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Floral elaiophores in *Lockhartia* Hook. (Orchidaceae: Oncidiinae): their distribution, diversity and anatomy

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• *Background and Aims* A significant proportion of orchid species assigned to subtribe Oncidiinae produce floral oil as a food reward that attracts specialized bee pollinators. This oil is produced either by glabrous glands (epithelial elaiophores) or by tufts of secretory hairs (trichomal elaiophores). Although the structure of epithelial elaiophores in the Oncidiinae has been well documented, trichomal elaiophores are less common and have not received as much attention. Only trichomal elaiophores occur in the genus *Lockhartia*, and their distribution and structure are surveyed here for the first time.

• *Methods* Flowers of 16 species of *Lockhartia* were studied. The location of floral elaiophores was determined histochemically and their anatomical organization and mode of oil secretion was investigated by means of light microscopy, scanning electron microscopy and transmission electron microscopy.

• *Key Results and Conclusions* – All species of *Lockhartia* investigated have trichomal elaiophores on the adaxial surface of the labellum. Histochemical tests revealed the presence of lipoidal substances within the labellar trichomes. However, the degree of oil production and the distribution of trichomes differed between the three major groups of species found within the genus. All trichomes were unicellular and, in some species, of two distinct sizes, the larger being either capitate or apically branched. The trichomal cuticle was lamellate, and often appeared distended due to the subcuticular accumulation of oil. The labellar trichomes of the three species examined using transmission electron microscopy contained dense, intensely staining cytoplasm with apically located vacuoles. Oil-laden secretory vesicles fused with the plasmalemma and discharged their contents. Oil eventually accumulated between the cell wall and cuticle of the trichome and contained electron-transparent profiles or droplets. This condition is considered unique to *Lockhartia* among those species of elaiophore-bearing Oncidiinae studied to date.

Key words: Anatomy, callus, elaiophore, *Lockhartia*, oil secretion, Oncidiinae, Orchidaceae, trichomes.

INTRODUCTION

The orchid genus *Lockhartia* Hook. comprises 29 species distributed from central-western Mexico to southeastern Brazil (M. A. Blanco, unpubl. res.). It is part of subtribe Oncidiinae, one of the largest and most diverse groups of Neotropical orchids (Chase, 2009). Like most other members of this subtribe, plants of *Lockhartia* are sympodial, caespitose epiphytes. However, they are easily recognized by their vegetative morphology. They have narrow stems bearing many short, laterally flattened, tightly clasping leaves which lack an abscission layer (with the exception of *L. genegeorgei* D.E. Benn. & Christenson, which has pseudobulbs and conduplicate, articulated leaves and is placed by some authors in the genus *Neobennettia* Senghas).

Owing to its unique vegetative architecture, *Lockhartia* was formerly placed by various authors in its own subtribe, Lockhartiinae Schltr. (e.g. Schlechter, 1914; Mansfeld, 1937; Senghas, 1995; Szlachetko, 1995), or even in its own tribe, Lockhartieae Schltr. (Schlechter, 1926). Others recognized it as a member of subtribe Oncidiinae (Dressler and Dodson, 1960; Dressler, 1981; Chase *et al.*, 2003; Chase, 2009), but until recently its precise relationship with other genera of that taxon remained a matter of debate (e.g. Garay, 1963; Dodson and Dressler, 1972; Chase, 1986; Dressler, 1993). Eventually, phylogenetic analyses of DNA sequence data confirmed the status of *Lockhartia* as an isolated lineage within Oncidiinae (Williams *et al.*, 2001; Neubig *et al.*, 2012).

The flowers of *Lockhartia* are 5–30 mm in length and lack fragrance perceptible to humans. Most species can be assigned to one of three groups on the basis of their gross floral morphology. Analyses of molecular data indicate that these groups are not monophyletic (M.A. Blanco, unpubl. res.), but nevertheless provide a useful framework for discussion of morphological

diversity within the genus. Informal names for these groups are used here for convenience (i.e. the Imbricata, Longifolia and Parthenocomos groups and are, in turn, based on the names of representative species).

Flowers of the Imbricata group are usually yellow [white in *L. acuta* (Lindl.) Rchb.f.], often spotted with brown, with a more or less elongate, trilobed labellum. Many of these species have elongate basal lobes to the labellum that curve forward, like embracing arms. The callus is a raised pad, either smooth or with numerous small tubercles or keels, and bears a tuft of minute, glistening, glandular hairs contained in a shallow depression at its base. Flowers of the Imbricata group closely resemble those of many yellow-flowered species of *Oncidium* Sw., but lack a tabula infrastigmatica (a pad-like structure found at the base of the column in most yellow-flowered species of *Oncidium*, that is ostensibly grasped by the mandibles of the bee as it attempts to collect oil from the flower with its legs). Most species of *Lockhartia* belong to this group. Representatives of the Longifolia group have an entire (non-lobed), flat or slightly convex labellum and a crateriform callus comprising a semicircular depression surrounded by a raised, variously toothed rim. All species of the Longifolia group have a small (~1 mm long), trapezoid projection at the base of the callus, which is partially covered with glistening hairs; these also occur on various parts of the callus rim. The flowers are yellow, except for *L. hercodonta* Rchb.f. ex Kraenzl., which has white flowers. Finally, species in the Parthenocomos group have campanulate, white or yellow flowers and a suborbicular, concave labellum with obscurely delimited, rotund, lateral lobes. The central region of the labellum is hirsute and the callus is represented by either a low, irregular, transverse thickening or an oblong patch of short hairs (as in *L. oblongicallosa* Carnevali & G.A. Romero).

Oil secretion by flowers of *Lockhartia* was first reported by Silvera (2002), but the morphology and anatomy of their elaiophores have not hitherto been studied in detail. That flowers of *Lockhartia* have trichomal elaiophores, while most other oil-secreting Oncidiinae have epithelial elaiophores, is noteworthy. Epithelial elaiophores, in contrast to trichomal elaiophores, have been extensively studied for the subtribe (Pacek and Stpiczyńska, 2007; Stpiczyńska *et al.*, 2007; Stpiczyńska and Davies, 2008; Davies and Stpiczyńska, 2008, 2009; Davies, 2009; Pacek *et al.*, 2012). In certain species of *Baptistonia* Barb. Rodr. (included in *Gomesa* R. Br. by Chase *et al.*, 2009 and Neubig *et al.*, 2012), both epithelial and trichomal elaiophores are known to occur (Aliscioni *et al.*, 2009; Chiron, 2010). Trichomal elaiophores in Oncidiinae are known mainly from genera of the *Ornithocephalus* Hook. clade (formerly subtribe Ornithocephalinae Schltr.). Indeed, members of the latter display diverse elaiophore morphology (Toscano de Brito, 2001; Pacek and Stpiczyńska, 2007; Pacek *et al.*, 2012), including epithelial, trichomal and intermediate types. However, trichomal elaiophores are not confined to the *Ornithocephalus* clade. For example, unicellular, capitate, oil-secreting hairs also occur on the callus of *Grandiphyllum pulvinatum* (Lindl.) Docha Neto (R. B. Singer, pers. comm., 2002). Similar hairs also occur on the lateral lobes of the labellum of *Trichocentrum pumilum* (Lindl.) M.W. Chase & N.H. Williams growing in gallery forests in the interior of São Paulo state in Brazil (Pansarin and Pansarin, 2011). These hairs secrete a lipoidal substance, and the non-fragrant flowers of this particular population of *T. pumilum* are said to be exclusively visited and pollinated by two species of bee: *Tetrapedia diversipes* and *Lophopedia nigrispinis*. As these bees collect the secretion, pollinaria are deposited on their mouthparts. Conversely, *Centris* bees were observed gathering oil from this same species further south in Brazil, but unlike the flowers observed by Pansarin and Pansarin (2011), these produced a honey-like fragrance and the elaiophores were of the epithelial type (R. B. Singer pers. comm., 2011). Given the widespread distribution of *T. pumilum*, some variation is to be expected; alternatively, it is possible that two different species are currently referred to by the same name.

Trichomal elaiophores composed of unicellular, secretory hairs, similar to those of *G. pulvinatum*, also occur in *Ornithocephalus ciliatus* Lindl. (Pacek and Stpiczyńska, 2007, as O. *kruegeri* Rchb.f.). These hairs contain a central nucleus, dense cytoplasm, oil droplets and plastids with few starch grains. The cuticle enclosing the trichome is thin and becomes distended as oil accumulates between it and the surface of the cell wall (Pacek and Stpiczyńska, 2007). *Ornithocephalus gladiatus* Hook., *Phymatidium falcifolium* Lindl., *Zygostates grandiflora* (Lindl.) Mansf. and *Z. lunata* Lindl. also display similar elaiophore anatomy (Pacek *et al.*, 2012). However, the elaiophore cuticle of *O. gladiatus* and *P. falcifolium* differs from that of the other species investigated in that it is bi-layered, the outer layer being lamellate, the inner reticulate.

Oil-filled blisters formed by distension of the trichomal cuticle have also been observed for several South American species of *Sisyrinchium* L. (Iridaceae; Cocucci and Vogel, 2001). Chiron (2010) reported the presence of a canal-like structure in the elaiophores of certain species of *Baptistonia*, and hypothesized that this functions as a conduit for the passage of oil to the surface. He did not, however, demonstrate conclusively that oil flows through this canal-like structure.

Reis *et al.* (2000) investigated the chemical composition of the floral oils of *Oncidium pubes* Lindl. [syn. *Gomesa pubes* (Lindl.) M.W. Chase & N.H. Williams]. These are produced by labellar, epithelial elaiophores and are collected by females of an unidentified species of *Tetrapedia* that simultaneously pollinate the flower (Singer, 2003). The oils consist mainly of diacylglycerols and triacylglycerols, with one or two acetyl residues and a long chain fatty acid. Similarly, preliminary results showed that the main components of the floral oils of *Oncidium sotoanum* R. Jiménez & Hágsater are hexadecanal, octadecanoic acid, 3-hydroxy-methyl ester, pentacosane and hexacosane (Silvera, 2002, as *Oncidium ornithorhynchum* Kunth), whereas that of *Gomesa* spp. formerly placed in *Baptistonia* consists of 40 % alkanes, 34 % fatty acids and 6–7 % each of dienes, alkenes and aliphatic compounds (Chiron *et al.*, 2009).

Here we describe the structure, position, anatomy and diversity of the floral elaiophores for a wide range of *Lockhartia* species, as well as the ultrastructure of secretory elaiophore cells and the process of oil secretion. Finally, we compare elaiophore structure and secretory activity in *Lockhartia* with that of other members of Oncidiinae and comment on the evolution of the elaiophore in this genus.

MATERIALS AND METHODS

Plant material

In total, flowers of 27 individual plants of *Lockhartia*, representing 16 species and all three major morphological groups, were investigated histochemically and by means of light microscopy, scanning electron microscopy and/or transmission electron microscopy (Table 1). These flowers were obtained from plants cultivated at the Florida Museum of Natural History (USA) and the Botanischer Garten München-Nymphenburg (Germany).

