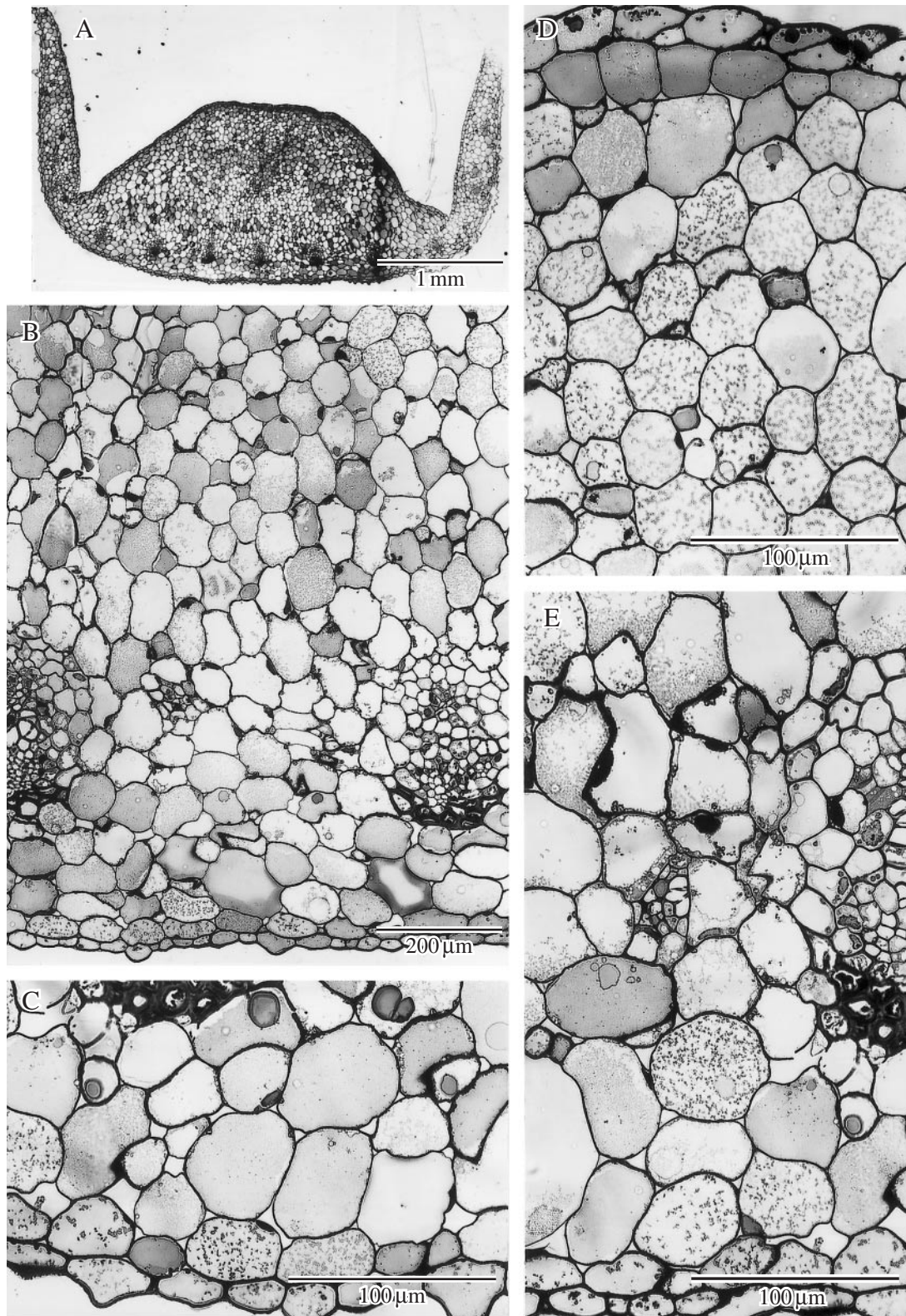
FIG. 3. (A) Transverse section of labellum showing vertical, lateral lobes and central, adaxial callus with abaxial vascular bundles. Scale bar = 1 mm. (B) Detail of parenchymatous labellum with abaxial, collateral, vascular bundles. Scale bar = 200 μm. (C) Detail of abaxial, labellar parenchyma showing flocculent, intravacuolar precipitates and spherical bodies that are thought to contain terpenoids. Scale bar = 100 μm. (D) Similar section through adaxial surface of callus showing flocculent, intravacuolar precipitates and raphides. Scale bar = 100 μm. (E) Detail of abaxial, collateral, vascular bundle and adjacent tissues. Scale bar = 100 μm.
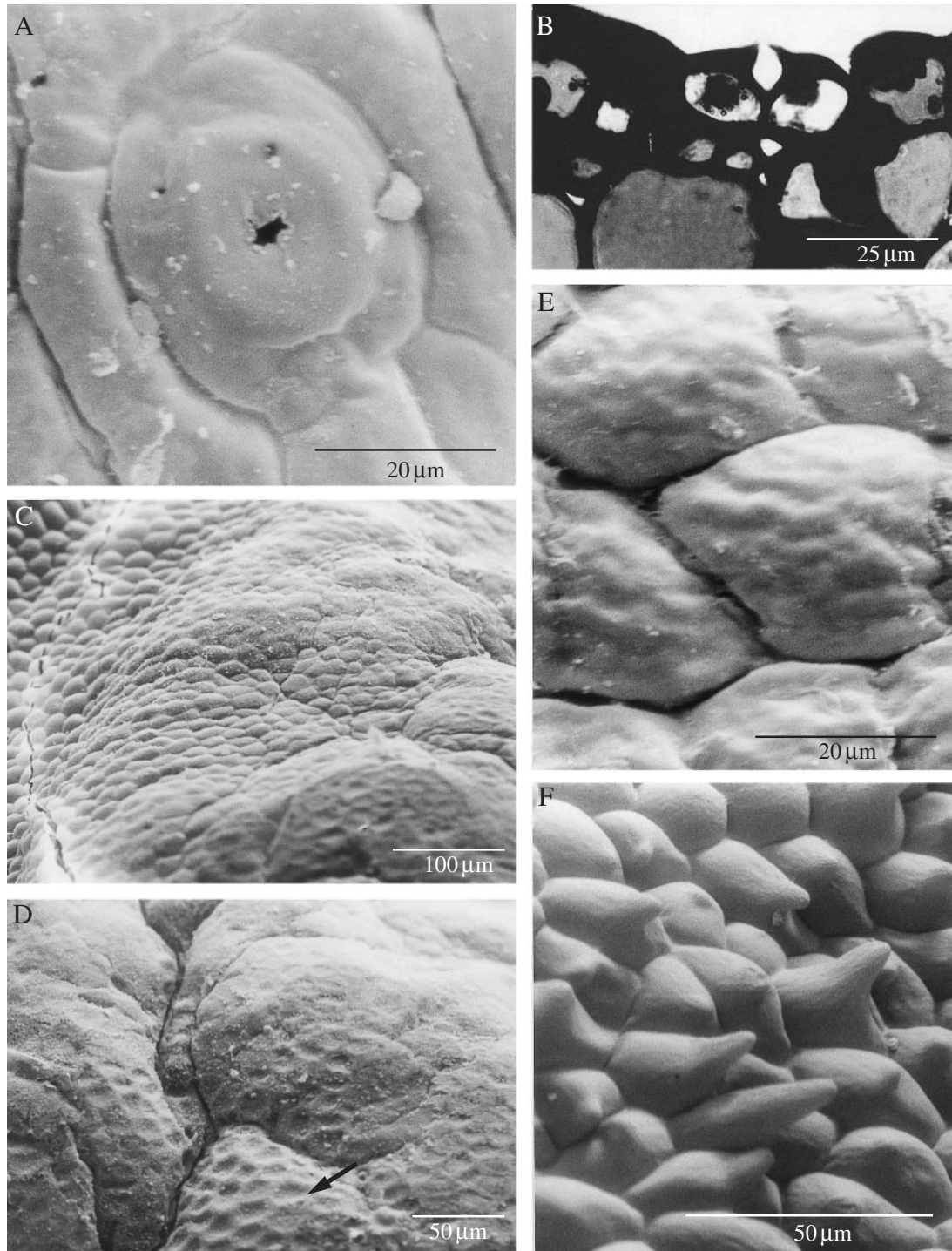
FIG. 4. (A) Detail of adaxial surface of labellar callus showing stoma. Note the peri-apertural ridge and the continuous, cuticular sheet that almost completely covers the stomatal aperture. Scale bar = 20 μm. (B) Transverse section through callus showing stoma. Note that the guard cells are nucleated, have dense cytoplasmic contents and thick, outer, tangential walls with cuticular projections adjacent to the stomatal aperture. Scale bar = 25 μm. (C) Labellar surface showing simple callus with glabrous surface. Scale bar = 100 μm. (D) Detail of callus surface of older flower. Here, compared with (C), the uninterrupted cuticle appears to have collapsed (arrow). Scale bar = 50 μm. (E) Detail of epidermal surface of callus showing a seemingly distended, cuticular layer. Scale bar = 20 μm. (F) Labellar surface remote from the callus showing typical, conical papillae. Scale bar = 50 μm.

