# Structure, ultrastructure and evolution of floral nectaries in the twinflower tribe Linnaeeae and related taxa (Caprifoliaceae)

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Linnaeeae is a small tribe of Caprifoliaceae consisting of six genera and *c.* 20 species. In Linnaeeae, floral nectaries are located on the corolla-filament-tube and nectar is produced from unicellular glandular hairs. We studied 23 taxa using scanning electron microscopy (SEM), light microscopy (LM) and transmission electron microscopy (TEM) and found two distinct nectary morphologies, zonate and gibbous types, and two distinct types of glandular hair, clavate and smooth base types. Plesiomorphic characters associated with the nectary and identified in the tribe include hypocrateriform corollas, dichogamous flowers, zonate nectaries, wet papillate stigmas, vestigial nectary disc and smooth pollen grains. Apomorphic characters include bilabiate corollas, homogamous flowers, bulging nectaries, dry papillate stigmas and echinulate pollen grains. The nectary structure is similar in *Vesalea* and *Linnaea* and differs from the rest of the tribe, in accordance with recent phylogenetic results. Nectar secretion is typically granulocrine with subcuticular accumulation of nectar, which we compared with the secretion in multicellular hairs of *Adoxa moschatellina*. The cuticle on the hair becomes detached from the cell wall and large subcuticular spaces filled with nectar are formed. Nectar is probably released in areas with a thin cuticle. In *Zabelia*, the smooth basal part of the hair could help to build up the hydrostatic pressure. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016, **181**, 37–69

ADDITIONAL KEYWORDS: flower – corolla filament tube – nectarial trichomes – nectary – pollination.

## INTRODUCTION

*Linnaea* L. belongs to the tribe Linnaeeae (Caprifoliaceae) which consists of six genera and *c.* 20 species (Graebner, 1901; Rehder, 1911; Christenhusz, 2013). Its centre of diversity is China, but it also occurs in Japan, North and South Korea, eastern Russia, Central Asia, the Himalayas and Mexico. *Linnaea borealis* L. ex Gronov. is a circumboreal species. Recent phylogenetic studies (Jacobs, Pyck & Smets, 2010; Landrein *et al.*, 2012; Wang *et al.*, 2015) have clarified the relationships among taxa in Linnaeeae. It emerged that the genus *Abelia* R.Br. is not monophyletic as a result of the position of *Vesalea* Martens & Galeotti and series *Serratae* Graebn., and that series was segregated as a new genus, *Diabelia* Landrein (Landrein, 2010). It also emerged that *Vesalea* is sister to *Linnaea* (Wang *et al.*, 2015). *Zabelia* (Rehder) Makino, which was segregated from

the tribe on the basis of several morphological characters as well as chromosome number, is sister to the *Morina* L. and *Linnaea* clade (Wang *et al.*, 2015).

Few studies of Linnaeeae have focused on the floral nectary and its systematic significance. Fukuoka (1968) was the first author to show how the nectary morphology differed between *Vesalea, Abelia* and *Zabelia*. Kurosawa & Hara (1955) studied the distinction and significance between the clavate nectary in *Diabelia spathulata* (Siebold & Zucc.) Landrein and the flat nectary in *D. serrata* (Siebold & Zucc.) Landrein. In this study, we provide a detailed analysis of the nectary in Linnaeeae using evidence from recent molecular work as a framework (Wang *et al.*, 2015) (Fig. 1).

### NECTARIAL TRICHOMES

Nectaries in Dipsacales are, with few exceptions, located on the inside of the corolla-filament-tube

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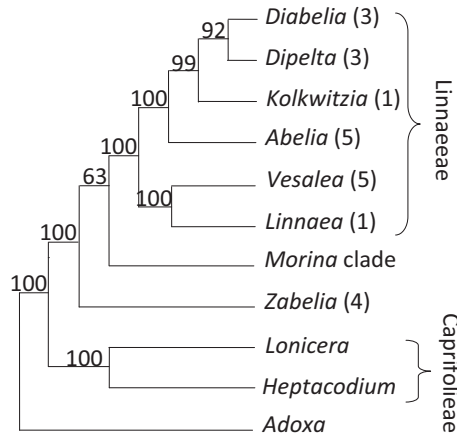*Corresponding author. E-mail: s.landrein@free.fr

**Figure 1.** Systematic relationships in Linnaeeae and close relatives (Wang *et al.*, 2015). One of the most parsimonious trees from an analysis of plastid data (*rbc*L, *trn*S-G, *mat*K, *trn*L-F, *ndh*A, *trn*D-*psb*M, *pet*B-D, *trn*L-*rpl*32 and *trn*H-*psb*A). Bootstraps consistent with the strict consensus tree are shown above the branches.

(stapet *sensu* Ritterbusch, 1991) and consist of unicellular epidermal hairs (nectaria trichomalia, *sensu* Smets, 1986) and an underlying nectary parenchyma. There may be five nectariferous areas or a continuous ring (*Cephalaria* Schrad., *Knautia* L.), but, in most cases, there is only one flat or cushion-like nectary in the abaxial position. The stapet can be gibbous, saccate or spurred at the base. Exceptions occur in Adoxaceae: in *Sambucus* L., nectaries are absent and secretion is restricted to the stigmatic region; in *Adoxa* L., nectar is secreted by multicellular hairs on the petals (Sturm, 1910); in *Viburnum* L., the nectary is a nectary disc (Weberling, 1977; Bentley & Elias, 1983; Wagenitz & Laing, 1984; Nicolson, Nepi & Pacini, 2007) (Fig. 2).

NECTARY ULTRASTRUCTURE

The nectary is made up of three tissue types: the nectary epidermis, the nectary parenchyma and the subnectary parenchyma (Nicolson *et al.*, 2007). The nectary epidermis may have trichomes as secretory structures and, in our case, it includes two types: unicellular hairs as found in *Lonicera* L. (Fahn & Rachmilevitz, 1970) and multicellular, capitate trichomes, such as in the extrafloral nectaries of *Vicia faba* L. (Davis, Peterson & Shuel, 1988).

The nectary parenchyma is composed of small cells, generally with thin walls, dense granular cytoplasm rich in ribosomes and mitochondria, small vacuoles and relatively large nuclei. Vacuole sizes in the cells change as the nectary develops, increasing in volume during secretion. The subnectary parench-

yma consists of larger cells, with larger vacuoles, less dense cytoplasm and larger intercellular spaces. Few ultrastructural changes take place in subnectary parenchyma cells when the nectary is nearing secretion.

Nectar secretion has been described in two related species: *Lonicera japonica* Thunb. (Fahn & Rachmilevitz, 1970) and *L. kamtschatica* Pojark (Weryszko-Chmielewska & Bożek, 2008). The prenectar is unloaded from the sieve elements to the adjacent phloem parenchyma cells. Prenectar passes through plasmodesmata to the nectary parenchyma by the symplastic route. Secretion is typically granulocrine, with the transport of sugar solution into vesicles derived from the dilated cisternae of endoplasmic reticulum (ER) or from dictyosomes that fuse with the plasmalemma, releasing nectar. Conspicuous wall protuberances can be observed in most epidermis cells, with vesicles developing from the swollen edges of the ER cisternae or, less frequently, dictyosomes seem to be in contact with the wall protuberances and could be released by reverse pinocytosis. The cuticle of the hair becomes detached from the cell wall and large subcuticular spaces filled with nectar are formed. Nectar is probably released in areas with a thin cuticle and, as observed by Weryszko-Chmielewska & Bożek (2008), through microchannels in the cuticle (Fig. 3).

Our study focuses on the nectary morphology and the question of whether the nectaries are homologous among the genera and taxa of Linnaeeae. Nectaries placed on corollas are typical of Dipsacales and represent the most derived state of nectaries in the angiosperms (Smets, 1986). These types of nectary have evolved in Dipsacales and display an array of morphological adaptations.

Smets (1986: 71) noted that, 'It is supposed that the role of the extending receptacle as a nectar-secreting tissue in certain Asterales has been taken over by the corolla, which also functions as a nectarotheca, these nectaries on the corolla are most manifest in the Dipsacales. This represents an evolutionary state which is more recent than that of the nectaries associated with stamens and even the disc nectaries'.

Later, Smets & Cresens (1988) updated this view, interpreting the mode of secretion as a secondary feature and defining the nectaries and the locality of the nectary in the flower as a primary criterion. The trichotamous nectary was thus called a caducous nectary (of the corolla or perigonal type) in relation to its localization.

Here, we present a detailed study of the ultrastructure of nectaries and how secretion and release mechanisms have also adapted in relation to floral morphology and pollination syndromes.
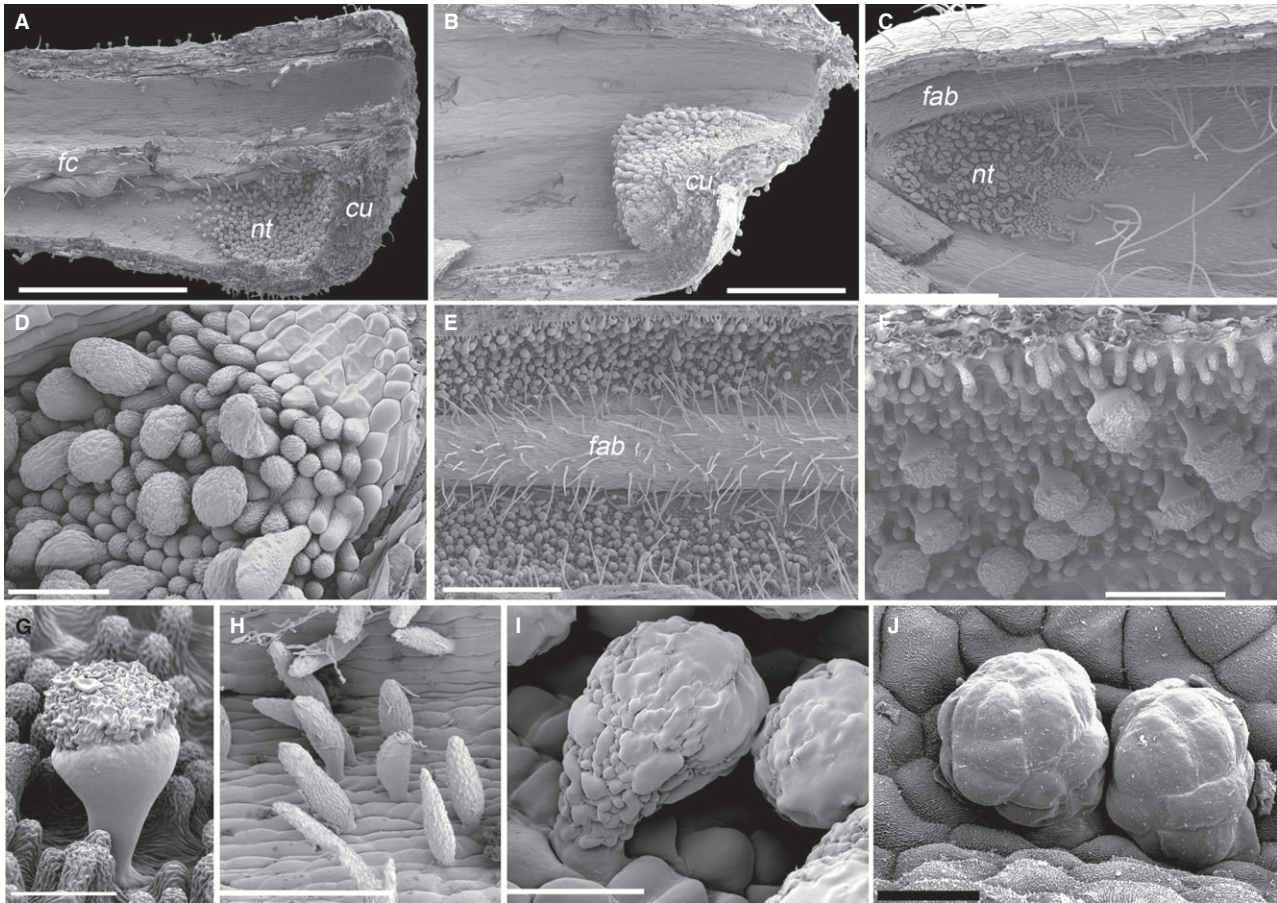
**Figure 2.** Nectary and nectaria trichomalia in tribe Linnaeeae (scanning electron microscopy, SEM). A, *Abelia forrestii* var. *forrestii*, longitudinal section of the corolla-filament-tube showing the gibbous base, nectaria trichomalia and filament curtain. B, *Diabelia spathulata* var. *spathulata*, longitudinal section of the stapet showing the gibbous base and clavate nectary cushion covered with glandular hairs. C, *Linnaea borealis*, longitudinal section of the corolla showing the zonate nectary with a single area in between the abaxial filaments. D, *Linnaea borealis*, nectaria trichomalia. E, *Vesalea floribunda* var. *floribunda*, longitudinal section of the stapet showing the zonate nectary with several areas in between the abaxial and adaxial filaments. F, *Zabelia tyaihyoni* var. *mosanensis*, longitudinal section of the stapet showing the nectaria trichomalia. G, *Zabelia tyaihyoni* var. *mosanensis*, nectaria trichomalia with a smooth base. H, *Heptacodium miconioides*, nectaria trichomalia and hairs. I, *Abelia forrestii* var. *forrestii*, clavate nectaria trichomalia. J, *Adoxa moschatellina*, multicellular glandular hair. Bars: A, B, C, E, 500 μm; F, H, 100 μm; D, J, 50 μm; G, 30 μm; I, 20 μm. cu, cushion; fab, abaxial filament; fc, filament curtain; nt, nectaria trichomalia.