Abbreviations for authors of plant names follow Brummitt and Powell (1992) throughout. Voucher specimens, when available, were deposited in the herbarium of the Florida Museum of Natural History (FLAS) and Botanische Staatssammlung München (M).

Elaiophore distribution

The presence and position of floral elaiophores or putative elaiophores were determined for 13 species of *Lockhartia* derived from the Imbricata and Longifolia groups (Table 1) by means of Sudan stains (Sudan III, Sudan IV and Sudan Black). These stains were applied by immersing the living flower for 1–2 min in an alcoholic solution of the stain (saturated solution of Sudan III, Sudan IV or 0·3 % (w/v) Sudan Black in 70 % ethanol). Sudan Black is particularly useful whenever red or brown floral pigmentation is likely to obscure the red staining that results from the use of Sudan III or Sudan IV. Sudan stains are much more soluble in lipids than in ethanol and consequently elaiophores become selectively stained. Unfortunately, living flowers of *L. bennettii* and *L. oblongicallosa* (the two species of the Parthenocomos group studied) were not available for testing with Sudan dyes. Freshly stained flowers were examined by means of a dissecting microscope and photographed.

Scanning electron microscopy

Flowers of nine species of *Lockhartia* (Table 1) were prepared for scanning electron microscopy. Flower parts bearing elaiophores (or putative elaiophores) were fixed in either 75 % (v/v) ethanol or FAA [0·5 parts formaldehyde, 0·5 parts glacial acetic acid, 9 parts 75 % (v/v) ethanol], dehydrated in a graded ethanol series, dried to critical point using liquid CO_2, sputter-coated with platinum or gold–palladium, and examined using a Hitachi S-4000 scanning electron microscope at an accelerating voltage of 6–8 kV. Elaiophores of *Lockhartia lunifera*, *L. oerstedii* and *L. verrucosa* were also fixed in 2·5 % glutaraldehyde/4 % formaldehyde in phosphate buffer (pH 7·4; 0·1 M) for 4 h at 4 °C and then washed three times in phosphate buffer. Following post-fixation in 1 % (w/v) osmium tetroxide solution at 0 °C for 1·5 h, the material was dehydrated in acetone, subjected to critical-point drying using liquid CO_2, sputter-coated with gold and examined using a Tescan/Vega LMU scanning electron microscope at an accelerating voltage of 30 kV.

Histology and histochemistry

Flowers of six species of *Lockhartia* (Table 1) were prepared for light microscopy by fixing in FAA. Floral tissues were then

TABLE 1. *Species of* Lockhartia *classified according to the morphological groups indicated in the text, with voucher or source information and method of study.*

Taxon	Voucher or source	Method of study
Imbricata group		
L. acuta (Lindl.) Rchb.f.	*Blanco 2567* (FLAS)	LM, SEM
L. acuta (Lindl.) Rchb.f.	*Blanco 3221* (FLAS)	Sudan IV
L. amoena Endres & Rchb.f.	*Blanco 2556* (FLAS)	Sudan IV
L. grandibractea Kraenzl.	*Blanco 2559* (FLAS)	Sudan IV
L. lepticaula D.E. Benn. & Christenson	*Blanco 2573* (FLAS)	SEM
L. lepticaula D.E. Benn. & Christenson	*Blanco 3237* (FLAS)	Sudan IV
L. lunifera (Lindl.) Rchb.f.	*Blanco 2688* (FLAS)	Sudan IV
L. lunifera (Lindl.) Rchb.f.	*Gerlach 1994/2969* (M)	Sudan III/Black, LM, SEM, TEM
L. micrantha Rchb.f.	*Blanco 2558* (FLAS)	Sudan IV
L. micrantha Rchb.f.	*Blanco 2562* (FLAS)	Sudan IV
L. micrantha Rchb.f.	*Blanco 3220* (FLAS)	Sudan IV
L. oerstedii Rchb.f.	*Blanco 2565* (FLAS)	Sudan IV
L. oerstedii Rchb.f.	*Blanco 2566* (FLAS)	LM
L. oerstedii Rchb.f.	*Gerlach X/0502* (M)	Sudan III/Black, LM, SEM, TEM
L. niesseniae Kolan. & O. Pérez	*Whitten 2382* (FLAS)	LM
L. serra Rchb.f.	*Blanco 2574* (FLAS)	SEM
L. serra Rchb.f.	*Whitten 2431* (FLAS)	Sudan IV
L. tenuiflora M.A. Blanco, ined.	*Blanco 3012* (FLAS)	Sudan IV
L. tenuiflora M.A. Blanco, ined.	*Blanco 3231* (FLAS)	Sudan IV
L. verrucosa Lindl. ex Rchb.f.	*Blanco 3230* (FLAS)	Sudan IV
L. verrucosa Lindl. ex Rchb.f.	*Gerlach X/0501* (M)	Sudan III/Black, LM, SEM, TEM
Longifolia group		
L. hercodonta Rchb.f. ex Kraenzl.	*Blanco 3232* (FLAS)	Sudan IV
L. longifolia (Lindl.) Schltr.	*Blanco 3215* (FLAS)	Sudan IV
L. obtusata L.O. Williams	*Blanco 2572* (FLAS)	SEM
L. obtusata L.O. Williams	*Blanco 3025* (FLAS)	Sudan IV
Parthenocomos group		
L. bennettii Dodson	*Blanco 2554* (FLAS)	LM, SEM
L. oblongicallosa Carnevali & G.A. Romero	*Gerlach 2006/2502* (M)	SEM

Different vouchers for the same species correspond to different clones.
FLAS, Florida Museum of Natural History herbarium; M, Botanische Staatssammlung München. LM, light microscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

dehydrated using a graded series of ethanol and tertiary butanol, before embedding in paraffin (56 °C melting point). Sections were cut at a thickness of 10 μm on an American Optical 820 rotary microtome and secured to microscope slides using Haupt's adhesive. The sections were stained using Heidenhain's iron alum haematoxylin and safranin, and the preparations, following dehydration and clearing, made permanent by mounting in Permount or Canada balsam. They were subsequently examined using a Zeiss Axioskop 40 microscope with attached Pixera Pro 150ES digital camera.

Semi-thin sections were also prepared of *L. lunifera*, *L. oerstedii* and *L. verrucosa*. Pieces of elaiophore tissue (~2 mm^3) were fixed in 2·5 % glutaraldehyde/4 % formaldehyde in phosphate buffer (as above), washed in distilled water and dehydrated using a graded ethanol series, before being infiltrated and embedded in LR White resin. Following polymerization at 60 °C, semi-thin sections (0·9–1·0 μm thick) were stained with 0·25 % toluidine blue O in 0·25 % (w/v) aqueous sodium tetraborate solution (TBO). Hand-cut sections of fresh material were also tested for the presence of lipids using Sudan III (Jensen, 1962) and auramine O (Gahan, 1984). Control sections were used in each case. Light microscopy observations of these three species were made using a Nikon Eclipse 90i microscope equipped with a FITC filter and measurements were made using NIS-Elements imaging software.

Ultrastructural studies

Flowers of *L. lunifera*, *L. oerstedii* and *L. verrucosa* were also examined using transmission electron microscopy. Following fixation in 2·5 % glutaraldehyde / 4 % formaldehyde in phosphate buffer (pH 7·4; 0·1 M) for 4 h at 4 °C and, three washes in phosphate buffer, tissue samples were post-fixed in 1 % (w/v) osmium tetroxide solution at 0 °C for 1·5 h before being dehydrated and embedded in LR White resin. Following polymerization at 60 °C, sections were cut at a thickness of 60 nm using glass knives and a Reichert Ultracut-S ultramicrotome. The sections were subsequently stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined using an FEI Technai G2 Spirit Bio TWIN transmission electron microscope, at an accelerating voltage of 120 kV.

RESULTS

Elaiophore distribution

Lockhartia displays considerable floral diversity (Fig. 1A–I). Testing with Sudan stains revealed the presence of oil in the floral trichomes (which are invariably located on the adaxial surface of the labellum) of all species investigated. These included representatives of the Imbricata (Fig. 1A–F) and Longifolia groups (Fig. 1G–H); no species of the Parthenocomos group (Fig. 1I) were tested with these reagents. The position and structure of the elaiophore, and the degree of oil secretion, varied according to the morphological group being investigated.

In most species of the Imbricata group, the secretory trichomes that form the elaiophore were arranged in a well-defined, transversely rectangular or oval to slightly triangular area, located in a shallow depression of the basal-most part of the callus (hereafter referred to as the 'elaiophore cushion'; Fig. 2A–F). The remainder (distal part) of the callus was composed of irregularly thickened areas, folds and/or tubercles that, in most species, extended to the middle of the labellum (e.g. Fig. 1B–F). In *L. acuta*, except for a tuft of longer trichomes just beneath the column (Fig. 2G, H), there was no elaiophore cushion, and the elaiophore trichomes were uniformly scattered over the entire surface of the raised, but otherwise smooth, bifid callus. In all three species of the Longifolia group, the secretory trichomes covered the central and basal areas of the trapezoid projection; in *L. longifolia* and *L. obtusata*, secretory trichomes also occurred on the distal half of the callus rim (Fig. 3A–D). The callus and hair structure of the two species of the Parthenocomos group included in this study (not tested with Sudan dyes) is described below in the section Scanning electron microscopy.

In all species of the Imbricata group investigated, the glandular trichomes of the elaiophore cushion stained selectively with Sudan dyes (Fig. 2A–H). The natural colour of these trichomes varied from very pale yellow (e.g. *L. acuta*, Fig. 2G) to yellow and to pale orange (e.g. *L. verrucosa*, Fig. 2E), but all stained pale to dark red with Sudan III or Sudan IV. In *L. acuta*, the shorter trichomes that clothe most of the callus also stained with these reagents (Fig. 2G, H). In species of the Longifolia group, trichomes on the rim and at the centre of the callus stained much less intensely than corresponding hairs of members of the Imbricata group (Fig. 3A–D); the natural colour of the trichomes in all three species was uniformly dark orange.

In contrast to most other floral tissues, the stigmatic surface of representatives of both groups often stained with Sudan dyes (e.g. Fig. 2F, H).

Scanning electron microscopy

In species of the Imbricata group, two different types of trichomes were present on the elaiophore (Figs 4A, B, D, E and 5B, C): (1) relatively long, capitate hairs [100–400 μm long and ~20 μm in diameter at the base and 40 μm in diameter at the swollen tip, the apex sometimes bifid or shortly branched, but then widening (as in *L. lepticaula* and *L. serra*); hereafter referred to as 'long hairs' or 'long trichomes']; and (2) relatively short hairs of uniform diameter (100–180 μm long and 10–15 μm in diameter; hereafter referred to as 'short hairs' or 'short trichomes'). The long hairs were restricted to the central part of the elaiophore, directly beneath the stigma, and were mostly arranged parallel to each other and aligned with the longitudinal axis of the labellum (Figs 4D, E and 5A, B). Although they were free for most of their length, they were seemingly attached to each other by their swollen tips (Figs 4B, E–G and 5C, D). Each flower contained some 80–100 long hairs. By contrast, the short hairs were erect and free, and covered the whole surface of the elaiophore, both at the elaiophore margin and intermingled with the long hairs. However, the short hairs were often obscured by long hairs.