counterparts, as well as the adaxial epidermal cells them-selves, contain plastids comprising a homogeneous stroma with few lamellae and plastoglobuli (Figs 5B and 6C). By day 4 of anthesis, the cytoplasm is less dense and has a parietal distribution, much of the cell being occupied by a single vacuole (Fig. 6C). During the early stages of anthesis, secretory cells contain small, intravacuolar, osmiophilic globular bodies (Fig. 5B) whereas, by day 4, these are larger
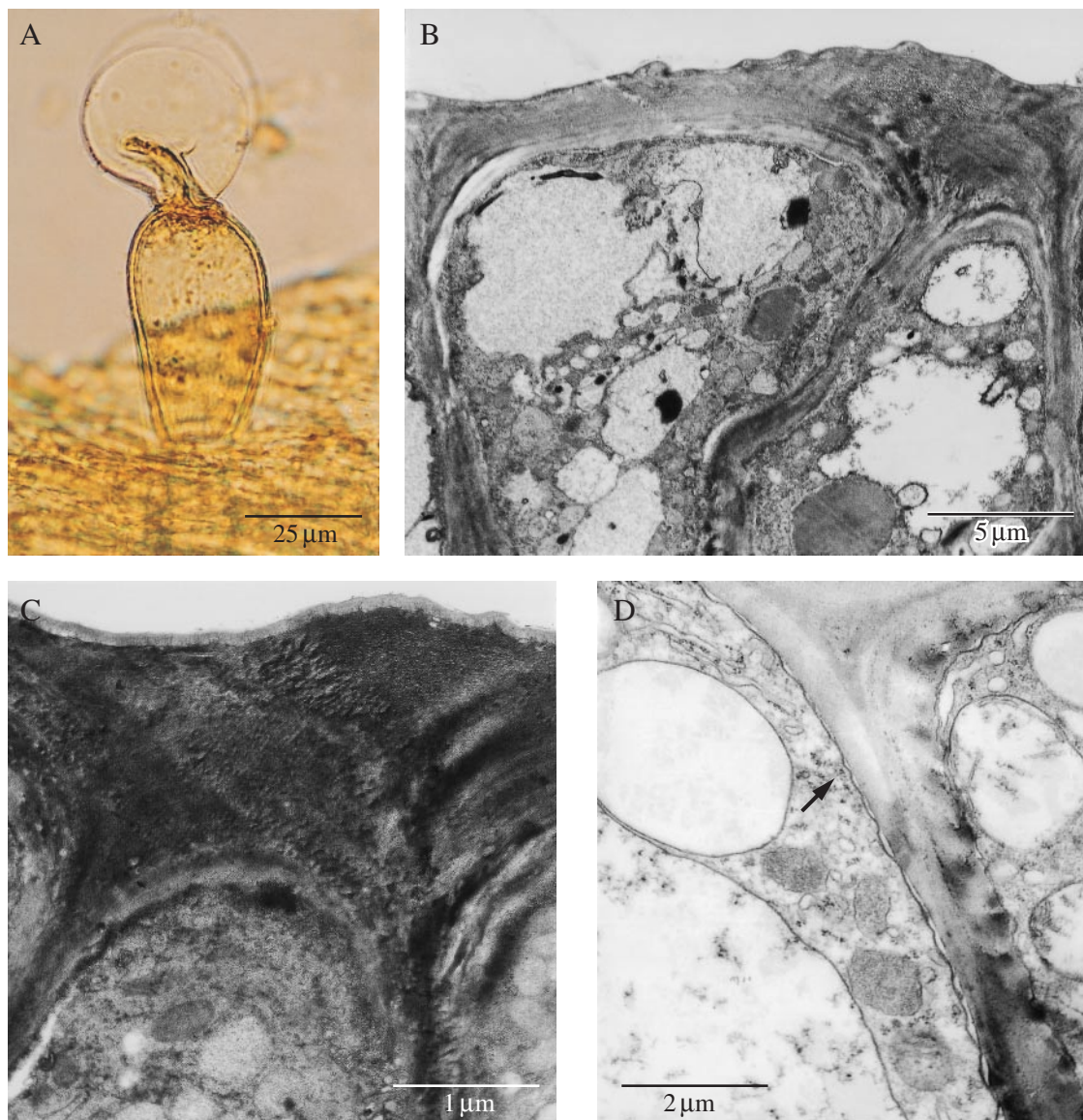
FIG. 5. (A) Stalked, glandular trichome on surface of labellar callus with exudate that possibly contains terpenes. Scale bar = 25 µm. (B, C) TEM of cells of secretory epidermis showing thick, outer, tangential wall and uninterrupted cuticle. Scale bars = 5 µm and 1 µm, respectively. (D) Secretory callus tissue during bud stage showing mitochondria, rough ER, components of the vacuome, tonoplast and coated vesicles (arrow). Scale bar = 2 µm.