## MATERIAL AND METHODS

### SCANNING ELECTRON MICROSCOPY (SEM)

Flower buds were collected from 23 taxa, mostly cultivated at the Royal Botanic Gardens Kew, China and Mexico (Appendix 1). The buds were fixed in FAA (ethanol 70%, acetic acid, formaldehyde 40%, 90 : 5 : 5), transferred to 70% ethanol and dissected under a Wild dissecting microscope. For SEM, the material was dissected in 70% ethanol, dehydrated to absolute alcohol and critical point dried using liquid $CO_2$ in an Autosamdri 815B CPD (Tousimis Research, Rockville, MD, USA). The dried material was mounted on aluminium stubs and coated with platinum using an Emitech K550 sputter coater (Emitech, Ashford, UK). Observations were made with a Hitachi cold field emission S-4700-II scanning electron microscope (Hitachi High Technologies, Tokyo, Japan).

### LIGHT MICROSCOPY (LM)

For anatomical studies, flower buds were dehydrated through an ethanol series and embedded in wax using standard methods. Specimens were sectioned using a Leica rotary microtome at 8–10 μm
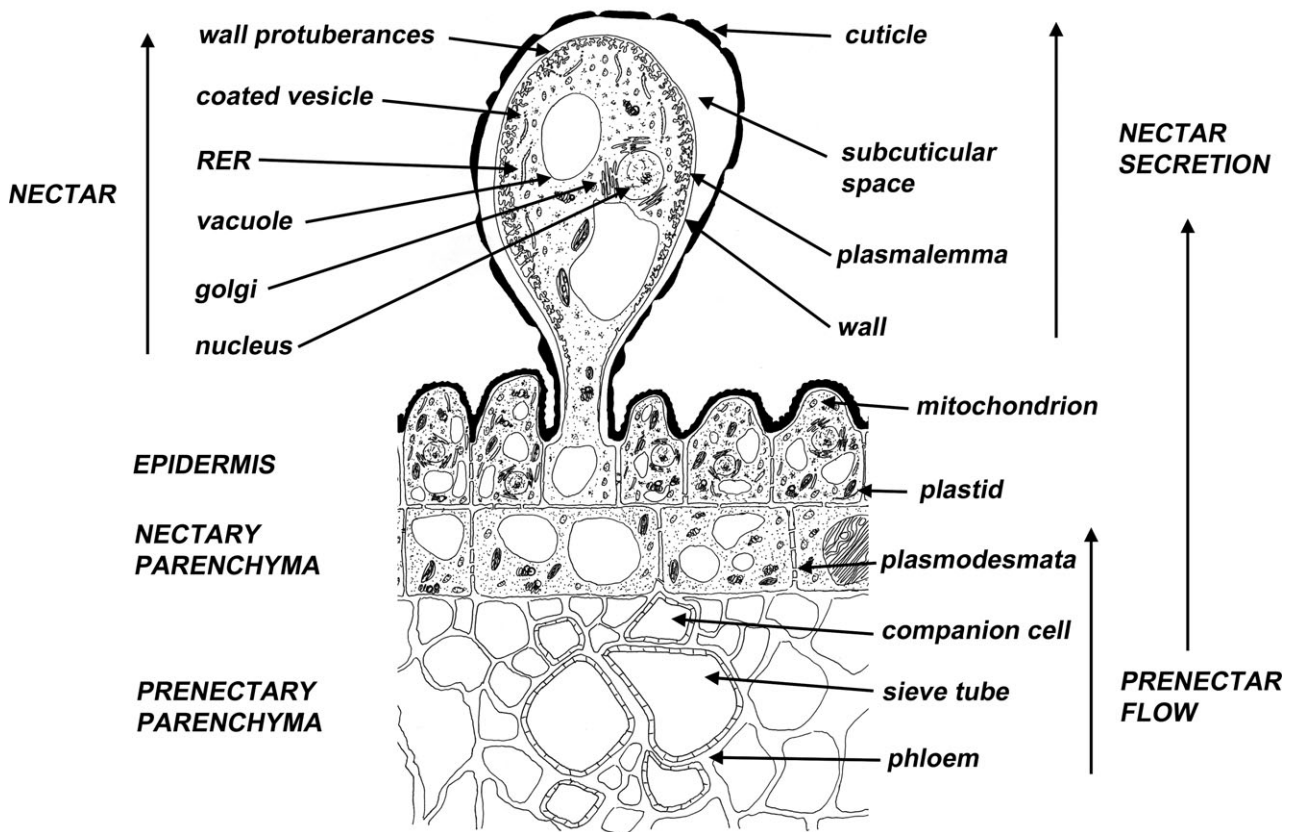
**Figure 3.** Nectar secretion and transport in *Lonicera japonica*. RER, rough endoplasmic reticulum.

thickness. Sections were stained in toluidine blue and mounted in DPX mountant (a mixture of distyrene, a plasticizer and xylene; Agar Scientific, Stansted, UK). Light micrographs were taken using a Leitz Diaplan photomicroscope, fitted with a digital camera. Nectary and flower diagrams were drawn using Microsoft Word 97-2002.

### Transmission electron microscopy (TEM)

Four species were studied [*Linnaea borealis* L., *Abelia macrotera* Rehder, *Zabelia biflora* (Turcz.) Makino and *Adoxa moschatellina* L.]. Nectary samples from floral buds from anthetic flowers were washed in 0.1 M Sörensen's phosphate buffer and fixed for 2 or 3 h in 1% aqueous osmium tetroxide. They were then washed in phosphate buffer and dehydrated through a graded ethanol series to 100% ethanol. The samples were fixed in Karnovsky solution (Karnovsky, 1965) for 24 h, postfixed in 1% osmium tetroxide (0.1 mol/L phosphate buffer, pH 7.2) and processed using standard methods (Roland, 1978). Samples were de-aerated under vacuum for *c.* 1 h. They were then stored in a refrigerator at 4 °C at least overnight before further

processing. Nectaries were embedded in medium-grade LR white resin (London Resin, Reading, UK) in gelatin capsules. Sectioning was carried out using a Reichert Ultracut. Semithin (*c.* 1 µm) sections were cut using a glass knife, stained with toluidine blue and examined using a Nikon Labophot optical microscope to determine the stage of nectar production. Ultrathin (gold) sections were cut using a Diatome diamond knife and stained with uranyl acetate and lead citrate in a Leica EM Gridstain. Sections were examined in a Hitachi H-7650 transmission electron microscope at an accelerating voltage of 100 kV. Images were taken with an AMT XR41 digital camera.

## RESULTS

### General organography

Flowers of Linnaeeae are pentamerous or tetramerous and zygomorphic. The perianth is composed of two to five free or partially fused sepals and four or five fused petals. Each flower has four stamens positioned opposite the lateral and abaxial sepals (antesepalous). Filaments are adnate to the corolla tube

between the lateral petals and the abaxial petals, forming a stapet (Ritterbusch, 1991). A zone of dense unicellular hairs forms in the abaxial part of the stapet in between the filaments (Fig. 4).

### Nectary vasculature

The vascular supply of the flower is derived from a very small stele. Four peripheral bundles branch off to the sterile half of the ovary; three are on the outside of the fertile locule. The central bundle becomes the ventral bundle, supplying the ovules. The fertile ovular bundle separates and becomes much larger. Seven to ten peripheral bundles continue into the apical sterile part of the ovary. Radial divisions of the peripheral bundles give rise to the sepal traces; five sepal–petal bundles and four sepal–stamen bundles supply the calyx. The fifth bundle of the stamen–sepal ring does not divide tangentially into stamen and sepal traces, but extends directly into the base of the calyx tube and divides into three

branches. The orientation of the flower is fixed by this bundle; the abaxial petal opposite this sepal trace is the petal which is glandular at the base. Its petal bundle usually divides into three branches, the middle of which often gives off a slight short strand to the glandular surface (Giger, 1912; Wilkinson, 1948, 1949) (Fig. 5).