The surface of both types of trichome was either smooth or possessed a striate and blistered cuticle (Figs 4B, C, F, G and 5C, D). Cuticular swellings were mainly present on the long trichomes. Amorphous deposits, probably oil residues, were present on the surface of some trichomes (Fig. 4B, C). The surface of the visible epidermal cells of the elaiophore cushion (i.e. those not obscured by trichomes) was smooth, the profile of individual epidermal cells being only slightly raised. Numerous stomata, however, were present (Fig. 4D). The

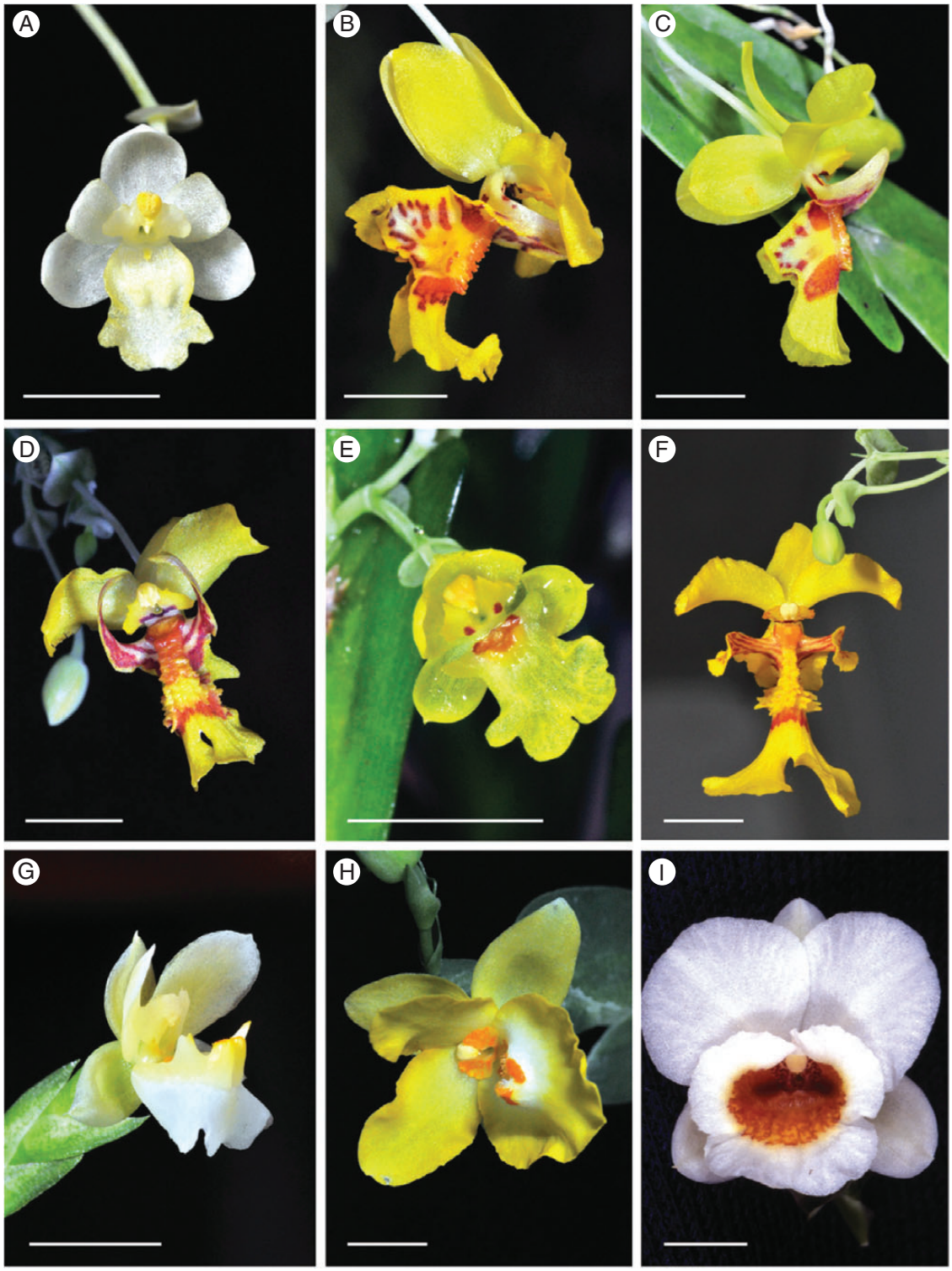

FIG. 1. Variation in floral morphology in the genus *Lockhartia*, illustrated by species included in the present study. (A) *L. acuta* (*Blanco 3221*). (B) *L. grandibractea* (*Blanco 2559*). (C) *L. lepticaula* (*Blanco 3237*). (D) *L. lunifera* (*Blanco 2688*). (E) *L. micrantha* (*Blanco 2562*). (F) *L. oerstedii* (*Blanco 2565*). (G) *L. hercodonta* (*Blanco 3232*); flower shown upright, but in this species the flowers are usually pendent. (H) *L. obtusata* (*Blanco 3025*). (I) *L. bennettii* (*Whitten 1704*, photograph courtesy of W. M. Whitten). Scale bars = 5 mm.

shallow depression of the elaiophore cushion measured 1·2 × 0·5 mm in *L. lunifera*, 1·4 × 1·1 mm in *L. oerstedii* and 1·4 × 0·5 mm in *L. verrucosa.*

The elaiophore of *L. acuta* differed from that of other species of the Imbricata group in that it lacked an elaiophore cushion, short hairs did not flank the tuft of long hairs at the base of the

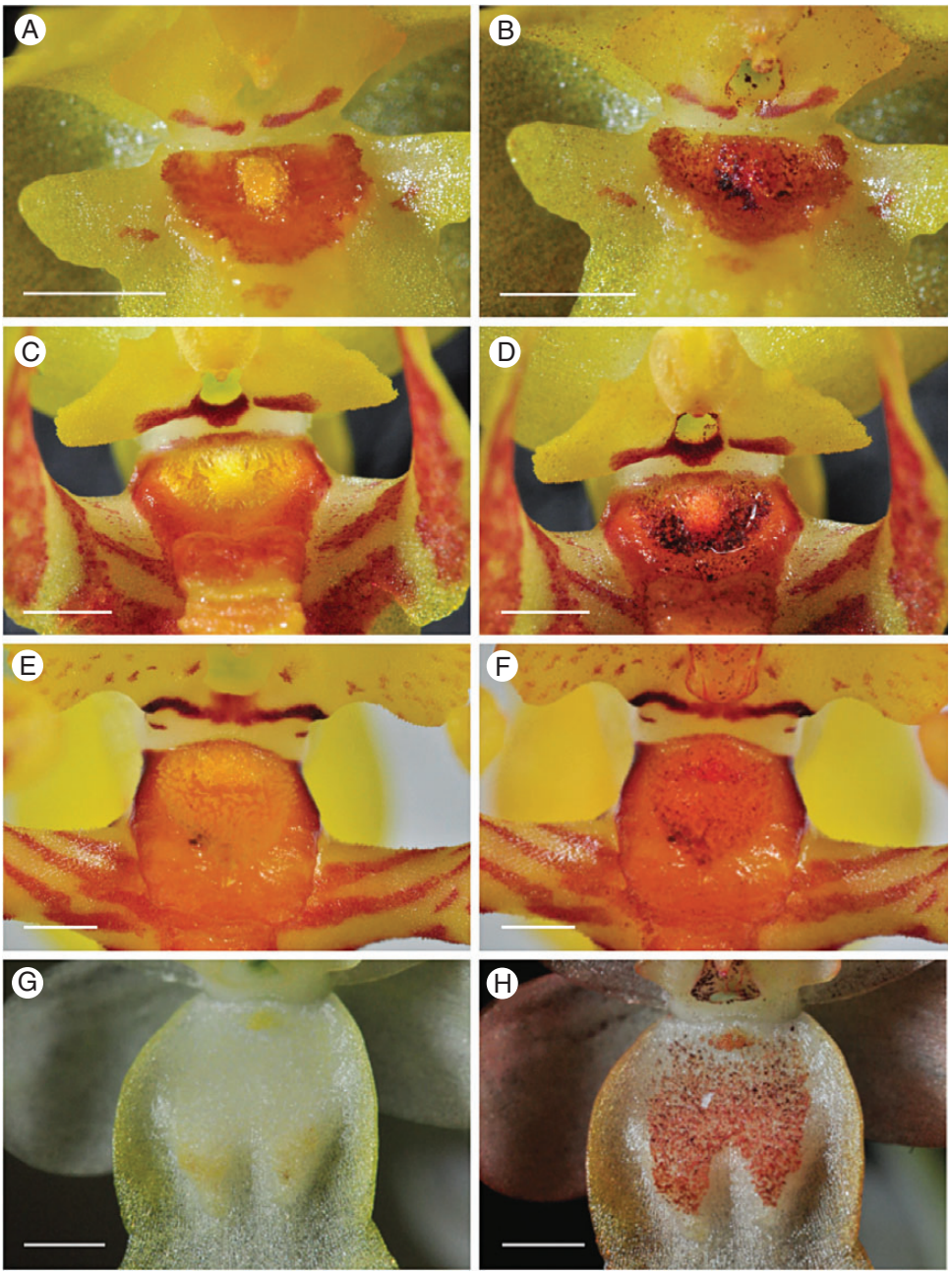

FIG. 2. Labellar trichomes of *Lockhartia* species from the Imbricata group prior to (left) and following (right) staining with Sudan dyes. (A, B) *L. micrantha* (*Blanco 2562*). (C, D) *L. lunifera* (*Blanco 2688*). (E, F) *L. verrucosa* (*Blanco 3230*). (G, H) *L. acuta* (*Blanco 3221*). In (A–F) the trichomes are located in a shallow concavity on the proximal part of the callus (i.e. the elaiophore cushion; see text for details). Scale bars = 1 mm.

labellum, and most of the callus surface (except for the base, where the tuft of long hairs was located) was clothed with short secretory hairs (Fig. 6A, B). As in other species of the Imbricata group, the long hairs lay parallel to the epidermis and were closely appressed to each other, but their expanded apices were globose, never bifid (Fig. 6B, C). The surface of the long hairs was longitudinally striate for most of its length, but the direction of the striations was more variable in the capitate tip. The short hairs on the callus surface were more or less uniform in diameter, or gradually became slightly narrower towards the apex; their surface was smooth. Scanning electron microscopy revealed that the tips of some of these short hairs were coated with a thin, cotton-like film, possibly lipoidal residues (Fig. 6D).