(Fig. 6C) and the vacuolar precipitates form annular profiles (Fig. 6D).

Nectar secretion and composition

Traces of nectar appear while flowers are still in bud (1 d before anthesis). On the first day of anthesis, these appear as small pin-pricks of nectar but by day 2, the rate of nectar secretion has increased to such an extent that not only is nectar visible as droplets upon the callus, but is also seen to collect as a relatively large drop beneath the distal end of the callus, where the latter joins the labellar surface. These drops of nectar continue to increase in size and by day 4 of anthesis, the volume of nectar has reached its maximum (approx. 28 µL) and has gathered at the base of the column-foot.

Pooled samples of nectar ($n = 5$ flowers) gave a refractometer reading of 66·5 % (w/w solution) and HPLC analysis showed that the nectar contained 62·36 % sucrose, 0·61 % glucose and 0·27 % fructose, as well as 0·76–1·53 mg cm^{-3} of free amino acids. The nectar fluoresced blue-green under UV light indicating that it probably contains terpenoids but no lipids were detected.

DISCUSSION

Nectary structure

Nectar is the principal reward offered by orchid flowers (Dressler, 1990) and its presence confers considerable evolutionary advantage (Johnson and Bond, 1997; Neiland
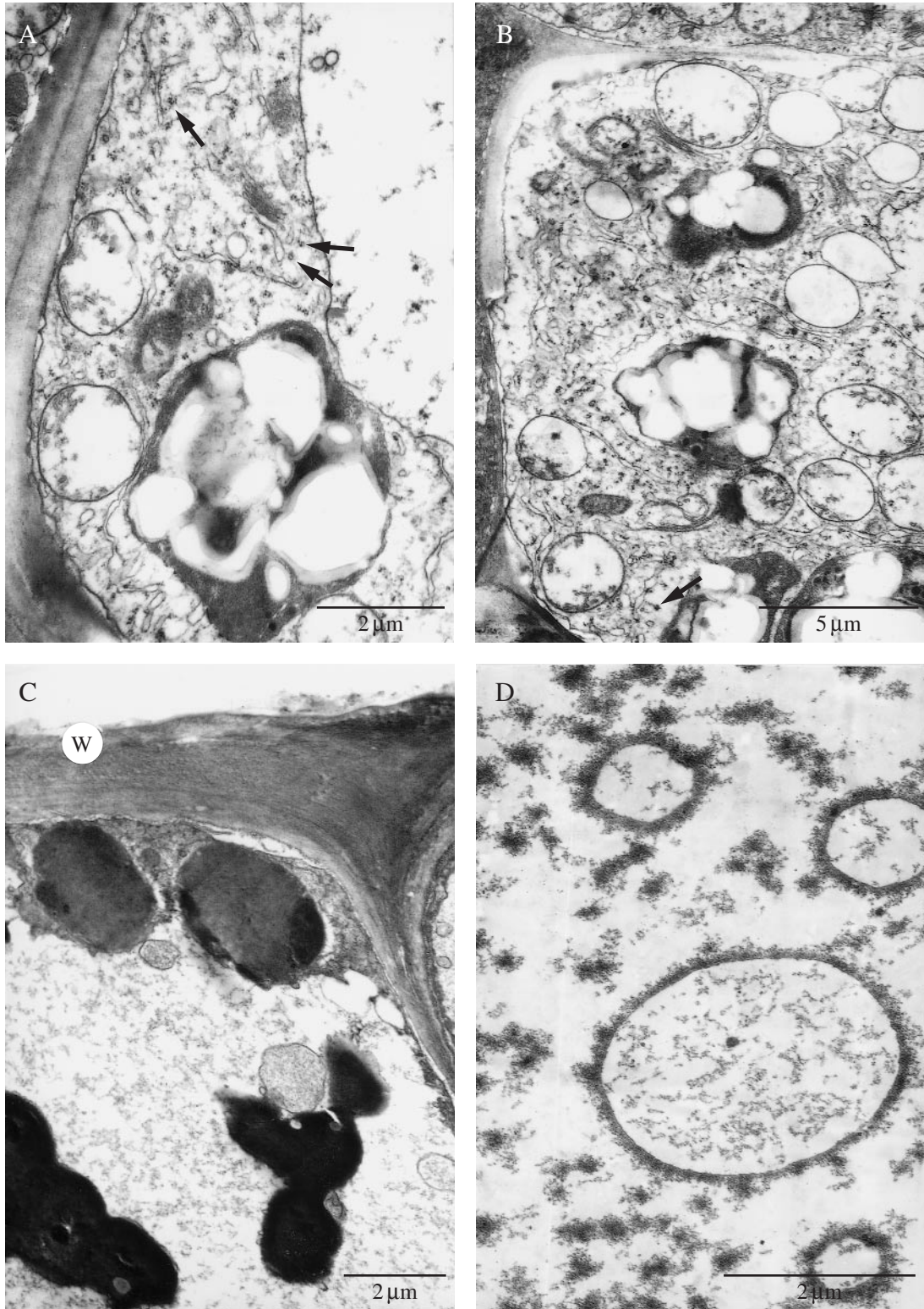
FIG. 6. (A, B) Abaxial, subepidermal, labellar cells with mitochondria, rough ER, amyloplasts with starch grains, dictyosomes, components of the vacuole and coated vesicles (arrows). Scale bars = 2 µm and 5 µm, respectively. (C) Secretory, epidermal cell 4 d into anthesis showing outer tangential wall (w), parietal cytoplasm and much of the cell volume occupied by a vacuole. Note the plastids and intravacuolar, osmiophilic bodies and compare them with those found in cells at the early stages of flowering (Fig. 5B). In both cases, the plastids have a homogeneous matrix but few plastoglobuli and lamellae, whereas 4 d into anthesis, the osmiophilic bodies are larger and occur more frequently. Scale bar = 2 µm. (D) Secretory cells 4 d into anthesis showing intravacuolar, annular profiles. Scale bar = 2 µm.

and Wilcock, 1998, 2000; Johnson and Nilsson, 1999). Indeed, notwithstanding the energy expenditure associated with nectar formation, it would appear that the production of nectar is the most effective way of increasing the incidence of successful pollination, even when there is a paucity of potential pollinators (Neiland and Wilcock, 1998).