### Linnaea borealis (Fig. 6)

The corolla of *L. borealis* is infundibuliform to campanulate, 5–15 mm long and fragrant (Scobie & Wilcock, 2009). The paired flowers are pendent and held at the end of a long peduncle, 3–7 cm above the ground. The calyx is reduced and adpressed to the base of the stapet. In *L. borealis* var. *longiflora* Torr., occurring on the west coast of North America, a basal tube is present and the corolla is infundibuliform, whereas it is campanulate in *L. borealis* var. *borealis* and intermediate in *L. borealis* var. *americana* (J.Forbes) Rehder.
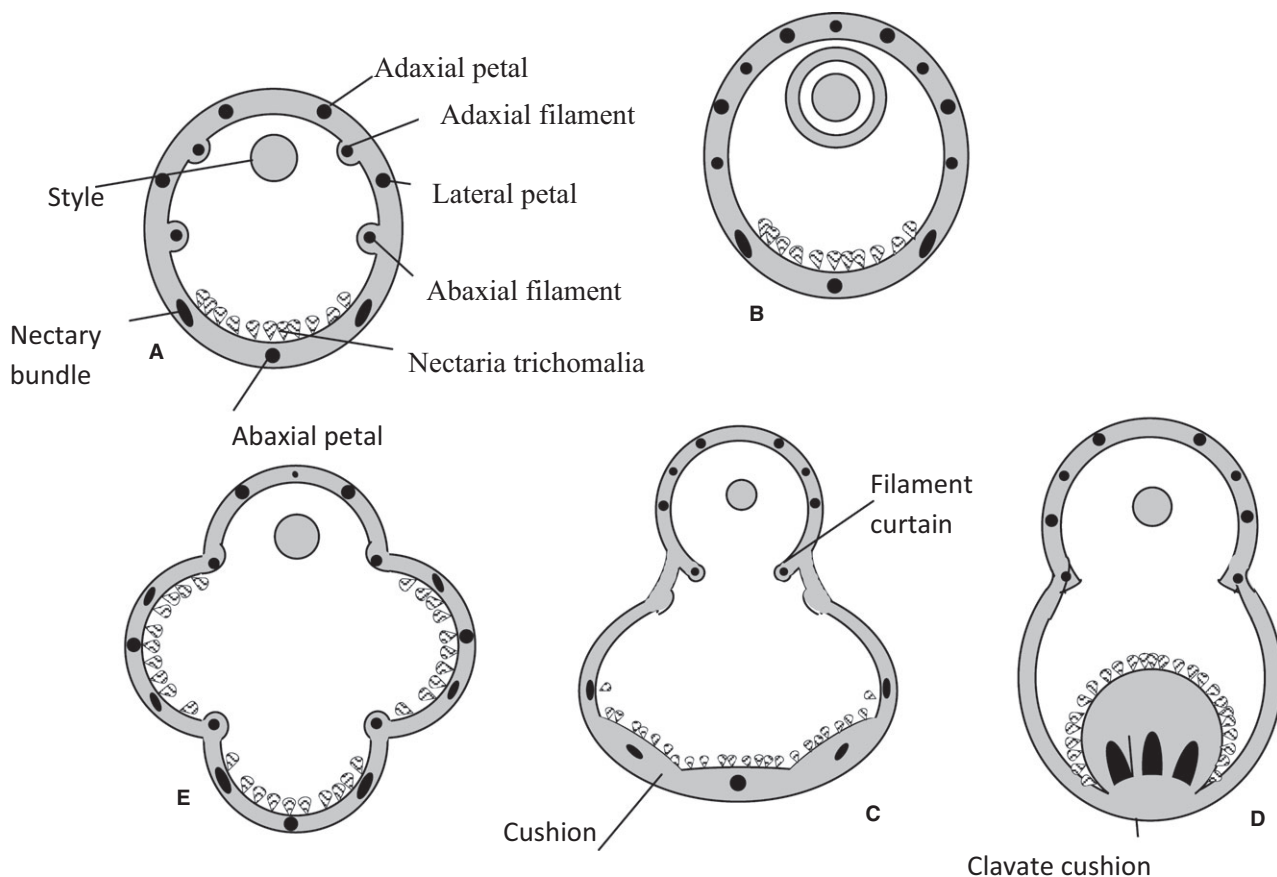


**Figure 4.** Diagrams of the stapet base and nectary in Linnaeeae and close relatives. A, Diagram with labels. B, *Lonicera* sect. *Caprifolium* with a nectary disc. C, *Abelia*, *Diabelia*, *Dipelta*, *Kolkwitzia*, gibbous with cushion base nectaria trichomalia, also note the filament curtain. D, *Diabelia spathulata* with a clavate cushion. E, *Vesalea floribunda* with abaxial, ventral and lateral nectaria trichomalia.
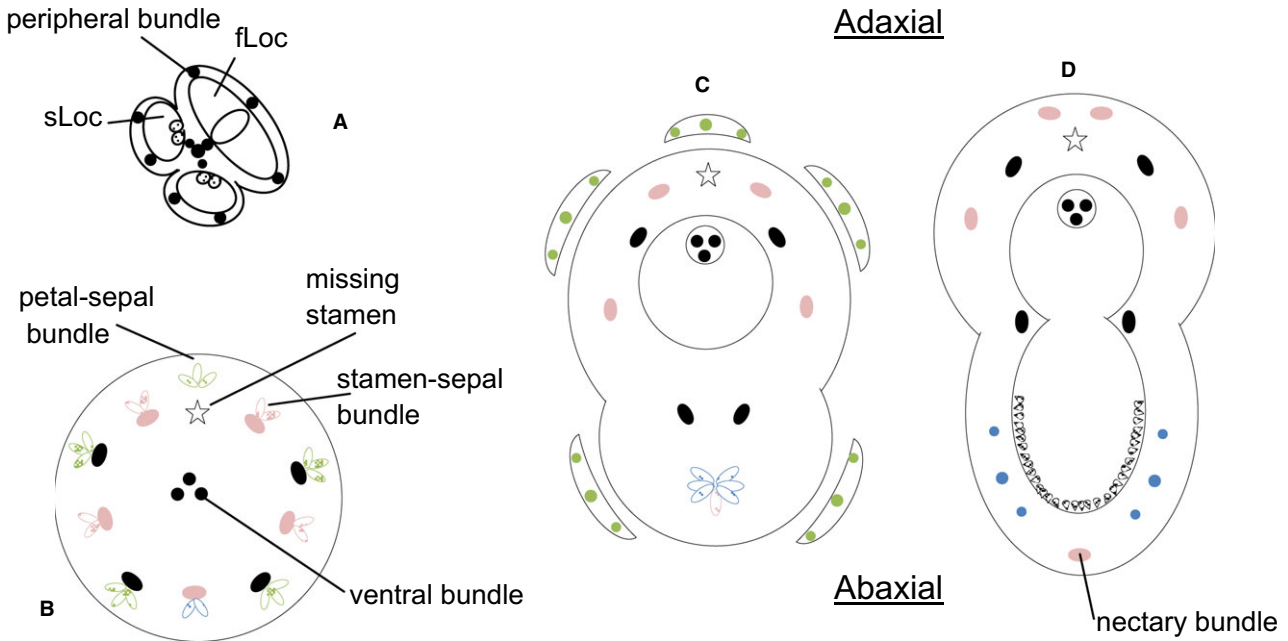
**Figure 5.** Vascular supply of the ovary, corolla and nectary. Schematic representation of four serial sections through: A, the ovary; B, the ovary sterile neck; C, the base of the corolla; D, the nectary. fLoc, fertile locule; sLoc, sterile locule.

The corolla mouth and lobes are almost regular with strong pink, red to yellow nectar marks and dense long hairs obstructing the mouth (Fig. 6A, B). The nectary is formed by dense unicellular glandular hairs located at the base of the stapet in between the abaxial filaments. The glandular part reaches one-third of the length of the stapet and is glabrescent (Fig. 6C, D). The glandular corolla part is not bulging and expands gradually as the stapet and corolla tube widen (Fig. 6C, E, F). Serial sections at the base of the stapet (Fig. 6F-1) show nine vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe. In the serial section 6F-3, the lower corolla lobe bundle branches into two short nectary traces, reaching the nectary parenchyma. The nectarial trichomes occupy the zone in between the abaxial filaments (Fig. 6F, G); sections through this zone show a well-developed parenchyma and vascular bundles (Fig. 6G). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands. The wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 6H).

### VESALEA FLORIBUNDA (FIG. 7)

The corolla of *V. floribunda* M.Martens & Galeotti is tubular, 2.5–4.5 cm long and red–purple (magenta or fuchsia). The stapet is straight in its lower third; it becomes slightly bent where the filaments become free and gradually widens towards the corolla tube and mouth (Fig. 7A). The flowers are pendent and grouped at the end of short branches. The calyx is large, spreading and later accrescent. The corolla mouth and lobes are almost regular with light yellow to white nectar marks. The corolla and the lobes are glabrous. The style and stamens are inserted and homogamous. The filaments are large, hairy and well developed in the lower part of the stapet and deep furrows are formed along them (Fig. 7G-3), creating distinct capillary chambers in which nectar can accumulate (Fig. 7G3–4). Serial sections at the base of the stapet (Fig. 7G1–2) show 16 vascular bundles corresponding to the lacking upper stamen (reduced bundle), upper corolla lobes, adaxial stamens, lateral nectary, lateral corolla lobes, abaxial stamens, abaxial nectary and the lower corolla lobe. Three capillary nectary chambers (two lateral and one abaxial) and the associated nectary bundles are discernible in serial section (Fig. 7G-3). Each nectary has three vascular bundles and a lacunar parenchyma (Fig. 7H). More distally, the filaments become free and the chambers are no longer present (Fig. 7G-5). The nectarial trichomes occupy three large zones in between the abaxial filaments, lower petal, the adaxial filaments and lateral petals. The nectary spreads along more than one-third of the corolla length and is densely covered
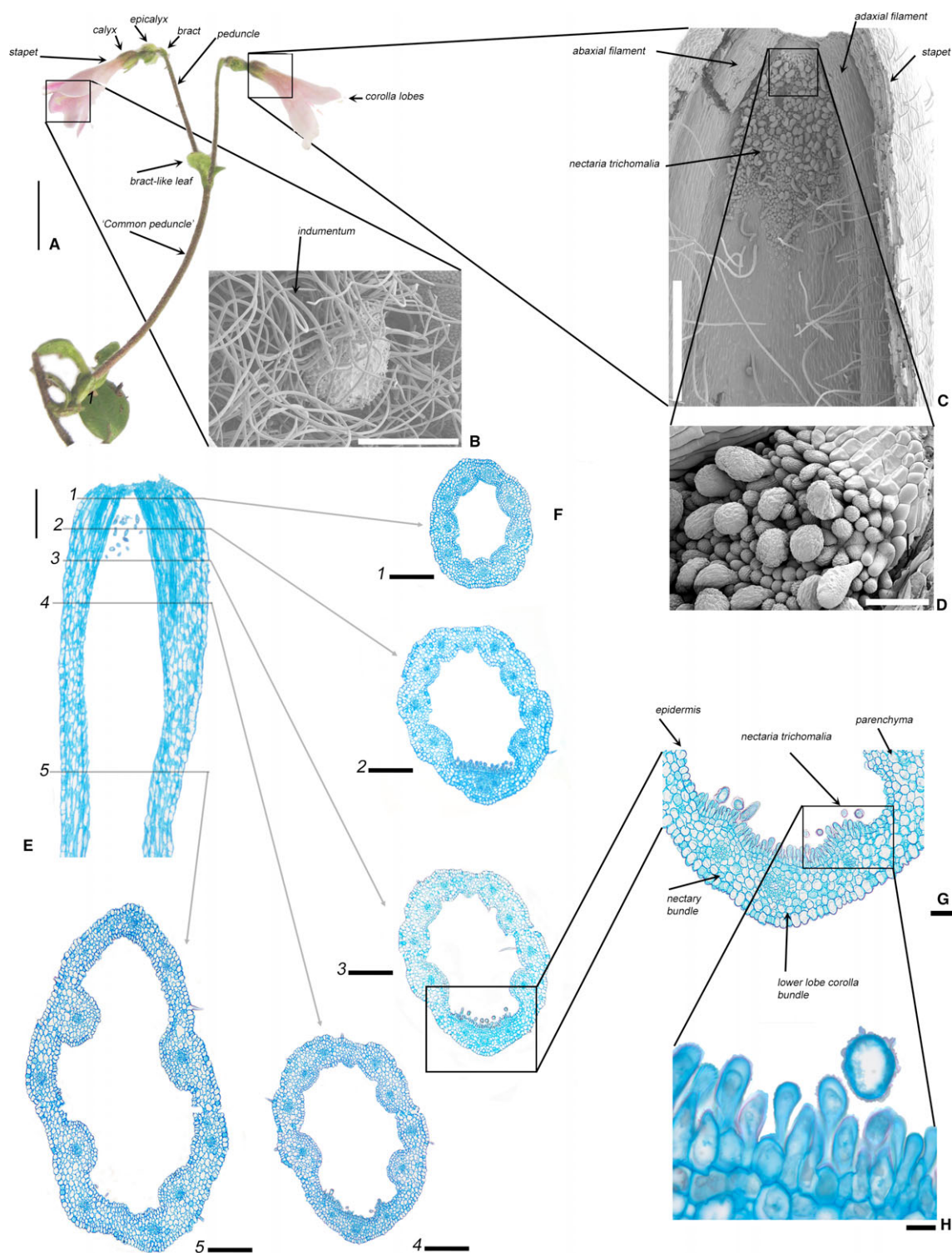
**Figure 6.** *Linnaea borealis*, zonate (one zone) nectary [scanning electron microscopy (SEM) and serial sections]. A, Inflorescence and flowers. B, Corolla mouth with dense indumentum and hidden stigma. C, Longitudinal section of the stapet showing the single zone of nectaria trichomalia in between the abaxial filaments in a ventral position. D, Nectaria trichomalia. E, Longitudinal section of the stapet. F, Five serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs. G, Transverse section of the ventral side of the stapet through the nectary. H, Detail of nectaria trichomalia. Scale bars: A, 6 mm; B, C, 500 μm; E, F, 200 μm; D, G, 50 μm; H, 10 μm.

with hairs (Fig. 7D–F). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands up to 100 μm long. The wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 7I).