The callus of *L. bennettii* (Parthenocomos group) consisted of a relatively large, slightly thickened area at the centre of the labellum, densely covered with hairs of uniform diameter (Fig. 6E). Trichome lengths, when plotted, revealed a continuum, the longest hairs (~550 μm long and 20 μm in diameter) being located towards the base of the labellum. Scattered, crystal- or

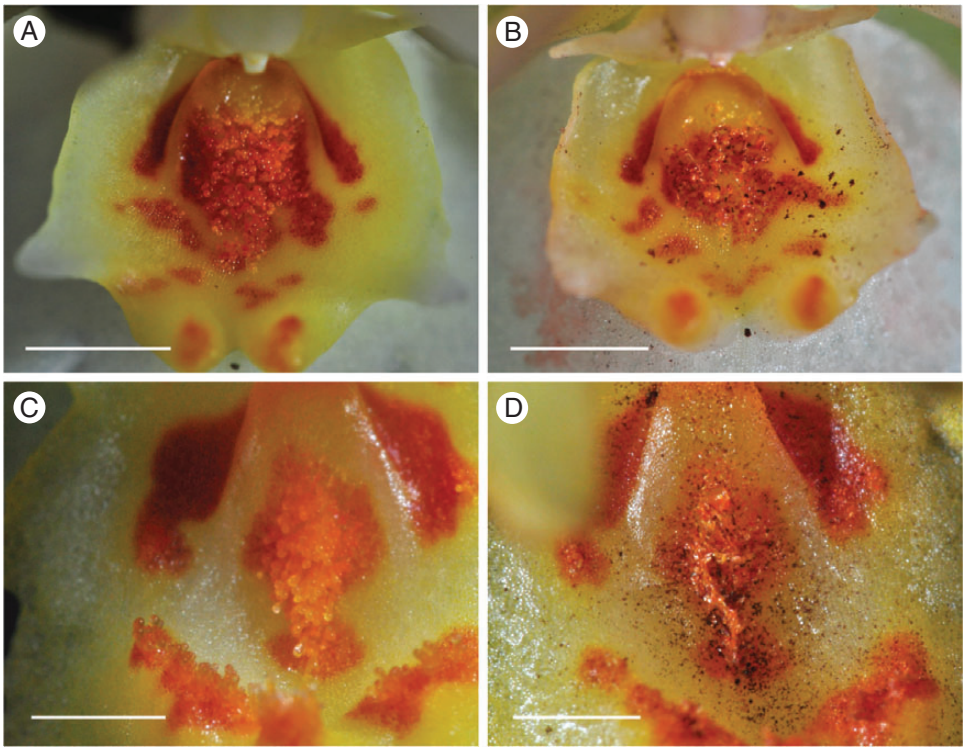

FIG. 3. Labellar trichomes of *Lockhartia* species from the Longifolia group prior to (left) and following (right) staining with Sudan dyes. (A, B) *L. hercodonta*. (C, D) *L. longifolia*. Scale bars = 1 mm.

wax-like deposits were present on the apices of these hairs (Fig. 6F).

Capitate hairs were present on various parts of the callus of *L. obtusata* (Longifolia group; Fig. 6G–I). These were found on (1) the central area of the basal trapezoid projection; (2) an apical projection (tooth) of the callus rim; (3) two rounded swellings on either side of the apical tooth and forming part of the callus rim; and (4) two zones flanking the trapezoid projection; here, the hairs were much shorter than elsewhere. The longest hairs were ~300 μm long and 25 μm wide at the base. The tips of these hairs were slightly swollen (~30 μm in diameter) and often coated with crystalline deposits (Fig. 6H, I). These crystals were elongate and formed bundles that projected more or less perpendicularly from the surface of the trichomal cuticle. Each hair arose as a projection of an epidermal cell, and even atrichomatous epidermal cells possessed a central papilla. Again, plotting papilla and trichome lengths revealed a continuum.

The callus of *L. oblongicallosa* consisted of two longitudinal, parallel, narrow keels that extended from the base to the middle of the labellum (Fig. 7A). The basal half of each keel, and the gap between them, were clothed with capitate hairs (to 250 μm in length and 70 μm in diameter at the apex; Fig. 7A–C). The apices of these hairs were globular and unbranched.

Histology and histochemistry

Transverse sections of the labellum across the trichome-bearing region (i.e. the elaiophore) of *L. niesseniae* (Fig. 8A, B), *L. acuta* (Fig. 8C), *L. bennettii* (Fig. 8D), *L. oerstedii* (Fig. 8E, F), *L. verrucosa* (Fig. 8G–I) and *L. lunifera* (Fig. 9A–J) were studied using light microscopy. The labellum was ~500 μm thick in each case, and consisted largely of ground parenchyma enclosed by a single-layered epidermis and two or three layers of subepidermal cells. The ground parenchyma contained collateral vascular bundles and idioblasts with raphides and/or phenolic content (Fig. 8E, G). The largest of the ground parenchyma cells measured ~50–75 μm in diameter, but the subepidermal cells were much smaller. The epidermal cells were rectangular in section and about twice as wide as deep. The trichomes of all species investigated were unicellular, each arising as an outgrowth of an epidermal cell (Figs 8B, D–G and 9B–D). The epidermal cell wall was cellulosic, regardless of whether the cells were trichomatous or atrichomatous. However, the thickness of the outer, tangential wall varied according to species (0·50–1·87 μm in *L. oerstedii*, 1·00–2·83 μm in *L. verrucosa* and 1·00–6·50 μm in *L. lunifera*), and in each case a relatively thin cuticle (~0·2 μm) was present.

In *L. acuta*, *L. bennettii*, *L. lunifera*, *L. niesseniae* and *L. oerstedii*, the trichomatous cells were similar in shape to adjacent atrichomatous epidermal cells (except for the trichomal projection) (Figs 8A–F and 9B, D). In all species investigated using light microscopy, the cytoplasm of trichomatous epidermal cells was often denser than that of adjacent atrichomatous cells, and the nucleus was frequently located within the trichome itself (Fig. 9B, D).

Each transverse section through the elaiophore region of *L. oerstedii* and *L. niesseniae* displayed ~200 long hairs, also cut transversely (owing to their orientation parallel to the longitudinal axis of the labellum; Fig. 8A, E). Similar sections through

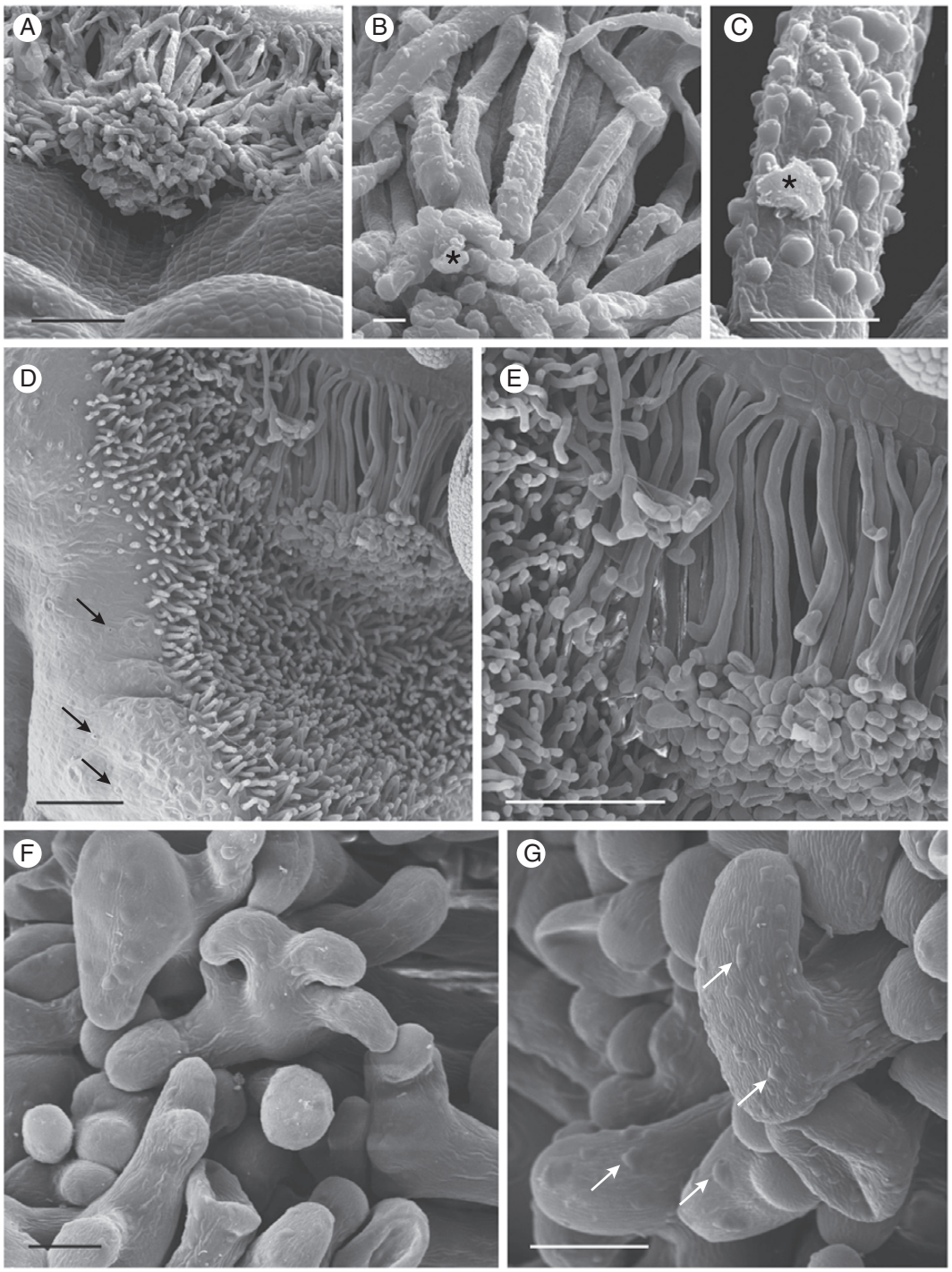

FIG. 4. Labellar trichomes of (A–C) *Lockhartia lunifera* and (D–G) *L. oerstedii* viewed by scanning electron microscopy. (A) Long trichomes at base of labellum. (B) Surface of trichomes with striate and blistered cuticle, and residues of secreted material visible at apex (asterisk). (C) Detail of middle portion of trichome with secretion (asterisk). (D) Long trichomes reclining on labellum surface and surrounded by shorter, erect trichomes. Stomata (arrows) occur in the epidermis directly behind the secretory area. (E) Long trichomes with branched tips. (F) Branched apices of long trichomes. (G) Long trichomes with striate and blistered cuticle towards apex (arrows indicate cuticle blisters). Scale bars (A, D, E) = 200 μm; (B, C, F, G) = 20 μm.

the basal elaiophore of *L. acuta* revealed only some 70 such hairs (Fig. 8C).