The nectaries of orchids are very diverse. The most obvious type of nectary is the nectar spur, which arises from one of the perianth segments. Such structures occur in *Calanthe* R. Br., *Comparettia* Poepp. & Endl., *Disperis* Sw., *Satyrium* Sw. and *Tipularia* Nutt. (Dressler, 1990), but in many moth-pollinated orchids (e.g. *Aerangis* Rchb.f., *Angraecum* Bory, *Gymnadenia* R. Br. and *Mystacidium* Lindl.), the spur nectary arises as an outgrowth from the proximal part of the labellum, whereas in Spiranthinae it may be formed from the lateral sepals, the labellum and the column-foot. In *Pelexia* Poit. ex Rich., the spur is partially free, in contrast to *Sarcoglottis* C. Presl., where it has fused to a greater extent with the ovary (Singer and Sazima, 1999). Likewise, in *Hexisea imbricata* (Lindl.) Rchb.f., the nectary is represented by a saccate spur formed by the fusion of the column and proximal part of the labellum (Stpiczyńska *et al.*, 2005). A similar arrangement is said to occur in *Systeloglossum* Schltr. (Dressler, 1990). Indeed, in orchids that have a column-foot (e.g. *Dendrobium* Sw. and *Scaphyglottis* Poepp. & Endl.), the nectar spur may arise either solely from that structure or from both the column-foot and the base of the labellum. In many Laeliinae such as *Brassavola* R. Br., *Epidendrum* L. and *Rhyncholaelia* Schltr., the spur is less obvious (cuniculus), lies parallel to and is fused with the ovary, and is only evident when the flower is cut longitudinally (Dressler, 1990).

Many orchids possess shallow, superficial nectaries upon their labella [e.g. *Listera* R. Br., *Stelis* Sw., *Pleurothallis* R. Br., *Cirrhopetalum* Lindl. (Dressler, 1990), *Bulbophyllum ipanemense* Hoehne, *B. involutum* Borba, Semir & F. Barros, *B. weddellii* (Lindl.) Rchb.f. (Teixeira *et al.*, 2004), *Epipactis atropurpurea* Raf. (Pais, 1987) and the entomophilous *Maxillaria parviflora* (Poepp. & Endl.) Garay (Singer and Koehler, 2004)]. In *Beadlea dutraei* (Schltr.) Garay (syn. *Cyclopogon dutraei* Schltr.), *Pelexia bonariensis* (Lindl.) Schltr. and *Stenorrhynchos orchioides* (Sw.) Rich. (syn. *Sacoila lanceolata* (Aubl.) Garay), the nectaries occur as two symmetric glands upon the adaxial labellar surface (Galetto *et al.*, 1997). In *Maxillaria coccinea*, the nectary is represented by a small protuberance upon the ventral surface of the column (Stpiczyńska *et al.*, 2004) and nectar has been observed to collect at the base of the column of *M. pendens* Pabst and *M. rigida* Barb. Rodr. (Singer and Koehler, 2004), although no protuberance was reported for these species.

By contrast, in *Maxillaria anceps*, nectar is secreted by the labellar callus. The latter consists of parenchyma delimited by a single-layered epidermis that contains modified stomata. The incidence of minute droplets of nectar upon the callus would appear to correspond with stomatal distribution. Indeed, the stomata have an affinity for very dilute aqueous solutions of neutral red and toluidine blue, thereby indicating that they are probably involved in secretion. A number of reports relating to the exudation of nectar

by modified stomata occur in the literature. This phenomenon is known to occur in a number of families including Rosaceae (Radice and Galati, 2003; Weryszko-Chmielewska *et al.*, 2003), Brassicaceae (Davis *et al.*, 1998), Scrophulariaceae (Gaffal *et al.*, 1998; Nepi *et al.*, 2003), Myrtaceae (O'Brien *et al.*, 1996; Davis 1997) and Fabaceae (Waddle and Lersten, 1973; Teuber *et al.*, 1980; Davis and Gunning, 1991; Razem and Davis, 1999). Members of these families, especially Fabaceae, however, have a greater stomatal density than *M. anceps* but like the latter species, possess stomata whose apertures become almost completely covered by a cuticular layer. Nectar-secreting stomata tend to differ from typical stomata in that, like hydathodes, they are unable to close fully since all the free surfaces of the guard cells are cuticularized. Also, ridges of circumferentially arranged microfibrils occur along the outer (and sometimes the inner) walls, and an unidentified osmiophilic wall material is often present (Davis and Gunning, 1991). However, whereas the stomatal guard cells borne upon the callus of *M. anceps* retain their nuclei and cytoplasm (Fig. 4B), much like those of typical stomata, the guard cells of hydathodes lose their living contents early in their development (Esau, 1965; Harder *et al.*, 1970). The presence of cytoplasmic contents within the guard cells, together with observations that the stomata are not associated with epithema (distinct groups of small, parenchymatous cells lacking chlorophyll), nor occur at the ends of xylem strands, compel us to classify them as stomata rather than hydathodes (Esau, 1965; Harder *et al.*, 1970) and it would appear that nectar probably passes from the phloem to the adaxial stomata along an apoplastic and/or a symplastic route (Fahn, 2000, and references therein). Although it is possible that nectar crosses the outer tangential wall of the epidermal cells and is secreted through the cuticle onto the surface of the callus, this hypothesis would not satisfactorily explain the selective uptake of stain by the stomata nor the more or less regular distribution of nectar droplets upon the callus surface. Sparse stomata also occur upon the nectary of *M. coccinea* but, in contrast to those of *M. anceps*, these are not involved in the secretion of nectar. Instead, in *M. coccinea*, nectar is secreted via a distended, permeable cuticle.