### VESALEA CORIACEA GROUP (FIG. 8)

The *Vesalea coriacea* (Hemsl.) T.Kim & B.Sun ex Landrein group consists of four morphologically similar species: *V. mexicana* (Villarreal) H.F.Wang & Landrein, *V. coriacea*, *V. occidentalis* (Villarreal) H.F.Wang. & Landrein and *V. subcoriacea* (Villarreal) Landrein. The corolla in this group is infundibuliform-bilabiate, 10–30 mm long, purple–violet and fragrant. The shape and length of the narrow part of the stapet are variable, and this is used to delimit taxa in the group (Fig. 8A–G). The calyx is large, spreading and later accrescent. The corolla mouth and lobes are almost regular, glabrous and without nectar marks or with faint orange to yellow lines. The style and stamens can be inserted to exserted and are homogamous. The filaments are large and hairy at the base of the stapet (Fig. 8B, C). Serial sections at the base of the stapet (Fig. 8M1–2) reveal 11 vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens, nectary and the lower corolla lobe. In the serial section in Fig. 8M-4, the nectary zone in between the abaxial filaments is expanded and slightly saccate, but only in the upper part of the stapet. The nectary has three vascular bundles and a lacunar parenchyma (Fig. 8N, O). The nectary occupies a single large zone between the abaxial filaments. It extends for more than one-third of the corolla length and is densely covered with hairs (Fig. 8B–L). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands which are up to 50 μm long. The wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 8I).

### ABELIA FORRESTII VAR. FORRESTII (FIG. 9)

The corolla of *A. forrestii* (Diels) W.W.Smith var. *forrestii* is infundibuliform-bilabiate, 35–40 mm long, pinkish-white, fragrant and has a long narrow cylindrical tube, up to 2 mm in diameter, and the stapet is half as long as the corolla (Fig. 9A). The base of the stapet is slightly gibbous and saccate, and it

contains the nectary (Fig. 9B, C). A longitudinal section through the stapet (Fig. 9C) reveals that the nectary and lower part of the stapet are partitioned by the winged filament (filament curtain); the capillary tube is 0.3 mm wide and could act as nectar marks. The calyx is large, consisting of five sepals, spreading and later accrescent. The corolla mouth and lobes are almost regular, glabrous and without nectar marks. The style and stamens are inserted to slightly exserted and are homogamous. Serial sections at the base of the stapet (Fig. 9F1–3) show a conspicuous bulge and nectary cushion with three vascular bundles; the lower corolla lobe bundle and two short branches. The same sections show 11 vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens, nectary and the lower corolla lobe. In the serial sections Fig. 9F4–6, the nectary zone in between the abaxial filaments is expanded and saccate, but only in the lower part of the stapet. The filaments form a conspicuous partition with the stylar chamber. The nectary has three vascular bundles, a dense prenectary parenchyma and a lacunar parenchyma (Fig. 9G). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands, up to 60 μm long. The wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 9D, H).

### ABELIA UNIFLORA GROUP (FIG. 10)

The *A. uniflora* R.Br. group consists of three morphologically similar species: *A. schumannii* Rehder, *A. macrotera* and *A. uniflora*. The corolla in this group is bilabiate, 11–40 mm long and pink–purple–violet. The shape and length of the narrow part of the stapet and the corolla mouth are variable and are used to delimit taxa in the group (Fig. 10A–D). The calyx is made up of two lobes; one lobe corresponds to the fused adaxial and lateral sepals and the other corresponds to the two abaxial sepals. The nectary is opposite the two abaxial fused sepals. The corolla mouth and lobes are asymmetric and bilabiate with a conspicuous lip forming a landing platform. The corolla mouth has strong yellow to orange nectar marks as well as long hairs. The style and stamens are inserted to slightly exserted and are deflexed towards the upper corolla lobes. The filaments are protruding inside and hairy at the base of the stapet (Fig. 10E). Serial sections at the base of the stapet (Fig. 10G-1, I-1) show nine vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe, and traces from the lower
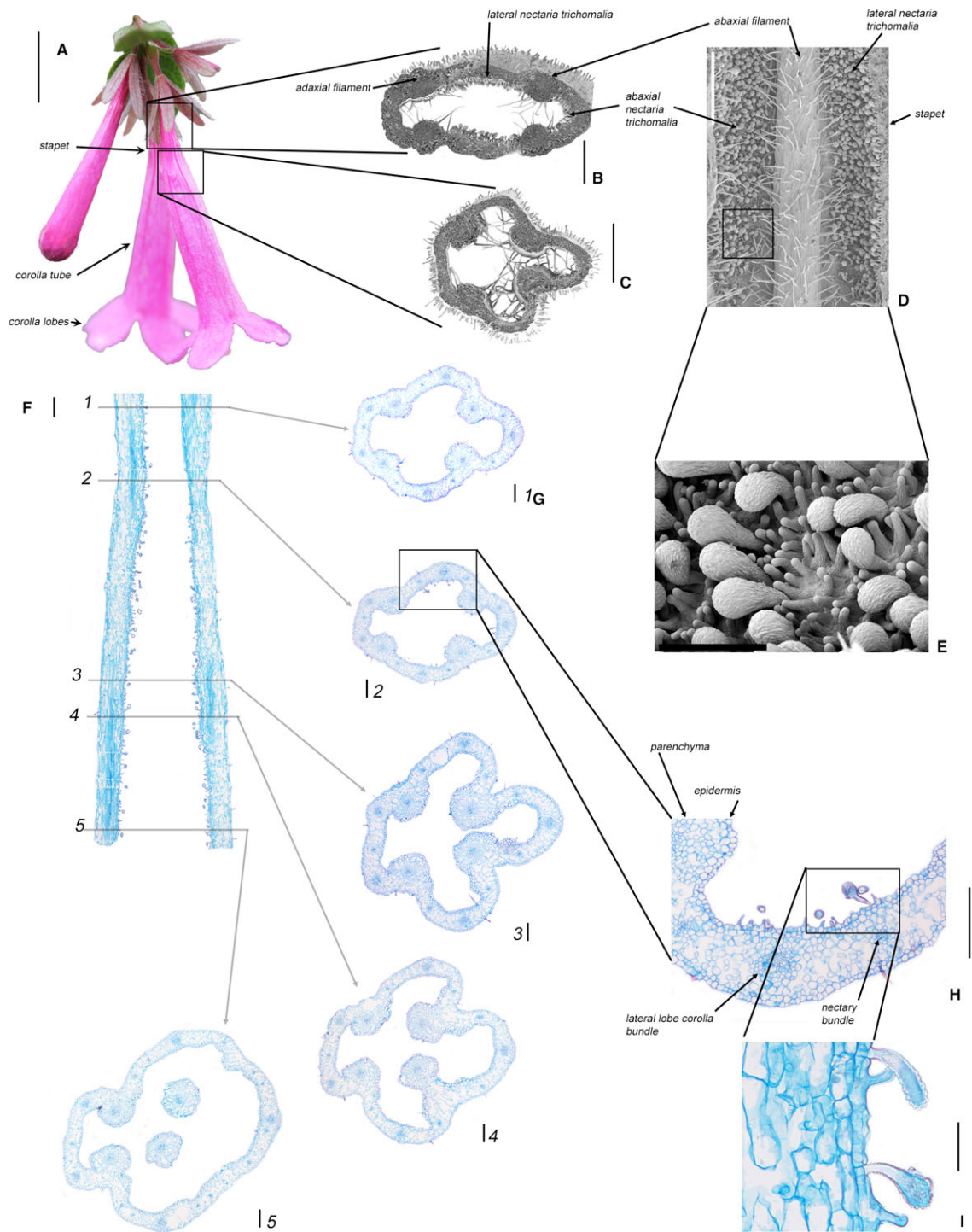
**Figure 7.** *Vesalea floribunda* var. *floribunda*, zonate (three zones) nectary [scanning electron microscopy (SEM) and serial sections]. A, Flowers. B, C, Two transverse sections of the stapet showing three zones of nectaria trichomalia in between the abaxial and adaxial filaments in ventral and lateral positions. D, Longitudinal section of the stapet showing nectaria trichomalia. E, Nectaria trichomalia. F, Longitudinal section of the stapet. G, Five serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs. H, Transverse section of the ventral side of the stapet through the nectary. I, Detail of nectaria trichomalia. Scale bars: A, 1 cm; B–D, 500 μm; F–H, 200 μm; E, 100 μm; I, 50 μm.

corolla lobe bundle to the nectary cushion. All serial sections show a conspicuous bulge and nectary cushion with up to 13 nectary bundles. The bulge is restricted to the basal part of the stapet and does not exceed 1 mm. In *A. macrotera* var. *deutziaefolia* (Lév.) Landrein, the bulge expands outwards and forces the opposite abaxial calyx lobe to spread further. In the same variety, there is no narrow zone between the nectary cushion and the abaxial filaments; this variety also has a more conspicuous clavate cushion protruding in between the abaxial filaments (Fig. 10I1–4). The filaments form a conspicuous partition with the stylar chamber. The nectary has a dense parenchyma (Fig. 10J, K). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands which are up to 80 μm long. The wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 10F, L).

### ABELIA CHINENSIS (FIG. 11)

The corolla of *A. chinensis* is infundibuliform-campanulate, 7–11 mm long, white and fragrant (Fig. 11A). The base of the stapet is slightly gibbous and saccate, and contains a small nectary (Fig. 11B). The calyx is large, made up of five spreading sepals, later accrescent. The corolla mouth and lobes are almost regular, glabrous and without nectar marks. The style and stamens are long exserted and the anthers are versatile. Serial sections at the base of the stapet (Fig. 11E1–2) show nine vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe, and traces from the lower corolla lobe bundle to the nectary cushion. All serial sections show a bulge and nectary cushion with up to three nectary bundles. The bulge is restricted to the basal part of the stapet and does not exceed 2 mm (Fig. 11D). In the lower part of the stapet, the filaments form a conspicuous partition with the stylar chamber. The nectary has a dense parenchyma

(Fig. 11F). The nectary is similar to the nectary of *A. uniflora*, but the bulge and cushion are not as well developed and the glandular zone extends further into the stapet. The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands which are up to 100 μm long. The cell wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 11G).