The epidermal surface and hairs were coated with a lipoidal secretion (Figs 8H, I and 9A–J). Following treatment with Sudan III, stained lipid droplets could also be seen within hairs and epidermal cells and also directly beneath cuticular blisters (Fig. 9C, F, H, I). The secreted material was heterogeneous and contained small, spherical profiles or droplets (Fig. 9B, D, E, G, H). In unstained sections, secreted material on the epidermal surface and intracellular droplets, both initially yellow, stained

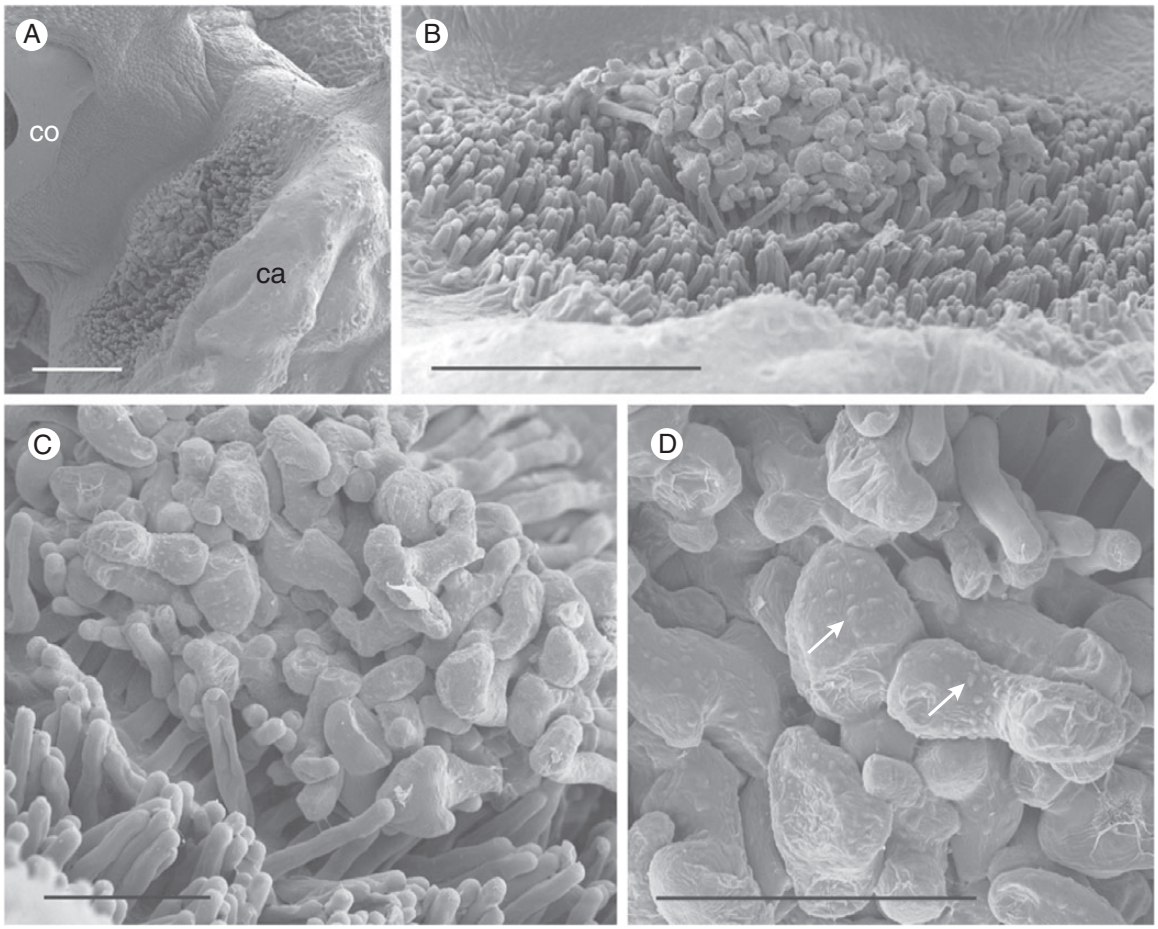

FIG. 5. Labellar trichomes of *Lockhartia verrucosa* viewed by scanning electron microscopy. (A) Elaiophore at base of labellum; base of callus (ca) and column (co) visible. (B) Detail of elaiophore; notice long trichomes surrounded by short trichomes. (C) Branched and rounded apices of long and short trichomes, respectively. (D) Cuticular blisters (arrows) at apex of long trichomes. Scale bars (A, B) = 400 μm; (C, D) = 100 μm.

blue–grey with TBO (Fig. 9A, B, D, E) and orange–red with Sudan III (Figs 8H and 9C, F, H, I). However, only the small droplets dispersed within the surface secretion of epidermal hairs, together with intracellular lipid droplets, fluoresced green–yellow with auramine O (Figs 8I and 9G, J). In *L. lunifera*, plastids (probably chromoplasts) were visible in the subepidermal parenchyma (Fig. 9F).

Ultrastructural studies

In all three species of *Lockhartia* investigated for ultrastructure using transmission electron microscopy (*L. lunifera*, *L. oerstedii* and *L. verrucosa*), both trichomatous and atrichomatous epidermal cells were nucleate and contained dense, intensely staining cytoplasm. Nuclei were generally centrally located (Fig. 10A, F; but sometimes located in the trichomal projection, as in *L. oerstedii*, see above), whereas vacuoles occurred either at the cell base or within the trichomal projection. Secretory cells also contained plastids, and these were present both in the perinuclear and parietal cytoplasm (Figs 10A, B, H and 11B–D), the typical epidermal cells containing numerous chromoplasts and leucoplasts.

SEM revealed that the cuticular surface of the hairs of the same three species was striate and clearly distended, whereas transmission electron microscopy revealed that the epidermal cell walls, including those of the hairs, lacked cavities and pores, but that both cell wall and cuticle were lamellate (Figs 10A, D, E, H and 11E). Moreover, primary pit-fields with plasmodesmata (Fig. 10B) connected the cytoplasm of adjacent epidermal cells and also that of epidermal and subepidermal cells. The parietal cytoplasm contained numerous lipid droplets or irregularly shaped lipid deposits (Figs 10C, F, G and 11A, B). Likewise, some leucoplasts in both trichomatous and atrichomatous epidermal cells contained several large starch grains and lipid globules, and were considered to be amyloplasts (Figs 10A, B, H and 11A–D). Furthermore, the cytoplasm of atrichomatous epidermal cells and secretory hairs contained abundant mitochondria, endoplasmic reticulum profiles, dictyosomes and secretory vesicles. These vesicles occurred mainly in the parietal cytoplasm, where stages in their fusion with the plasmalemma were observed (Figs 10D, E and 11E). Some vacuoles also contained cytoplasmic enclaves and membranous intravacuolar bodies in the form of small myelin-like figures (Fig. 10D).

DISCUSSION

The earliest recorded microscopical observation of elaiophores in *Lockhartia* known to us was that made by H. G. Reichenbach,

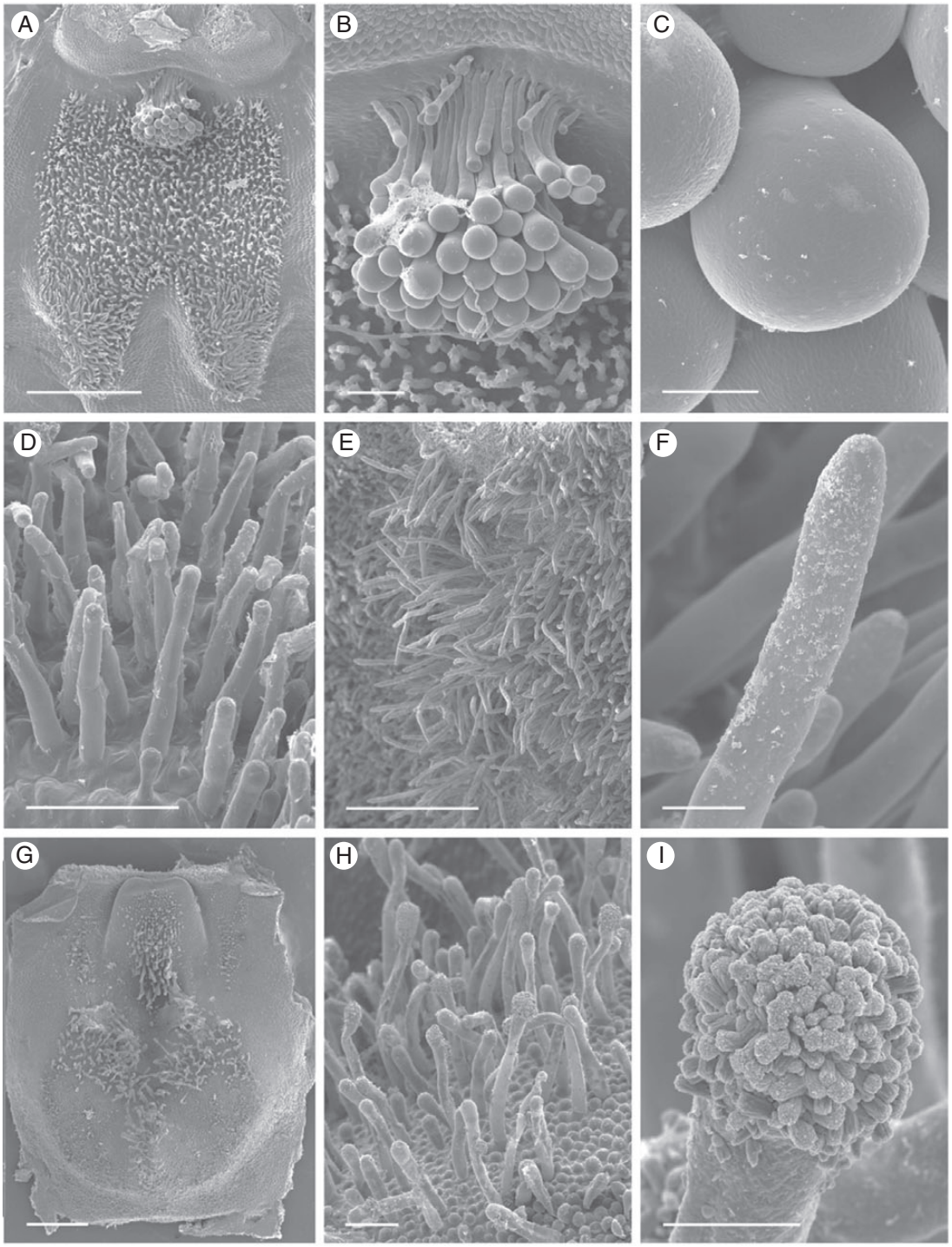

FIG. 6. Labellar trichomes of (A–D) *Lockhartia acuta*, (E, F) *L. bennettii* and (G–I) *L. obtusata* viewed by scanning electron microscopy. In A–C and G, the base (proximal part) of the labellum is towards the top of the image. (A) Callus clothed with short trichomes; note the tuft of longer, capitate trichomes at base of labellum. (B) Tuft of capitate trichomes at base of labellum. (C) Detail of apical portion of capitate trichomes. (D) Detail of short trichomes from main part of the callus. (E) Part of the callus, epidermis obscured by trichomes. (F) Apical portion of trichome with secretory residues. (G) Callus excised from the rest of the labellum, showing basal trapezoid projection, semi-circular rim and apical tooth, all of which are partly clothed with trichomes. (H) Group of trichomes on rim surface. (I) Apex of trichome, showing unidentified crystalline deposits. Scale bars (A, E, G) = 750 μm; (B, D, H) = 100 μm; (C, F, I) = 25 μm.