The secretory cells of *M. anceps*, like those of *M. coccinea* (Stpiczyńska *et al.*, 2004), are nucleated and contain abundant mitochondria. However, they differ from those of the presumed ornithophilous species *M. coccinea* and *Hexisea imbricata* (Stpiczyńska *et al.*, 2004, 2005) in that they are parenchymatous not collenchymatous, presumably since they are not required to withstand contact with a hard beak. Like the secretory cells of *M. coccinea*, those of *M. anceps* contain plastids with few plastoglobuli and lamellae, and the cell walls of both species contain plasmodesmata. Although rough ER is common in *M. anceps* (as in *M. coccinea*), it is the smooth type that predominates, as in *Hexisea imbricata* (Stpiczyńska *et al.*, 2005). Intravacuolar protein bodies are lacking in *M. anceps* and, instead, the vacuoles contain annular profiles. Dictyosomes and coated vesicles also occur within the secretory cells of *M. anceps* but not those of *M. coccinea*, and the osmiophilic globular bodies observed in the former species during the very early

stages of anthesis become larger as anthesis progresses. A similar complement of organelles also occurs in unrelated orchids such as *Limodorum abortivum* (L.) Sw. (Pais and Figueiredo, 1994) and *H. imbricata* (Stpiczyńska *et al.*, 2005). Osmiophilic bodies have also been recorded for the nectary cells of *Bulbophyllum* spp. (Teixeira *et al.*, 2004) but their function is not fully understood.

A distinctive feature of the nectary cells of *M. anceps* is the presence of presumed terpenoids. This may explain the yellow-grey, globular bodies seen in fresh material under light microscopy and the osmiophilic globules observed using TEM. This class of substance has also been recorded for the labella of other species of *Maxillaria* such as *M. cerifera* Barb. Rodr. and *M. friedrichsthalii* Rchb.f. (Flach *et al.*, 2004) and it is likely that these compounds are the source of the blue-green fluorescence observed when nectar droplets of *M. anceps* are examined using UV light. Nectar fluorescence under UV light has also been reported for *Allium porrum* L. and *Prunus persica* (L.) Batch together with many other non-orchidaceous species (Thorp *et al.*, 1975; Peumans *et al.*, 1997; Radice and Galati, 2003), and it is possible that this fluorescence, whether produced by the nectar or by the perianth, enables pollinators to locate and recognize rewards and to distinguish between reward-bearing and reward-less flowers (Thorp *et al.*, 1975). Remarkably, Holtzmeier *et al.* (1998) have reported bicellular and multicellular glandular hairs similar to those in Fig. 5A upon the leaves and pseudobulbs of a number of species of *Maxillaria*, and Dell and McComb (1978) have shown that the exudate produced by similar hairs often contains terpenes as well as essential oils. Although it is not possible to be certain of their role, Levin (1973) and Wagner (1991) have suggested that the primary function of such glandular trichomes is the production and storage of compounds that discourage herbivory.