### DIABELIA (FIG. 12)

*Diabelia* comprises four species that differ in their nectary morphology: *D. serrata*, *D. spathulata*, *D. stenophylla* (Honda) Landrein and *D. sanguinea* (Makino) Landrein (Fig. 12A, 12). The corolla in this group is bilabiate and 10–45 mm long. Although the flowers display a similar morphology to those of the related genera *Abelia*, *Dipelta* Maxim. and *Kolkwitzia* Graebn., the flower colour is much more variable from white, purple–pink to yellow and red. The calyx is made up of two to five lobes, which is a diagnostic character for the differentiation of the species. The corolla mouth and lobes are asymmetric and bilabiate with a conspicuous lip forming a landing platform. The corolla mouth has strong yellow to orange nectar marks and long hairs. The style and stamens are inserted to slightly exserted and deflexed towards the upper corolla lobes. The filaments are fused to the corolla at the base and do not form a partition between the stylar chamber and nectary chamber (Fig. 12E, F). Serial sections at the base of the stapet (Fig. 12E-1, F-1) show nine vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe, as well as traces from the lower corolla lobe bundle to the nectary. All serial sections show a small bulge and nectary cushion with up to five nectary bundles. The bulge is restricted to the basal part of the stapet and does not exceed 1 mm in *D. serrata* and 0.3 mm in *D. spathulata*. *Diabelia spathulata* is distinct from

**Figure 8.** *Vesalea coriacea* group of species, zonate (one zone) nectary [scanning electron microscopy (SEM) and serial sections]. A–C, *Vesalea mexicana* var. *grandifolia* flowers, longitudinal and transverse section of the stapet showing a single zone of nectaria trichomalia in between the abaxial filaments. D, *Vesalea coriacea* flowers. E, F, *Vesalea occidentalis* flowers and transverse section of the stapet showing a single zone of nectaria trichomalia in between the abaxial filaments. G–K, Flowers of *Vesalea subcoriacea* var. *subcoriacea*, longitudinal and transverse section of the stapet showing a single zone of nectaria trichomalia in between the abaxial filaments. L–O, *Vesalea mexicana* var. *grandifolia*. L, Longitudinal section of the stapet. M, Four transverse sections through the base of the stapet showing the occurrence of the nectary hairs. N, Transverse section of the ventral side of the stapet through the nectary. O, Details of nectaria trichomalia. Scale bars: A, D, E, G, 8 mm; B, 2 mm; H, 1 mm; C, F, I, 500 μm; J, 300 μm; L–N, 200 μm; K, O, 50 μm.
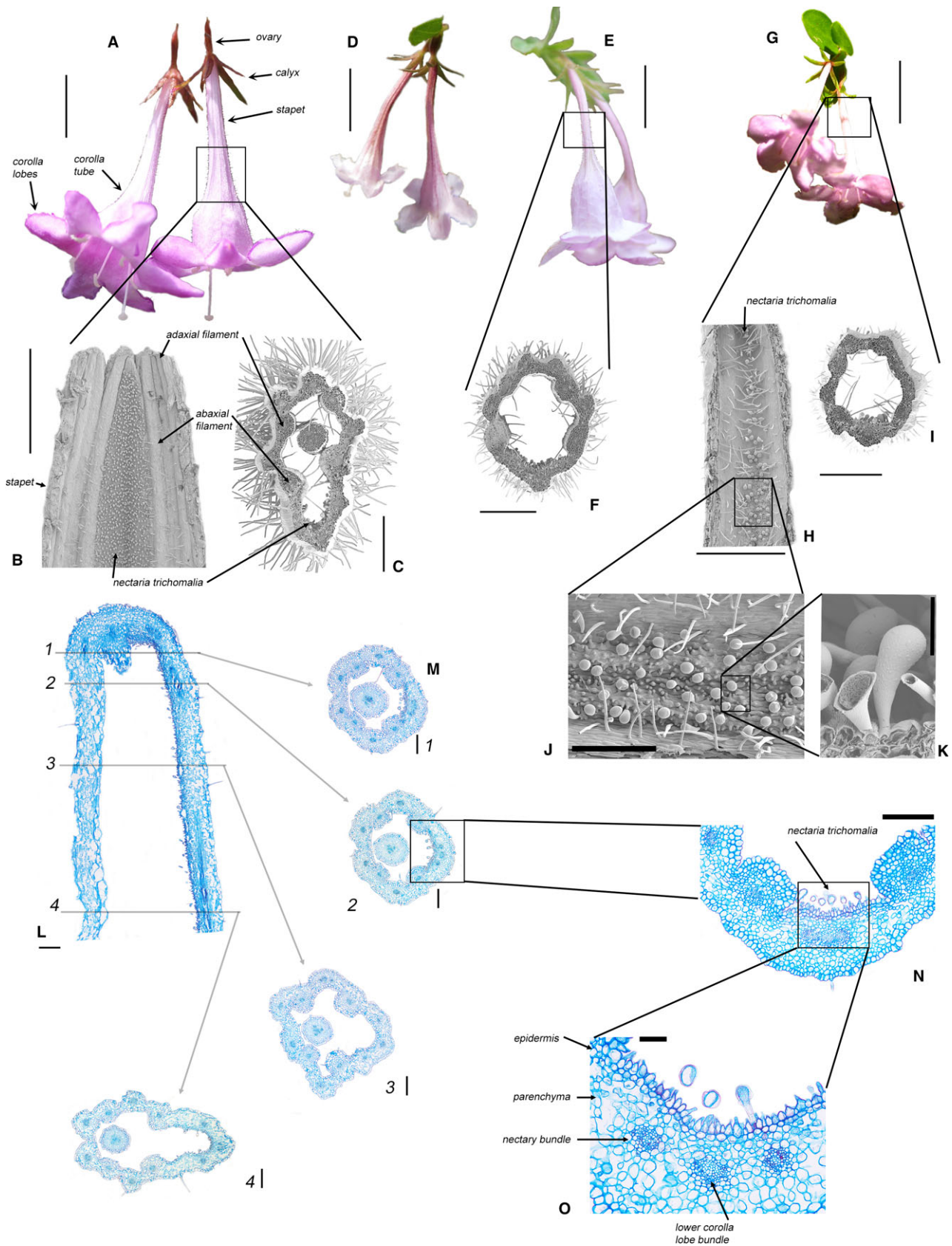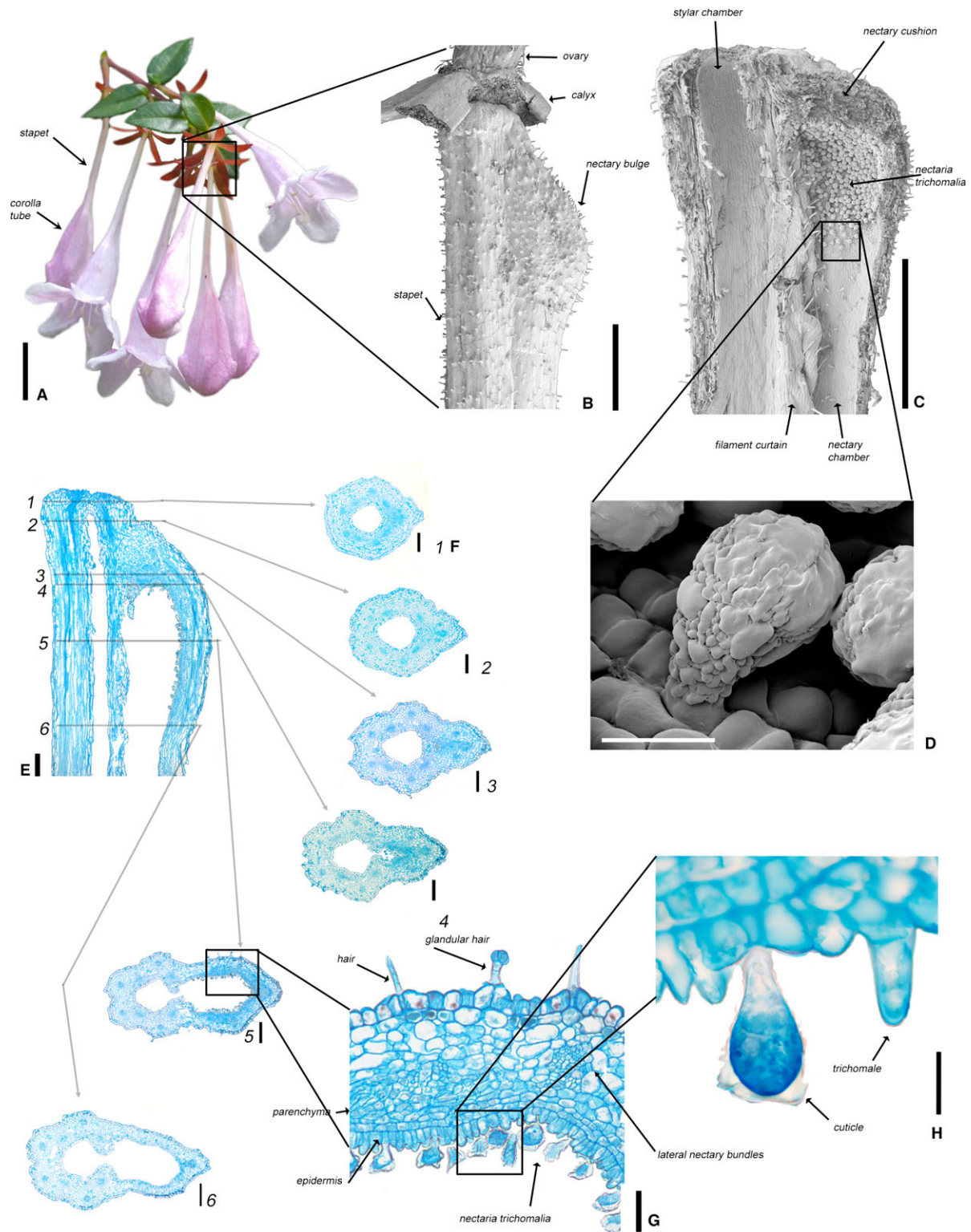
**Figure 9.** *Abelia forrestii var. forrestii*, gibbous nectary [scanning electron microscopy (SEM) and serial sections]. A, Flowers. B, C, Gibbous base and longitudinal section of the stapet. D, Nectaria trichomalia. E, Longitudinal section of the stapet. F, Six serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs. G, Transverse section of the ventral side of the stapet through the nectary. H, Detail of nectaria trichomalia. Scale bars: A, 1 cm; B, C, 1 mm; E, F, 200 μm; G, 50 μm; D, H, 20 μm.

the rest of the tribe because it forms a conspicuous clavate nectary cushion in the abaxial position (Fig. 12F2–3, G, H). This cushion can be up to 0.4 mm long and has a vascular bundle which branches from the lower corolla lobe. The nectariferous zone is restricted to the cushion in *D. spathulata*, but extends onto the stapet in *D. serrata*. The nectary has a dense parenchyma (Fig. 12H, I). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands which are up to 60 μm long. The wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 12C, I).