who, in 1875, prepared drawings of the long secretory trichomes of *L. cladoniophora* Rchb.f. from a plant cultivated at the Hamburg Botanic Garden. These drawings are now attached (along with other sketches of the plant) to the type specimen deposited at the herbarium of the Naturhistorisches Museum in Vienna (W). Reichenbach coined the specific epithet '*cladoniophora*'

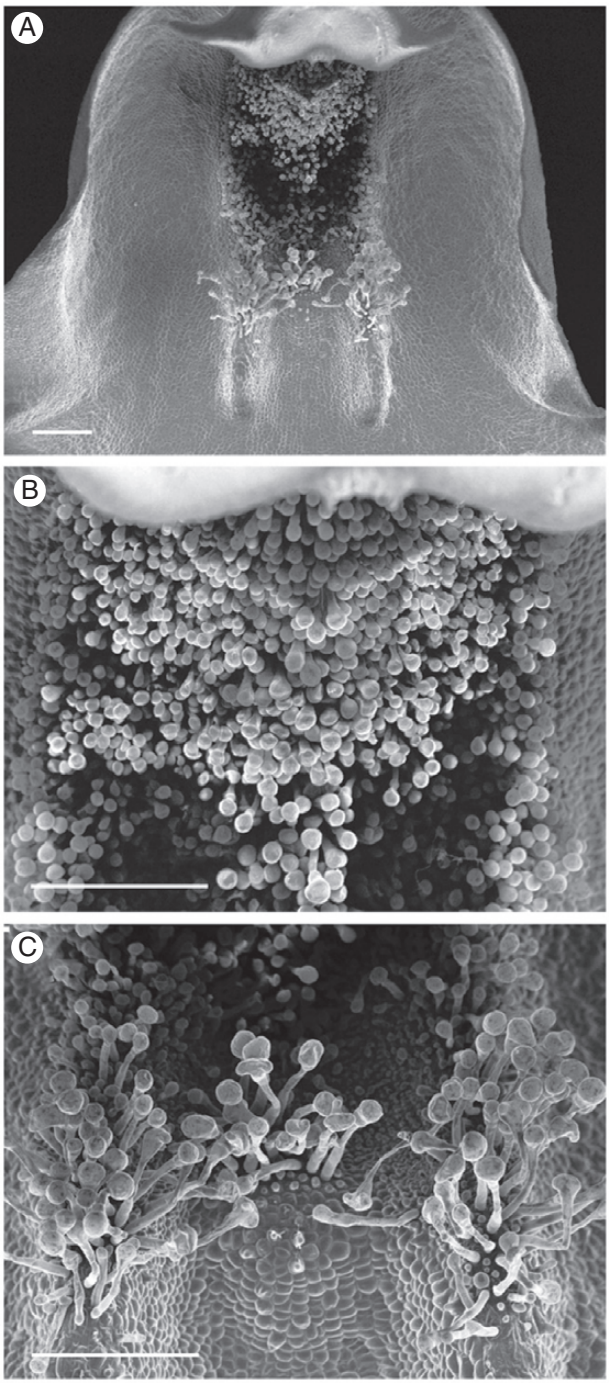

FIG. 7. Labellar trichomes of *Lockhartia oblongicallosa* viewed by scanning electron microscopy. The proximal part (base) of the labellum is towards the top of the images. (A) Base of labellum showing two parallel keels and intervening area partly clothed with trichomes. (B) Trichomes at base of callus. (C) Detail of capitate trichomes near the central region of callus. Scale bars = 500 μm.

(meaning 'branch-bearing') in allusion to these branched trichomes, which he compared to moose antlers in the species protologue (Reichenbach, 1888). The Costa Rican orchidologist Rafael Lucas Rodríguez subsequently illustrated the long elaiophore trichomes of *L. grandibractea* Kraenzl. in a botanical painting (posthumously published as *L. amoena* Endres & Rchb.f. in Rodríguez *et al.*, 1986). Singer *et al.* (2006) published a photograph of the base of the labellum of *L. lunifera*, but they erroneously classified the elaiophore as epithelial (probably because of the shiny surface of the remainder of the callus, which does not secrete oil). The present study is the first to characterize the elaiophores of the genus *Lockhartia* in detail.

Both the elaiophore and stigmatic surface of *Lockhartia* species stained with Sudan dyes. However, the latter is probably caused by the presence of stigmatic lipids, as demonstrated for other species of Oncidiinae (Clifford and Owens, 1990). Unlike elaiophore oils, these compounds are not pollinator rewards but probably contribute to the viscosity of the stigmatic fluid, thus facilitating the adhesion of pollinia to the insect, as well as possibly providing nutrients for the developing pollen tubes.

Of those members of the Oncidiinae whose elaiophore structure has been thoroughly investigated, that of *Lockhartia* most closely resembles the trichomal elaiophore of *Ornithocephalus ciliatus* (as *O. kruegeri*; Pacek and Stpiczyńska, 2007). For example, the oil-secreting, floral hairs of *O. ciliatus*, *P. falcifolium* (Pacek *et al.*, 2012) and many *Lockhartia* species are unicellular and have capitate tips. However, whereas the elaiophore hairs of *O. ciliatus* and *P. falcifolium* are unbranched, those of several species of *Lockhartia* in the Imbricata group are apically branched. Moreover, in *Lockhartia* spp., *O. ciliatus*, *O. gladiatus*, *P. falcifolium*, *Z. grandiflora* and *Z. lunata*, the trichomal surface becomes distended as oil accumulates between the cuticle and the cell wall (Pacek and Stpiczyńska, 2007; Pacek *et al.*, 2012). Cuticular distension is also known to occur in Oncidiinae that have epithelial elaiophores (Pacek and Stpiczyńska, 2007; Stpiczyńska *et al.*, 2007; Stpiczyńska and Davies, 2008; Davies and Stpiczyńska, 2009), such as *Oncidium cheirophorum* Rchb.f., *O. sotoanum* (as *O. ornithorhynchum*; Davies and Stpiczyńska, 2009), *Trichocentrum cavendishianum* (Bateman) M.W. Chase & N.H. Williams and *Gomesa radicans* (Rchb.f.) M.W. Chase & N.H. Williams (as *Ornithophora radicans* (Rchb.f.) Garay and Pabst). The cuticle of *Lockhartia*, however, unlike that of most Oncidiinae studied to date, is lamellate, (an exception being *Gomesa loefgrenii* (Cogn.) M.W. Chase & N.H. Williams [as *Oncidium loefgrenii* Cogn., Stpiczyńska *et al.*, 2007]), as is the cell wall, which contains abundant plasmodesmata. Similarly, in *Gomesa bifolia* (Sims) M.W. Chase & N.H. Williams, a species that has epithelial elaiophores, the cuticle of the callus and lateral lobes is striate (Aliscioni *et al.*, 2009). The oil-secreting hairs of *Ornithocephalus gladiatus*, *P. falcifolium*, *Z. grandiflora* and *Z. lunata* also have a lamellate cuticle, which in *O. gladiatus* and *P. falcifolium* is bi-layered, having an outer lamellate and an inner reticulate layer (Pacek *et al.*, 2012). In *Lockhartia*, *O. ciliatus*, *O. gladiatus*, *P. falcifolium*, *Z. grandiflora* and *Z. lunata*, the cell wall of the oil-secreting trichomes lacks cavities (Pacek and Stpiczyńska, 2007; Pacek *et al.*, 2012). Cavities are also absent from the outer tangential, epidermal wall of the epithelial elaiophores of *Oncidium sotoanum*, *Gomesa recurva* Lodd., *G. radicans* and *Rudolfiella picta* (Schltr.) Hoehne (Maxillariinae) (Pacek and Stpiczyńska, 2007; Stpiczyńska and Davies, 2008; Davies and Stpiczyńska, 2009). In common with other oil-producing Oncidiinae, the elaiophore hairs of *Lockhartia* have an organelle complement typical of secretory cells. These hairs have dense cytoplasm