Nectar composition

The nectar-sugar concentration of *M. anceps* relative to other bee-pollinated species (Baker and Baker, 1983, 1990; Cruden *et al.*, 1983; Wyatt, 1983) is high (>66 %). However, it is known that certain *Melipona* spp. (stingless bees or Meliponini) such as *M. fasciata* (syn. *M. panamica*) from Costa Rica (one of the countries in which *M. anceps* occurs) can cope with nectar of comparable (>60 %) concentrations, but usually take nectar of lower sugar concentrations (20–50 %) (Roubik and Buchmann, 1984; Biesmeijer *et al.*, 1999; Roubik, 2000). This may be particularly significant since Meliponini are thought to be the main pollinators of *Maxillaria* spp. (Singer and Cocucci, 1999; Roubik, 2000; Singer and Koehler, 2004). Similarly, the nectar-sugar concentration of melittophilous *Beadlea dutraei* also approaches 60 % (Galetto *et al.*, 1997; Galetto and Bernardello, 2003). It is, of course, possible that the high nectar-sugar values recorded may simply be due to the way that the nectar accumulates and/or evaporation and that the sugar concentration of newly secreted nectar is much lower. Even so, potential pollinators must be capable of utilizing such elevated sugar concentrations, regardless of how they arise.

HPLC analysis revealed that during anthesis, sucrose is the predominant nectar-sugar in *M. anceps* but that glucose and fructose (as well as free amino acids) are also present at much lower concentrations. This confirms the results of preliminary tests using glucose-sensitive test-sticks. Such tests performed on nectar from newly-opened flowers proved negative, indicating that at first, glucose is probably either present at extremely low concentrations or absent, and that it is gradually produced from sucrose by microbial or invertase activity. A number of studies have suggested that a relationship exists between the chemical composition of nectar and the type of pollinator. For example, the nectar of flowers pollinated by Hymenoptera and sunbirds tends to be hexose-dominant whereas sucrose predominates in the nectar of sphingophilous and hummingbird-pollinated species (Heinrich, 1975; Cruden *et al.*, 1983; Wyatt, 1983; Baker and Baker, 1990; van der Cingel, 2001; Nicolson and Fleming, 2003). That such a relationship also occurs amongst the Orchidaceae has been established for *Epipactis atropurpurea* Raf., *Limodorum abortivum* (L.) Sw. (Pais *et al.*, 1986) and *Mystacidium venosum* Harv. ex Rolfe (Luyt and Johnson, 2001). However, there are exceptions. For example, the nectar of the bee-pollinated orchids *Beadlea dutraei* (Schltr.) Garay and *Pelexia bonariensis* (Lindl.) Schltr. is sucrose-dominant (Galetto *et al.*, 1997; Galetto and Bernardello, 2003). Indeed, recent investigations of floral nectar in the tribe Synningieae (Perret *et al.*, 2001) and in species of *Ipomoea* L. (Galetto and Bernardello, 2004) have revealed that the relationship between nectar composition and the type of pollinator is not as simple nor as clear as was once supposed in that the nectar of most species studied to date is sucrose-dominant. In fact, it would appear that the composition of nectar is phylogenetically biased, irrespective of the type of pollinator (Percival, 1961; Jeffrey *et al.*, 1970; Bernardello *et al.*, 1999; van Wyk, 2002).

The nectary of *M. anceps* thus, exhibits a remarkable set of important features. It would appear that in this species, nectar is secreted via modified stomata found upon the labellar callus and, although this feature has been observed for a number of plant species, it is the only example hitherto reported for the genus *Maxillaria* and, as far as is known, for any orchid. Although this is the first detailed account of nectary structure in a presumed melittophilous species of *Maxillaria*, it is predicted, given the apparently polyphyletic nature and diverse morphology of the genus, that nectaries of other nectariferous, bee-pollinated orchids currently assigned to *Maxillaria* will also reflect this diversity. The nectar of *M. anceps* fluoresces under UV light indicating that it probably contains terpenoids. This may help potential pollinators to recognize the nectar as a source of food and help them locate it within the flower. Since the resinous, labellar secretions found in a number of nectarless *Maxillaria* spp. are also known to contain triterpenoids (Flach *et al.*, 2004), this may support the hypothesis that during the course of evolution, resinous labellar secretions may have replaced nectar as the main reward (Davies *et al.*, 2003*a*).

Finally, the nectar, like that of *Beadlea* and *Pelexia* (Galetto *et al.*, 1997; Galetto and Bernardello, 2003), is sucrose-dominant and, notwithstanding the complexity of

nectar composition–pollinator relationships, this strengthens the possibility that *M. anceps* is melittophilous. Furthermore, the high sugar concentration would suggest that, like the majority of entomophilous *Maxillaria* spp., *M. anceps* is perhaps pollinated by stingless bees—a claim that can only be confirmed or refuted by careful field studies.

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