### DIPELTA AND KOLKWITZIA (FIG. 13)

*Dipelta* comprises four species (*D. elegans* Batalin, *D. floribunda* Maxim., *D. ventricosa* Hemsl. and *D. yunnanensis* Franch.) and *Kolkwitzia* is monospecific. The corolla in this group is bilabiate, fragrant, purple–pink and 15–35 mm long (Fig. 13A–C, F). The stapet is variable and a useful diagnostic character to differentiate species, which may also be linked to pollination syndromes. The tube is narrow and cylindrical in *D. elegans* (Fig. 13B), but extremely short in *D. yunnanensis* (Fig. 13C). The calyx is distinct and has three different morphologies: reduced and with free lobes in *D. floribunda*; fused at the base with lobes recurved and incised adaxially leaving a basal gap in which the nectary is inserted in *D. yunnanensis*; and fused and cupular in *D. elegans*. Most species, except *D. elegans*, have a large nectary bulge that extends beyond the base of the stapet and protrudes in between the abaxial sepals. *Dipelta elegans* is the only species in Linnaeeae that has fused sepals, and this also explains why the nectary is not as developed as in other species because of the lack of available space in the calyx (Fig. 13B). The corolla mouth and lobes are zygomorphic and bilabiate with a conspicuous lip forming a landing platform. The corolla mouth has strong yellow to orange nectar marks as well as long hairs. The style and stamens are inserted to slightly exserted and are deflexed towards the upper corolla lobes. The filaments are fused to the corolla at the base and form a partition between the stylar chamber and nectary chamber (Fig. 13H). Serial sections at the base of the stapet (Fig. 13H-2) show nine vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe, and traces from the lower corolla lobe bundle to the nectary cushion. All serial sections show a large bulge and nectary cushion with up to 15 nectary bundles. The bulge is restricted to the

basal part of the stapet and does not exceed 1.5 mm in length, but extends laterally and can be up to 6 mm wide (Fig. 13G). The nectary has a dense parenchyma (Fig. 13I–L). The abaxial filaments are more developed than the adaxial ones and later become didynamous. The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands up to 60 μm long. The cell wall and cuticle of the hairs are irregular and create a space in which nectar accumulates prior to secretion (Fig. 13L).

### ZABELIA TRIFLORA GROUP (FIG. 14)

*Zabelia* is a genus of four to eight species which can be divided into two groups: the *Z. triflora* (R.Br.) Makino group with *Z. triflora*, *Z. corymbosa* (Regel & Schmalh.) Makino and *Z. tyaihyoni* (Nakai) Hisauti & Hara; and the *Z. biflora* group consisting of *Z. integrifolia* (Koidz.) Makino ex Ikuse & Kurosawa, *Z. coreana* (Nakai) Hisauti & Hara and *Z. dielsii* (Graebn.) Makino. The groups are mainly separated on the basis of their inflorescence morphology, but their flowers are similar. The corolla in the *Z. triflora* group is hypocrateriform, strongly fragrant, white to pinkish-white and 10–15 mm long (Fig. 14A–C). The corolla lobes are spreading and regular. The calyx is similar to the calyx in Linnaeeae and varies from four lobes in *Z. corymbosa* to five in the rest of the species. The style and stamens are inserted to slightly exserted and the stigma is conspicuously green and mucilaginous. The base of the style is thickened with a distinct ring of thick-walled cells which could correspond to a vestigial nectary disc (Fig. 14H). This was also observed in *Lonicera*, which has species with a nectary disc and species lacking a disc, but with a vestigial thickening at the base of the style. The filaments are fused with the corolla at the base, but do not form a partition between the stylar chamber and nectary chamber (Fig. 14H). Serial sections at the base of the stapet (Fig. 14H-1) reveal nine vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe. The nectary is present between the abaxial filaments as well as between the adaxial filaments, and extends from the base of the tube to more than 6 mm or half the length of the corolla (Fig. 14G). The lower corolla lobe bundle and the lateral corolla lobes branch slightly towards the nectary (Fig. 14I). The nectary has a dense lacunar parenchyma and a dense prenectary parenchyma (Fig. 14I). The prenectary parenchyma is stained red around the vascular bundles (tannins). The epidermis in the glandular zone is papillate with a ridged cuticle, and some cells expand to form large clavate glands which

are up to 60 μm long. The cell wall and cuticle of the hairs are smooth at the base, but irregular in the apical part, and create a space in which nectar accumulates prior to secretion (Fig. 14E, F).

### Zabelia biflora group (Fig. 15)

The corolla in the *Z. biflora* group is hypocrateriform, strongly fragrant, white to pinkish-white and 10–15 mm long (Fig. 15A–C). The corolla lobes are reflexed and regular. The calyx is similar to the calyx in Linnaeeae and is tetramerous. The style and stamens are inserted to slightly exserted and the stigma is conspicuously green and mucilaginous. The base of the style is thickened with a distinct ring of thick-walled cells which could correspond to a vestigial nectary disc (Fig. 15I2, J). The filaments are fused to the corolla at the base, but do not form a partition between the stylar chamber and nectary chamber (Fig. 15G–I). Serial sections at the base of the stapet (Fig. 15I-1) show eight vascular bundles corresponding to the upper corolla lobe, adaxial stamens, lateral corolla lobes, abaxial stamens and the lower corolla lobe. The second upper corolla lobe is lacking and the corolla is also tetramerous. A conspicuous bulge is also present, although this is not located at the base of the stapet, but in its middle part (Fig. 15G–I). The nectary is present in three to four zones; in between the abaxial filaments, the adaxial filaments and on the upper corolla lobe (Fig. 15I-3). It extends from the base of the tube to more than 6 mm or half the length of the corolla (Fig. 15G–I). The vasculature of the lower corolla lobe as well as the lateral corolla lobes is conspicuously branched towards the nectary, which forms three protruding cushions around the bundles (Fig. 15G, I-4). The lateral and adaxial nectaries are missing in *Z. integrifolia* and *Z. coreana*, but present in *Z. dielsii* and may represent a diagnostic character. The nectary has a dense lacunar parenchyma and a dense prenectary parenchyma (Fig. 15K, L). The prenectary parenchyma is stained red around the vascular bundles (tannins). The epidermis in the glandular zone is papillate with a ridged cuticle, and some cells expand to form large clavate glands which are to 70 μm long. The cell wall and cuticle of the hairs are smooth at the base, but irregular in the apical part, and create a space in which nectar accumulates prior to secretion (Fig. 15F, M).

### Related taxa in Dipsacales (Fig. 16)

The corolla of *Lonicera tragophylla* Hemsl. ex F.B.Forbes & Hemsl. is tubular-bilabiate, fragrant, yellow and 40–70 mm long (Fig. 16A). The corolla lobes are strongly bilabiate and recurved, leaving the stamens and style exserted. The stigma is green and mucilaginous, as in *Zabelia* and *Heptacodium* Rehder. The calyx is reduced to a rim and pentamerous. The base of the style is surrounded by a nectary disc which is apically free from the style (Figs 16J, 17J2–3). This nectary disc has not been recorded in previous studies and seems to occur only in *Lonicera* subgenus *Periclymenum* Rehder. Secretion of this nectary disc has not been studied here and needs to be investigated. The filaments are fused to the base of the corolla where, together, they form the stapet, but the filaments do not form a partition between the stylar chamber and nectary chamber (Fig. 16J). Serial sections at the base of the stapet (Fig. 16J1–2) show ten vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, lateral stamens, abaxial stamen and the lower corolla lobe. A bulge is also present, although this is located in the middle part of the stapet, rather than at its base (Fig. 16J). The nectary is present in a single area in between the abaxial filaments (Fig. 16J-4). It extends from 1.5 mm above the base of the tube to > 6 mm above the base (Fig. 16J). The lower corolla lobe bundle is conspicuously branched towards the nectary and forms three protruding cushions around the bundles (Fig. 16J4, K). The nectary has a lacunar parenchyma and a dense prenectary parenchyma (Figs 16K, 17L). The epidermis in the glandular zone is papillate and some cells expand to form large clavate glands which are up to 100 μm