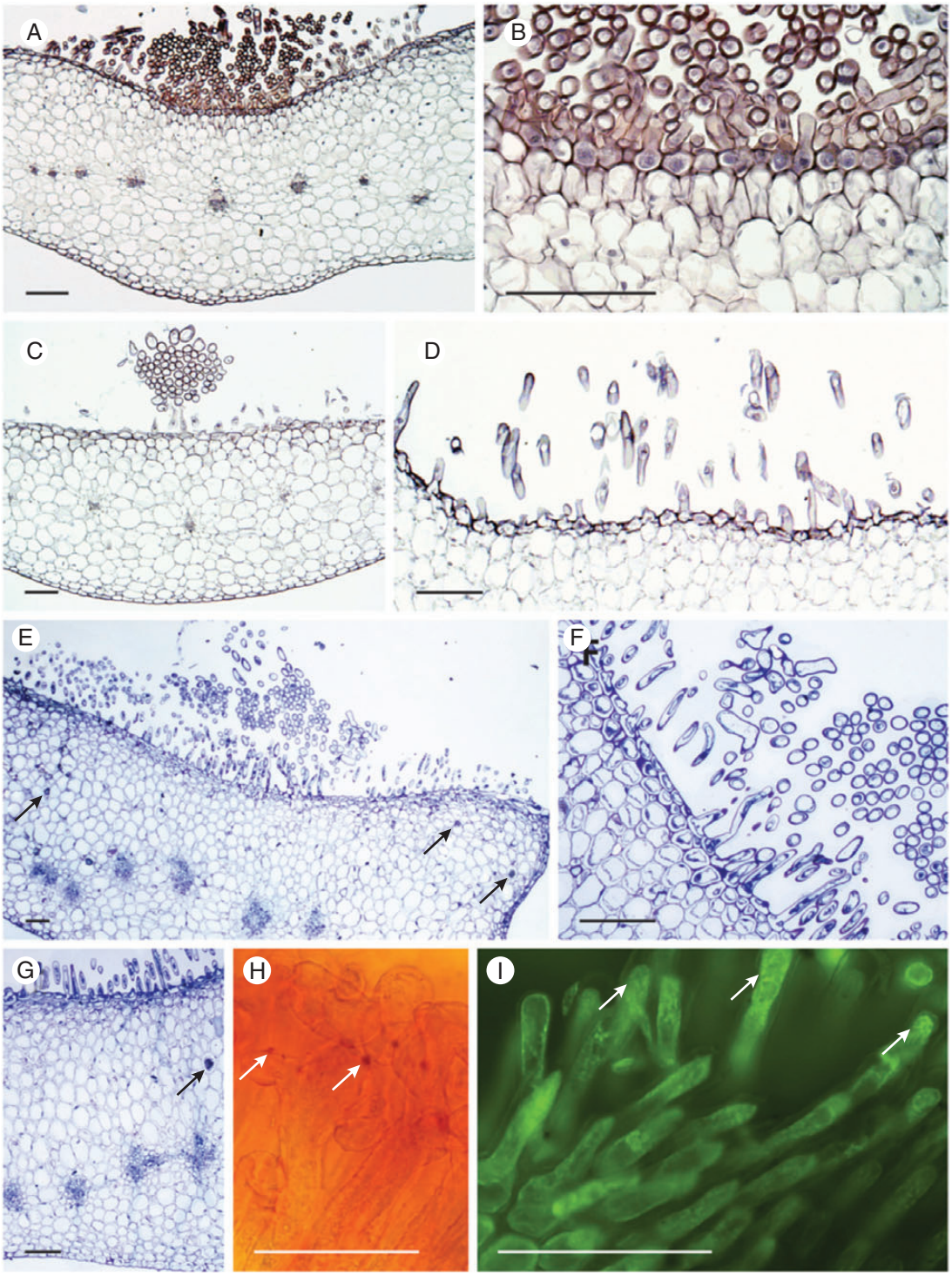

FIG. 8. Transverse sections of the trichome-bearing region of the labellum (i.e. the elaiophore) of (A, B) *Lockhartia niesseniae*, (C) *L. acuta*, (D) *L. bennettii*, (E, F) *L. oerstedii* and (G) *L. verrucosa*, and secretory trichomes of *L. verrucosa* (H, I) viewed by light microscopy. (A) Base of labellum of *L. niesseniae* showing elaiophore trichomes, subepidermal parenchyma and ground parenchyma with vascular bundles. The concavity that contains the trichomes is part of the 'elaiophore cushion'. (B) Detail of (A): secretory trichomes and adaxial epidermal cells. (C) Base of labellum of *L. acuta*: section through the group of long trichomes. In (A–C) the long elaiophore trichomes lie parallel to the longitudinal axis of the labellum and are shown in transverse section; the short elaiophore trichomes are shown in oblique section. (D) Central part of labellum of *L. bennettii* showing adaxial epidermal cells, many of which are trichomatous; the trichomes mostly lean towards the labellum apex and are cut obliquely. (E) Base of labellum of *L. oerstedii* showing sections through long and short trichomes. Idioblasts with phenolic contents and raphides (arrows) occur in the ground parenchyma. (F) Detail of E, showing trichomatous epidermis and subepidermal parenchyma composed of small, densely packed cells. (G) Base of labellum of *L. verrucosa* with trichomatous adaxial epidermis and subepidermal parenchyma, both tissues intensely stained with TBO. An idioblast with raphides is indicated by the arrow. (H) Lipid droplets on the surface of branched apex of long trichomes (arrows), stained with Sudan III. (I) Intracellular lipids following staining of trichomes with auramine O (arrows). Scale bars = 100 μm.

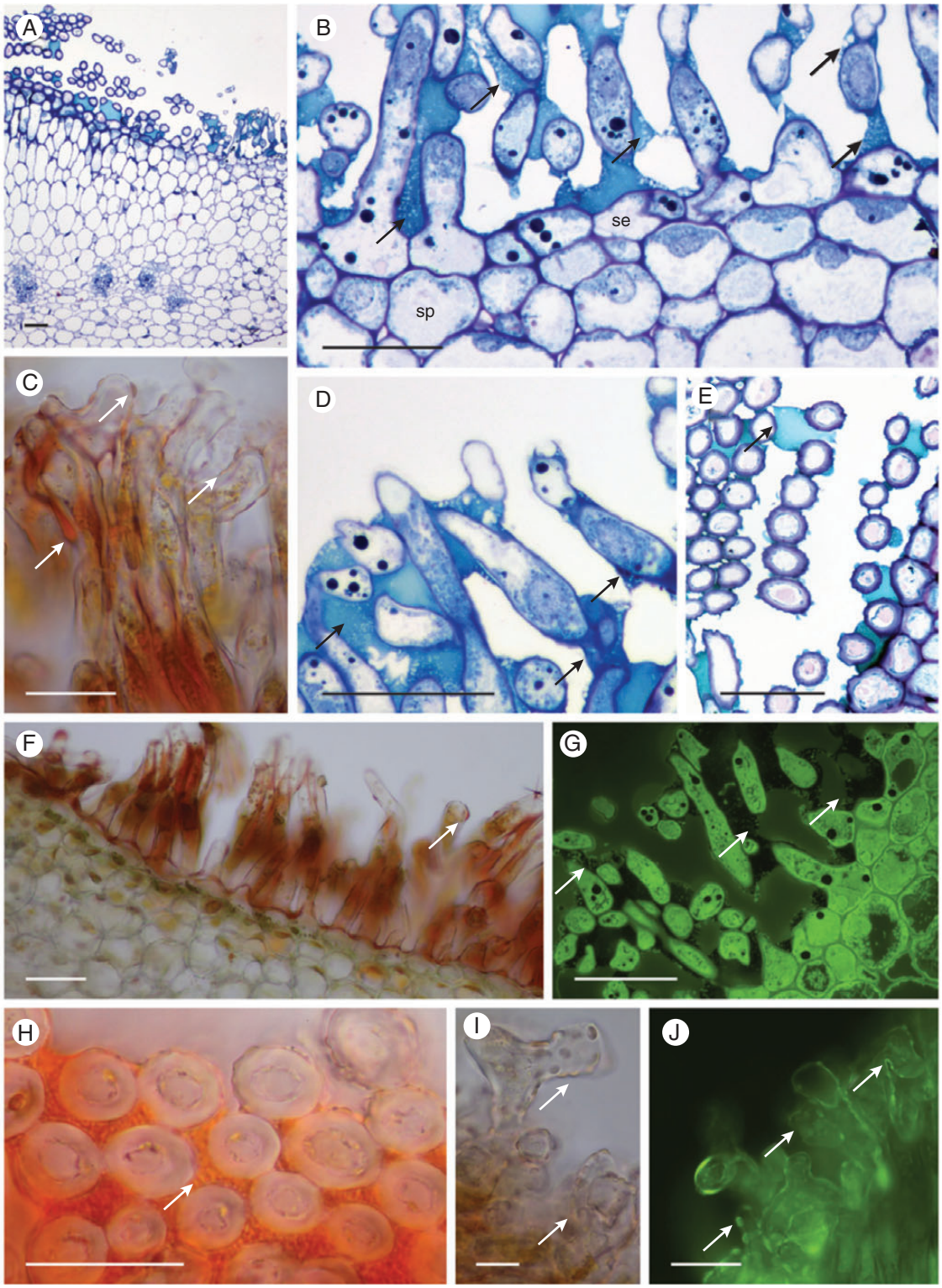

FIG. 9. Secretory trichomes of *Lockhartia lunifera* viewed by light microscopy. (A) Transverse section of labellum base stained with TBO and showing trichomatous epidermis, subepidermal parenchyma and ground parenchyma with vascular bundles. Note long elaiophore trichomes cut transversely above adaxial epidermis. (B) Short elaiophore trichomes with dense cytoplasm and nuclei, their surface coated with heterogeneous secretion (arrows). (C) Long trichomes with surface lipids stained with Sudan III (arrows). (D) Heterogeneous secretion (arrows) coating surface of short trichomes. (E) Transverse section of long trichomes with thick cellulose cell wall coated with secretion (arrow). (F) Hand-cut section of labellum stained with Sudan III showing trichomatous epidermis and plastids in subepidermal parenchyma. Lipids are present in trichomes (arrow) and subepidermal parenchyma. (G) Trichomes surrounded by heterogeneous secretion (arrows) following staining with auramine O. (H) Secretory, long trichomes in transverse section with thick cell walls coated with secretion (arrow) that stains with Sudan III. (I) Apex of trichome stained with Sudan III showing blistered cuticle and subcuticular secretion (arrows). (J) Blistered cuticle and secretion stained with auramine O (arrows). Abbreviations: se, secretory epidermis; sp, subepidermal parenchyma. Scale bars = 50 μm.