**Figure 10.** *Abelia uniflora* group, gibbous nectary [scanning electron microscopy (SEM) and serial sections]. A, Flowers of *Abelia schumanii*. B, Flowers of *Abelia macrotera* var. *macrotera*. C, Flowers of *Abelia macrotera* var. *deutziaefolia*. D–F, Flowers of *Abelia macrotera* var. *mairei*. E, Longitudinal section of the gibbous part of the stapet. F, Nectaria trichomalia. G, *Abelia schumanii*, longitudinal and five serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs. H, *Abelia macrotera* var. *macrotera*, longitudinal and five serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs. I–L, *Abelia macrotera* var. *deutziaefolia*. I, Longitudinal and four serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs and cushion. J, Longitudinal section of the stapet showing clavate nectary cushion. K, Transverse section of the ventral side of the stapet through the nectary, L. Detail of nectaria trichomalia. Scale bars: A–D, 1 cm; E, 1 mm; G–K, 200 μm; F, 50 μm; L, 20 μm.
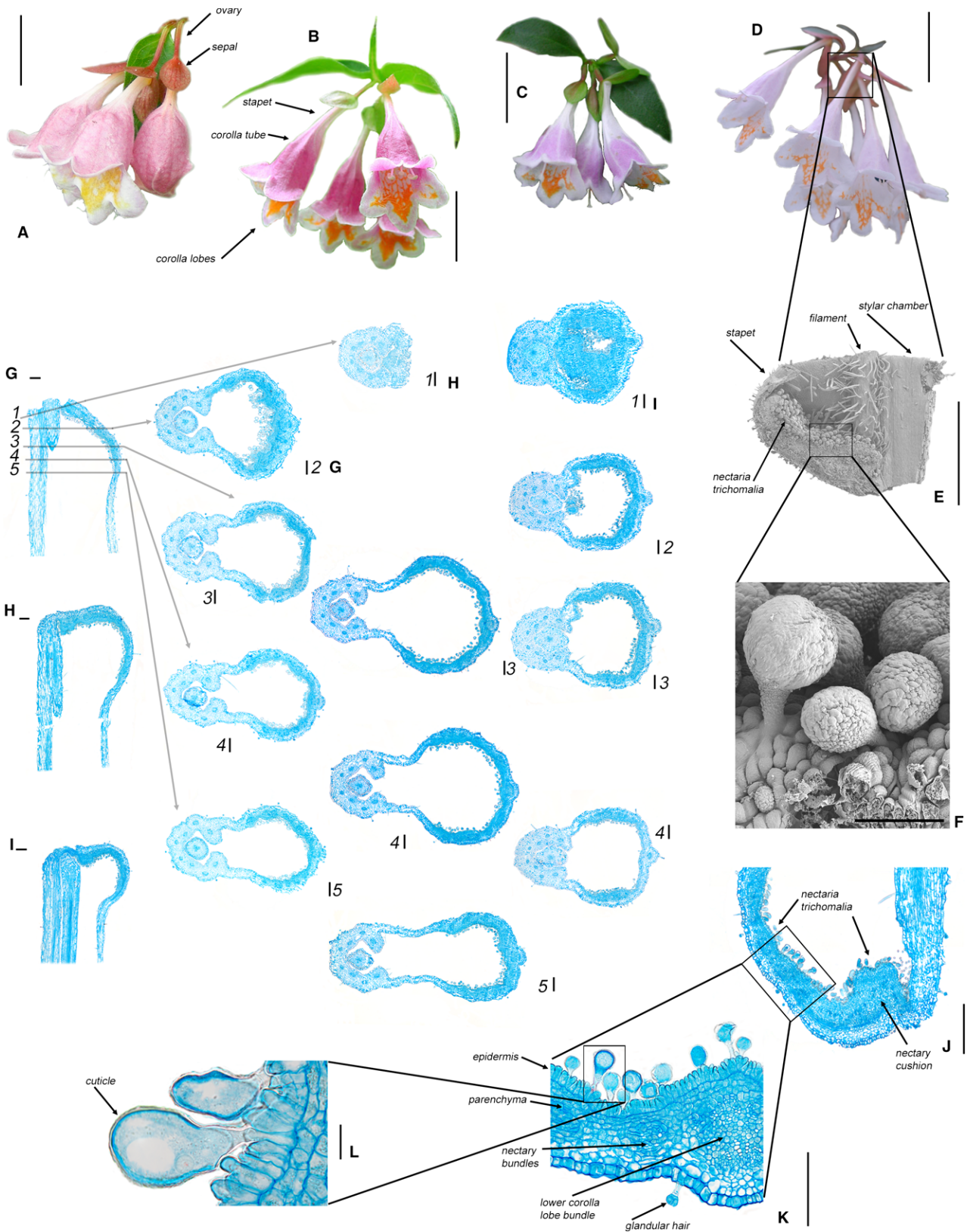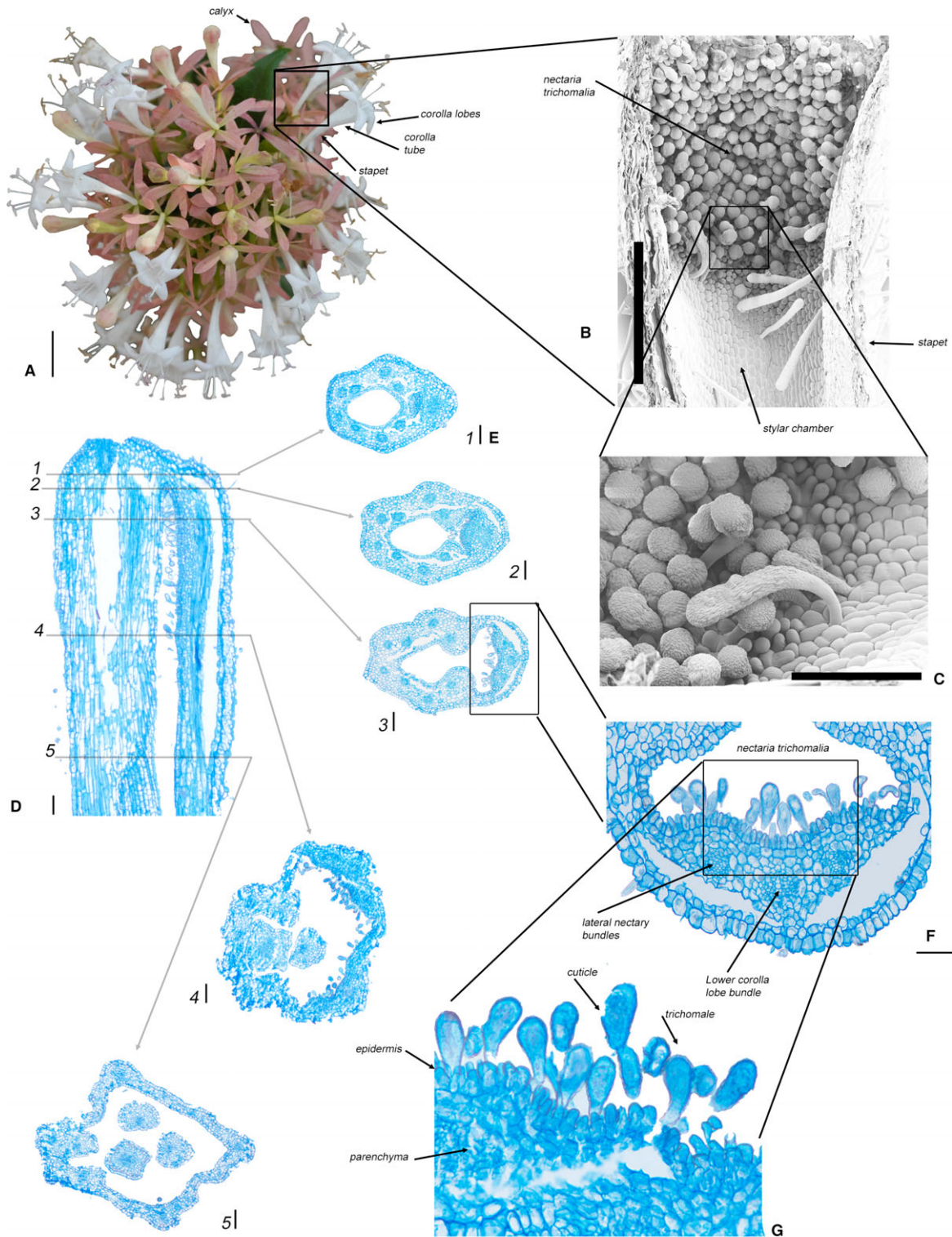
**Figure 11.** *Abelia chinensis* var. *chinensis*, flowers and gibbous nectary [scanning electron microscopy (SEM) and serial sections]. A, Inflorescence and flowers. B, Longitudinal section of the gibbous part of the stapet showing the nectaria trichomalia. C, Nectaria trichomalia. D, Longitudinal section of the stapet. E, Five serial transverse sections through the stapet showing the occurrence of the nectary hairs. F, Transverse section of the ventral side of the stapet through the nectary; G, Nectaria trichomalia. Scale bars: A, 1 cm; B, 300 μm; D, E, 200 μm; C, 100 μm; F, G, 50 μm.

long. The cell wall and cuticle of the hairs are irregular in the apical part and create a space in which nectar accumulates prior to secretion (Fig. 16C).

The corolla in *Heptacodium miconioides* Rehder is hypocrateriform, fragrant, white and 10–15 mm long (Fig. 16D). The corolla lobes are regular and spreading. The stamens and stigmas are exserted and, as in *Zabelia* and *Lonicera*, the stigma is green and mucilaginous. The calyx is well developed, pentamerous and similar to the calyx in *Abelia*. The filaments are fused to the corolla at the base, but do not form a partition between the stylar chamber and nectary chamber (Fig. 16I). Serial sections at the base of the stapet (Fig. 16I-1) show ten vascular bundles corresponding to the upper corolla lobes, adaxial stamens, lateral corolla lobes, lateral stamens, abaxial stamen and the lower corolla lobe. The nectary is present in a single zone in between the abaxial filaments (Fig. 16I2–3) and is localized in a small area extending 0.5 mm from the base of the tube to 1.5 mm from the base (Fig. 16I). The lower corolla lobe bundle and the lateral corolla lobes branch slightly towards the nectary (Fig. 16I-2). The epidermis in the glandular zone is papillate with a ridged cuticle, and some cells expand to form large glands which are up to 70 μm long.

The corolla in *A. moschatellina* is isomerous (tetramerous in terminal, pentamerous in lateral flowers) and regular with petals only fused at the base (Fig. 16E). About 15–30 multicellular hairs form a cushion-like nectary at the base of the adaxial side of each corolla lobe and in between the paired thecae (Fig. 16F, G). The calyx is di- to tetramerous and reduced. The nectaries are not accompanied by an underlying nectary tissue, but have an underlying vascular petal bundle (Fig. 16M, N). It should be noted that Roels & Smets (1996) and Erbar (2014) observed a vascular bundle below the nectary. The multicellular hairs are clavate, have 10–20 cells and are up to 100 μm long.

## ULTRASTRUCTURE

### *Linnaea borealis* (Fig. 17)

At the earliest developmental stage, the epidermis cells have a thin cuticle, a dense cytoplasm rich in ribosomes and few mitochondria/plastids. The vacuoles at this stage are relatively small and the nucleus is large with a conspicuous nucleolus (Fig. 17E).

Figure 17B represents the stage at which the secretory cell has reached its definitive shape. The ribbed cuticle is completely developed and consists of a network of thick ribs and thin areas (Fig. 17C). When the cuticle withdraws from the cell wall, it creates a subcuticular space filled with nectar and lipid droplets

(Fig. 17B). At this stage, an inner layer of wall protuberances is well developed. In addition to a large number of vesicles, mitochondria and plastids, ER and rough lamellar ER can be observed. This stage is also characterized by the appearance of many coated vesicles budding off cisternae and becoming attached to the plasmalemma (Fig. 17C, D, G, H). There are plasmodesmata connecting neighbouring nectar parenchyma cells (Fig. 17F). Lipid droplets can be seen in the subcuticular space (Fig. 17B, H). The plastids are large and can take various shapes, often having round plastoglobuli in the interior (Fig. 17B, C). After secretion stops, the vacuole increases in size until it occupies most of the cell and starch grains no longer appear in the plastids (Fig. 17A).

### *Abelia macrotera* (Fig. 18)

At the earliest developmental stage, the epidermis cells have a thin cuticle, a dense cytoplasm rich in ribosomes and few mitochondria/plastids. The vacuoles are relatively small and the nucleus is large (Fig. 18B). Figure 18A represents the stage at which the secretory cell has reached its definitive shape. The ribbed cuticle is completely developed and consists of a network of thick ribs and thin areas (Fig. 18C). When the cuticle withdraws from the cell wall, it creates a subcuticular space (Fig. 18A). By this stage, an inner layer of wall protuberances is well developed. In addition to a large number of vesicles, mitochondria and plastids, rough lamellar ER can be observed. This stage is also characterized by the appearance of many coated vesicles budding off cisternae and becoming attached to the plasmalemma (Fig. 18C, D). There are plasmodesmata connecting neighbouring nectar parenchyma cells (Fig. 18F). The plastids are large and have various shapes, and starch grains are prevalent in the interior of the plastids (Fig. 18E). After secretion stops, the vacuole increases in size until it occupies most of the cell and starch grains no longer appear in the plastids (Fig. 18A).

### *Zabelia coreana* (Fig. 19)

At the earliest development stage, the epidermis cells have a thin cuticle, a dense cytoplasm rich in ribosomes and few mitochondria/plastids. The vacuoles at this stage are relatively small and the nucleus is large (Fig. 19B). The nectary parenchyma cells contain a single large vacuole occupying most of the cell and filled with tannins (Fig. 19A, C).

Figure 19F represents the stage at which the secretory cell has reached its definitive shape. The ribbed cuticle is completely developed and consists of a network of thick ribs and very thin areas. When

the cuticle withdraws from the wall, it creates a sub-cuticular space filled with nectar and lipid droplets (Fig. 19F). By this stage, an inner layer of wall pro-tuberances is well developed. In addition to a large number of vesicles, mitochondria and plastids, rough lamellar ER can be observed (Fig. 19E). This stage is also characterized by the appearance of many coated vesicles budding off cisternae and becoming attached to the plasmalemma (Fig. 19I, J). Lipid droplets can be seen in the subcuticular space (Fig. 19F, H). The plastids are large and can take various shapes, often with round plastoglobuli (Fig. 19I).

### ADOXA MOSCHATELLINA (FIG. 20)

The multicellular, capitate trichome shows ten cells in cross-section (Fig. 20A). The cell wall of the cen-tral cell or stalk is rich in plasmodesmata and mito-chondria (Fig. 20B, C). The tip cells have a thin cuticle and a large number of vesicles, mitochondria and rough lamellar ER; an inner layer of wall protu-berances is well developed (Fig. 20D–F). The subcu-ticular space is not filled with nectar and is attached to the wall (Fig. 20G). The surrounding petal cells have a thicker cuticle and consist of a network of thick ribs (Fig. 20F).

## DISCUSSION

### NECTARIES AND PHYLOGENETICS OF LINNAEEAE

The nectaries in Dipsacales consist of a vascularized parenchyma with trichomes through which the nec-tar is secreted and which are located on the stapet. However, there are notable exceptions in Adoxaceae: nectaries are absent in *Sambucus* and secretion is in the region of the stigmas; in *Adoxa*, nectaries are multicellular hairs on the petals and *Viburnum* shows a typical nectary disc. *Heptacodium* has inter-mediate morphological characters between Linnaeeae and Caprifolieae and, especially, *Lonicera* subgenus *Periclymenum* (Pyck & Smets, 2000; Zhang, Zhou & Gu, 2002; Landrein & Prenner, 2013). *Adoxa* is the only example with multicellular glandular hairs (Sturm, 1910).