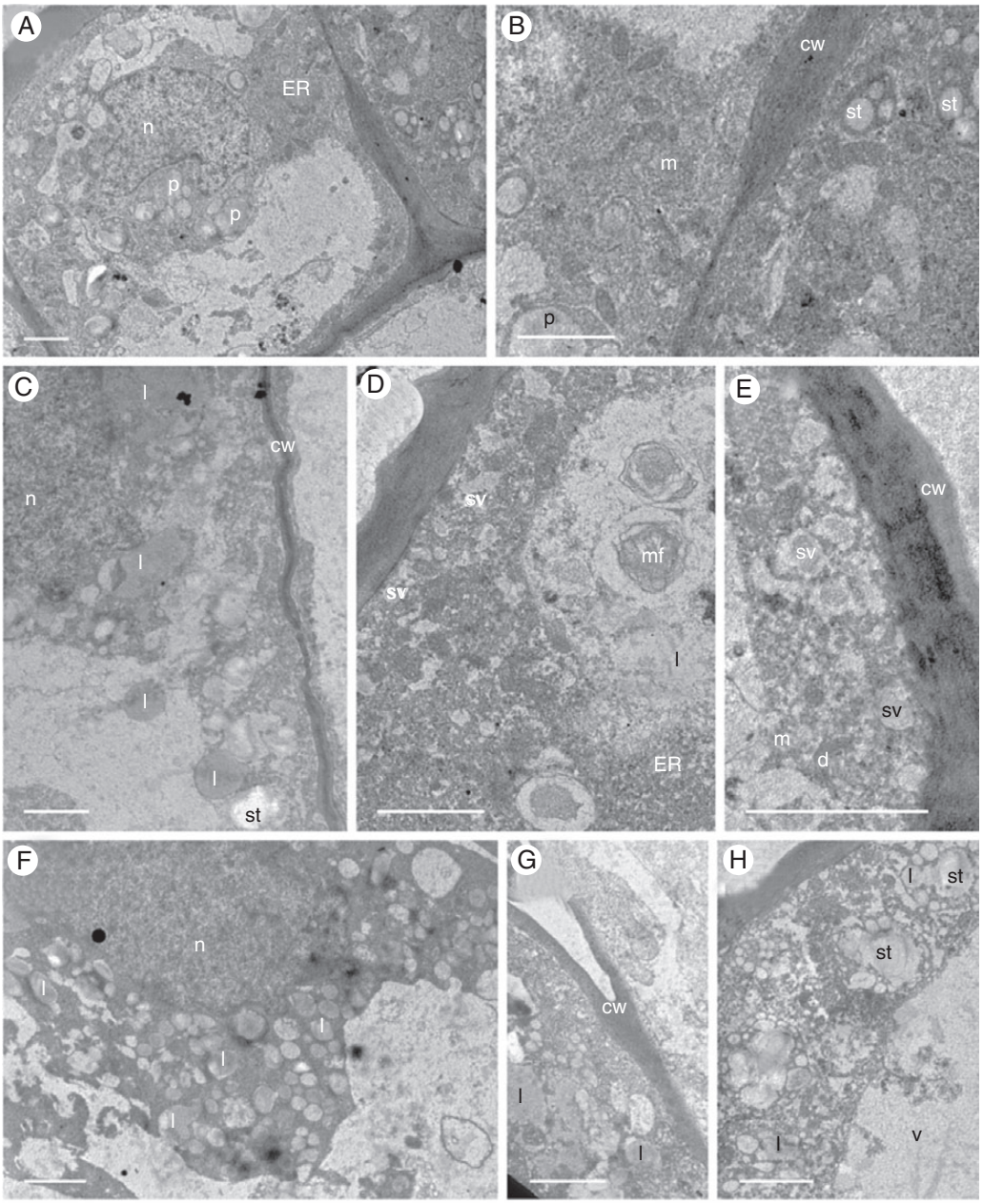

FIG. 10. Ultrastructure of secretory cells of (A–E) *Lockhartia oerstedii* and (F–H) *L. lunifera* viewed by transmission electron microscopy. (A) Atrichomatous, adaxial epidermal cell showing centrally positioned nucleus, plastids with starch grains, and vacuole towards the bottom of the image. (B) Detail of granular cytoplasm with plastids, mitochondria and endoplasmic reticulum. The cell wall contains primary pit-fields with plasmodesmata. (C) Parietal cytoplasm containing numerous lipid droplets and plastid with starch grain. (D) Secretory vesicles adjacent to cell wall, and vacuoles with myelin-like figures. (E) Secretory vesicles near plasmalemma. Note lamellate structure of the cuticle overlying the wall. (F) Numerous lipid droplets in perinuclear cytoplasm. (G) Lipid droplets in parietal cytoplasm towards base of trichome. (H) Plastids containing starch grains and lipid droplets. Abbreviations: cw, cell wall; d, dictyosome; ER, endoplasmic reticulum; l, lipid-filled vesicle; m, mitochondrion; mf, myelin-like figure; n, nucleus; p, plastid; st, starch; sv, secretory vesicle; v, vacuole. Scale bars = 2 μm.

containing a nucleus, plastids, mitochondria, lipid droplets or deposits, endoplasmic reticulum profiles, dictyosomes and secretory vesicles. Some plastids contain starch grains and oil droplets. Plastids with numerous lipids droplets (elaioplasts) also occur in *Ornithocephalus gladiatus*, *Z. grandiflora* and *Z. lunata*. Secretory vesicles present in the cytoplasm fuse with the plasmalemma and thereby discharge their contents (i.e. oils). The oil-secreting hairs of *Lockhartia*, like those of *Ornithocephalus ciliatus* and the epithelial elaiophores of *Oncidium sotoanum*, *Oncidium cheirophorum*, *Gomesa venusta* (Drapiez) M.W. Chase & N.H. Williams (as *Oncidium trulliferum* Lindl.) and *T. cavendishianum*, have vacuoles that contain cytoplasmic enclaves and membranous intravacuolar bodies or myelin-like configurations. These are thought to be formed by autolysis and invagination of the tonoplast or, if formed in the cytoplasm, by rapid membrane turnover or

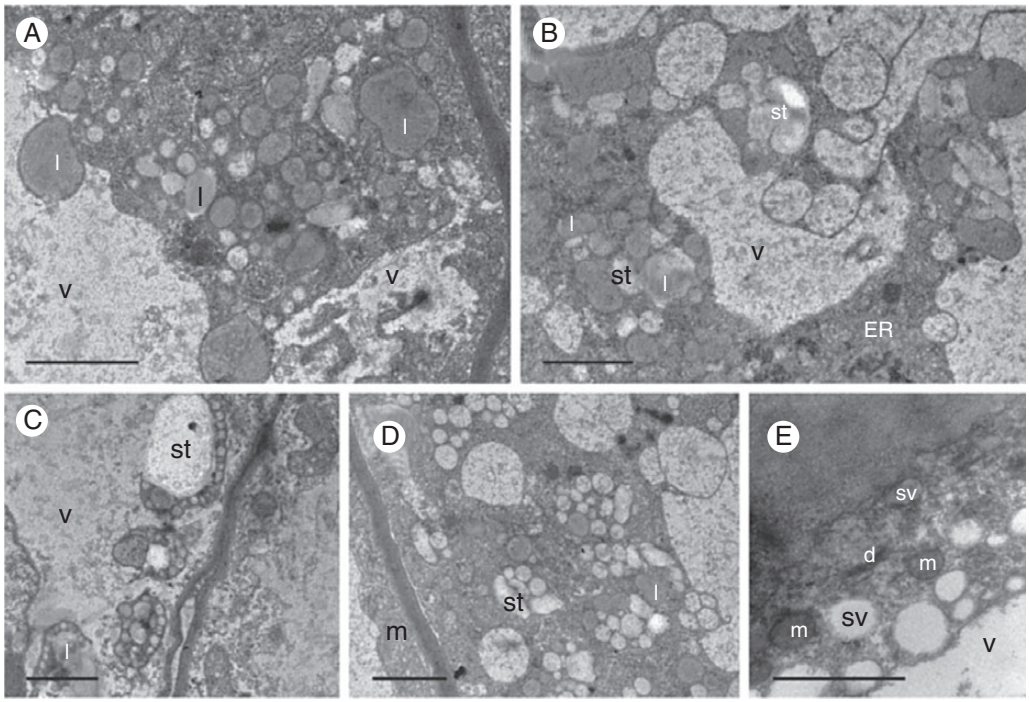

FIG. 11. Ultrastructure of secretory hairs of *Lockhartia verrucosa* viewed by transmission electron microscopy. (A) Large lipid droplets and endoplasmic reticulum profiles in parietal cytoplasm. (B) Lipid droplets and numerous small vacuoles. (C) Plastids in parietal cytoplasm containing starch and small lipid droplets. (D) Mitochondria, plastids and small vacuoles in basal part of trichome. (E) Secretory vesicles, mitochondria and dictyosomes adjacent to plasmalemma. Abbreviations: ER, endoplasmic reticulum; l, lipid-filled vesicle; m, mitochondrion; st, starch; sv, secretory vesicle; v, vacuole. Scale bars = 2 μm.

invagination of the plasmalemma during tissue differentiation or senescence (Davies, Davies and Francis, 1992; Stpiczyńska *et al.*, 2007).

A remarkable feature of the floral oil of *Lockhartia*, which makes it unique among those members of Oncidiinae investigated to date is that, in transmission electron microscopic sections, it is seen to be heterogeneous and contains small, electron-transparent droplets. Hitherto, such heterogeneity has been reported only for the resin-secreting flowers of *Rhetinantha divaricata* (Barb. Rodr.) M.A. Blanco (as *Maxillaria* cf. *notylioglossa* Rchb.f.), a member of subtribe Maxillariinae (Davies, Turner and Gregg, 2003). This suggests that floral oil and resin production may have evolved along similar chemical pathways in these two orchid subtribes.

The production of floral oils by various species of *Lockhartia* strongly suggests that they are visited and pollinated by oil-collecting bees. In the New World, these specialized insects are represented by several different genera of the bee family Apidae, derived from the tribes Centridini, Tapinostapidini and Tetrapediini, and formerly assigned to a separate family, Anthophoridae (Vogel, 1973, 1988; Roubik, 1989; Alves dos Santos *et al.*, 2007). Based on the chemical composition of the oils, Silvera (2002) proposed that *Lockhartia* flowers are pollinated by bees of the genus *Centris*. These bees are known to pollinate a number of floral oil-producing New World plants assigned to the families Calceolariaceae, Iridaceae, Krameriaceae, Malpighiaceae, Plantaginaceae, Solanaceae and Orchidaceae (van der Pijl and Dodson, 1966; Vogel, 1973, 1988; Buchman, 1987; Rasmussen and Olesen, 2000; Cocucci and Vogel, 2001; Silvera, 2002; Alves dos Santos *et al.*, 2007; Pansarin and Pansarin, 2011; Renner and Schaefer, 2010; Torreta *et al.*, 2011). The only published record of an insect visitor to flowers of *Lockhartia* is that of the euglossine bee *Eulaema meriana* visiting flowers of *L. oerstedii* Rchb.f. (van der Pijl and Dodson, 1966). However, this is likely to be erroneous (C. H. Dodson, pers. comm., 2002), since identification of the bee, which was not collected, was based on a brief observation of the event from several metres away.

In most species of *Lockhartia*, the column is very short (2–4 mm long) and more or less perpendicular to the labellum, and the elaiophore is located at or near the base of the labellum. Thus, the pollinarium, whose viscidium is presented on the ventral surface of the column, probably becomes attached to the head or front legs of the pollinator as it collects oil. As far as is known, specialized hairs on the legs or abdomen (but not the mouthparts) of oil-gathering bees are used to collect oils, and the latter are then used as food for larvae (Vogel, 1973, 1988; Buchman, 1987; Alves dos Santos *et al.*, 2007). Pollinaria of *Lockhartia* are characteristically small (typically 0·7–1·3 mm long) and have a bifid, tegular stipe. To date, however, their attachment to the bodies of bees has not been reported. This may be due to the fact that the thin stipe collapses upon drying and this obfuscates identification of the pollinarium to generic level. The situation is further exacerbated by the fast-flying and extremely timid nature of oil-collecting bees. As a result, they are much more difficult to capture or observe from short distances than male euglossine bees, for which an abundance of observational data exists.

The marked difference in staining intensity of elaiophore trichomes with Sudan dyes between members of the Imbricata

and Longifolia groups indicates that floral oil is secreted to differing degrees in these taxa. The extent of oil production (if any) by members of the Parthenocomos group remains unknown. However, since the labellar trichomes of the latter do not appear to glisten when examined under magnification, it is possible that members of this group produce food-deceptive flowers. Furthermore, species assigned to the Parthenocomos group appear to be sister to the rest of the genus (M. A. Blanco, unpubl. res.), indicating that the common ancestor of *Lockhartia* may well have had relatively simple, food-deceptive flowers and that the more elaborate type of elaiophore found amongst species of the Imbricata group is a derived character.

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