### NECTARY MORPHOLOGY

The nectary and inflorescence are important morpho-logical characters which have been utilized to under-stand and study the phylogeny of Linnaeeae (Fukuoka, 1968; Landrein & Prenner, 2013). Phylo-genetics based on molecular markers have redefined circumscriptions in Linnaeeae (Landrein *et al.*, 2012; Wang *et al.*, 2015), and it is also important to char-acterize these new groups morphologically (Endress & Matthews, 2012).

Two types of nectary morphology exist, classified after Fukuoka (1968). Gibbous nectaries form an external bulge, glandular hairs are tightly packed and the nectary is inserted on a cushion at the base and on the ventral side of the stapet. Zonate nec-taries have spreading glandular hairs in between the abaxial filaments, but also in between the adaxial and abaxial filaments.

The gibbous nectary seems to be a synapomorphic character shared by the clade including *Abelia*, *Dia-belia*, *Dipelta* and *Kolkwitzia* (Fig. 1, Table 1). The zonate nectary seems to be a plesiomorphic character found in *Heptacodium* and *Zabelia* and *Linnaea* and *Vesalea* in the Linnaeeae clade (Fig. 1, Table 1). The gibbous nectary is associated with strongly bilabiate, unscented corollas with a landing platform and nec-tar marks. However, *Abelia chinensis* (Fig. 11) has an infundibuliform fragrant corolla without nectar marks, and its nectary is notably smaller than the bulging nectaries found in *A. macrotera* (Fig. 10) with unscented, bilabiate corollas. The nectaries in *Dipelta* and *Kolkwitzia* are almost saccate (Fig. 13) and are the largest in the tribe; the flowers are also slightly fragrant. In *Zabelia coreana*, the zonate nec-tary becomes inflated above the base of the corolla and a bulge with three conspicuous vascular bundles is formed, but this is located above and not at the base of the stapet (Fig. 15).

The zonate nectary is a shared character between *Linnaea* and *Vesalea*. This is in accordance with recent phylogenetic results, which show that *Lin-naea* and *Vesalea* form a clade (Fig. 1) (Wang *et al.*, 2015). If we consider the size of the corolla in rela-tion to the size of the glandular zone, the nectary of *L. borealis* is shorter than that in the *Vesalea cori-*

**Figure 12.** *Diabelia*, flowers and gibbous nectary [scanning electron microscopy (SEM) and serial sections]. A–C, *Dia-belia spathulata* var. *spathulata*. A, Flowers. B, Longitudinal section of the gibbous part of the stapet. C, Nectaria tri-chomalia. D, *Diabelia serrata* var. *serrata*. E, *Diabelia serrata* var. *serrata*, longitudinal and four serial transverse sections through the base of the stapet showing the occurrence of the nectary hairs. F, *Diabelia spathulata* var. *spathu-lata*, longitudinal and five serial transverse sections through the stapet showing the occurrence of the nectary hairs. G–I, *Diabelia spathulata* var. *spathulata*. G, Longitudinal section of the stapet showing the clavate nectary cushion. H, Transverse section of the stapet showing the clavate nectary cushion. I, Nectaria trichomalia. Scale bars: A, D, 1 cm; B, 500 μm; E, F, 200 μm; G, H, 100 μm; C, I, 50 μm.
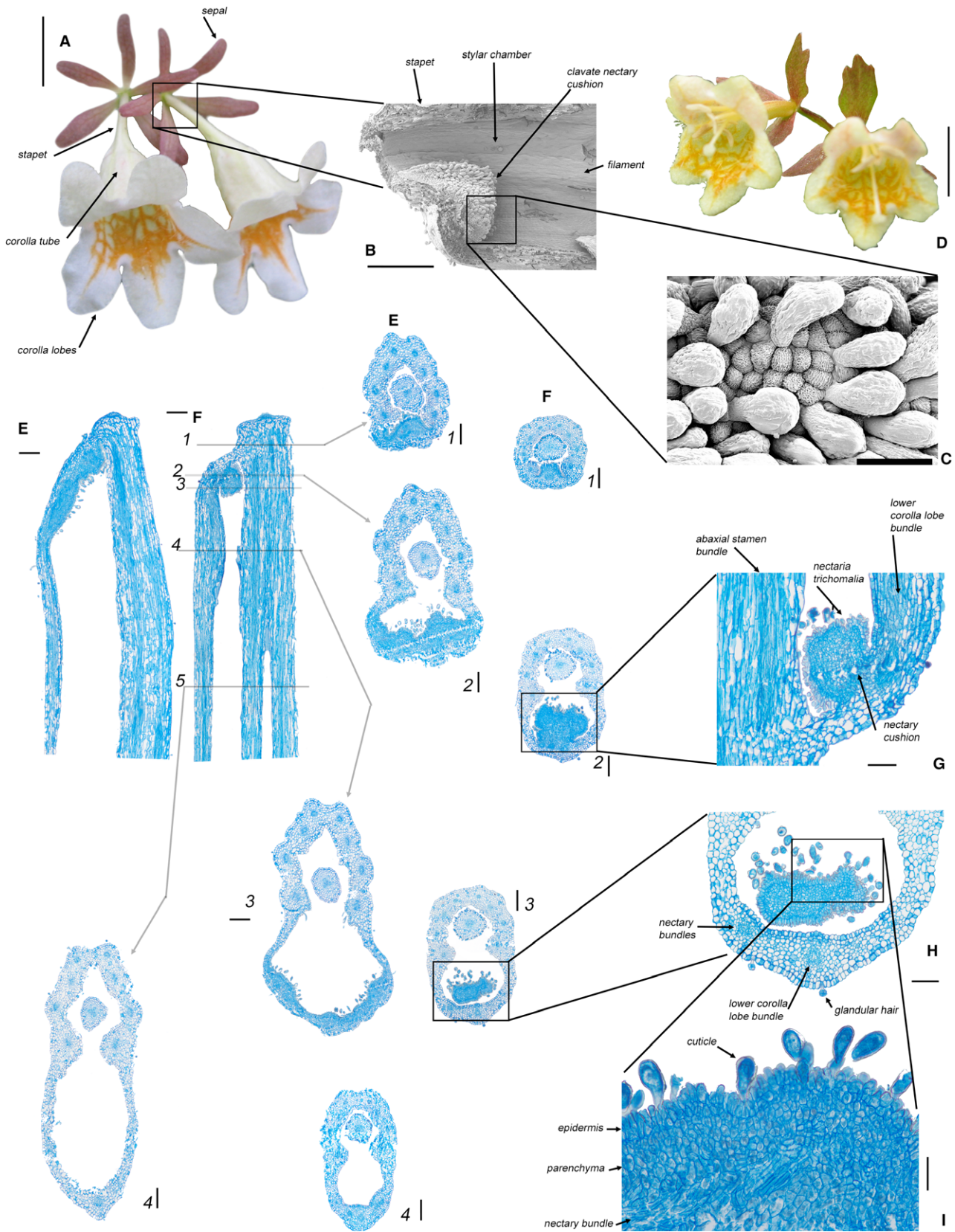
*acea* group. The nectary of *L. borealis* var. *americana* is 1 mm long and its corolla is 8 mm long: 1/8 (stapet up to 2.5 mm long). In *Vesalea mexicana* var. *grandifolia*, the nectary is 7 mm long and its corolla is 14 mm long: 1/2. The nectary in *Linnaea* is nevertheless distinct from those in *Abelia*, *Diabelia*, *Dipelta* and *Kokwitzia* because no bulge is formed at the base of the tube.

## NECTARIAL TRICHOMES

In this study, we identified three different types of secreting trichomes. (1) The clavate type is the most common glandular hair; it is unicellular with an irregular cell wall and cuticle. The subcuticular space is filled with nectar and it either bursts open or nectar seeps through the permeable membrane (Fig. 2). (2) Nectary hairs with a smooth base were only found in *Zabelia*. This hair type is similar to the previous type, but the wall is smooth at the base and becomes very irregular at the apex (Fig. 2). (3) The multicellular type is only found in *Adoxa*; it is composed of 10–15 cells and the cuticle is thin without a subcuticular space (Fig. 2).

The clavate hair is plesiomorphic and is also found in most other Caprifoliaceae (Wagenitz & Laing, 1984). The smooth base type is a synapomorphy of *Zabelia*. The smooth base type is unique and could be linked to a specialized secretion mechanism, in which secretion is apical and the smooth basal part increases the hydrostatic pressure. This last character forms a synapomorphy which distinguishes *Zabelia* from Linnaeeae. Finally, the multicellular hair is only found in *Adoxa* and has been used as an argument that this genus belongs to its own family: Adoxaceae *s.s.*

## THE FILAMENT CURTAIN

The hemisynstapetal region (with decurrent filaments; Ritterbusch, 1991) is found frequently in Asterales. In some genera, however, the hemisynstapetal region may extend to the base of the corolla. Such well-developed filament ridges are important in the pollination biology of the flower. They may form channels that lead down to the nectar. Examples of such channels are found in families with actinomorphic corollas (Solanaceae; Endress, 1994) and zygomorphic corollas (Scrophulariaceae; Hilliard, 1994). Their role may include evaporation limitation in taxa with pendent flowers (capillary tube), or they could act as a nectar guide (Manktelow, 1999).

The filaments in Linnaeeae are fused to the corolla and become detached in the upper half. In most species, the filaments are swollen and decurrent, separating the stylar chamber from the nectary chamber. Few species develop a true filament curtain, creating a distinct capillary or nectary guide for pollinators. In *Abelia forrestii* var. *forrestii*, the nectary and the lower part of the stapet are partitioned by the winged filament and could act as a nectar guide or to limit evaporation as the flowers are pendent (Fig. 9). In *Vesalea floribunda*, the filaments are hairy and well developed in the lower part of the stapet and, along them, deep furrows are formed. The large filaments form curtains and furrows, creating distinct capillary chambers in which nectar can accumulate and which could act as nectar guides (Fig. 7).

The filament curtain seems to be associated with two specialized corolla types that are both hanging and are adapted to distinct pollinators. Hummingbirds require large amounts of nectar which would flow out of the flower if no capillary tube was present. In *Abelia forrestii*, the pollinator is not known, but the unique shape of the corolla with its exceptionally long and thin tube suggests an insect with a long proboscis for which a nectar guide would be required.

## CLAVATE CUSHION

Members of Linnaeeae generally possess a nectary cushion (Kurosawa & Hara, 1955), which is a tissue containing the prenectary parenchyma, vascular bundle, nectary parenchyma and the epidermis with the glandular hairs. Nectaries with clavate cushions feature extremely developed cushions, with an increased number of vascular bundles. Clavate cushions protrude into the tube and are apically free from

**Figure 13.** *Dipelta* and *Kokwitzia*, gibbous nectary [scanning electron microscopy (SEM) and serial sections]. A, Flower of *Dipelta floribunda*. B, *Dipelta elegans*, flower. C–E, *Dipelta yunnanensis*, flowers, longitudinal section of the gibbous part of the stapet and nectaria trichomalia. F, *Kolkwitzia amabilis*, flowers. G, *Kolkwitzia amabilis*, longitudinal section through the base of the stapet, showing the position of the nectary. H, *Dipelta yunnanensis*, longitudinal and three serial transverse sections through the base of the stapet showing the position of the nectary. I, J, *Dipelta yunnanensis*. I, Transverse section of the base of the stapet showing the nectary cushion. J, Transverse section of the base of the stapet showing the vasculature of the nectary. K, L, *Kolkwitzia amabilis*. K, Longitudinal section of the base of the stapet showing the nectary bulge and nectaria trichomalia. L, Detail of nectaria trichomalia. Scale bars: A, B, C, F, 1 cm; D, 400 μm; G–I, 200 μm; E, J, K, 100 μm; L, 50 μm.
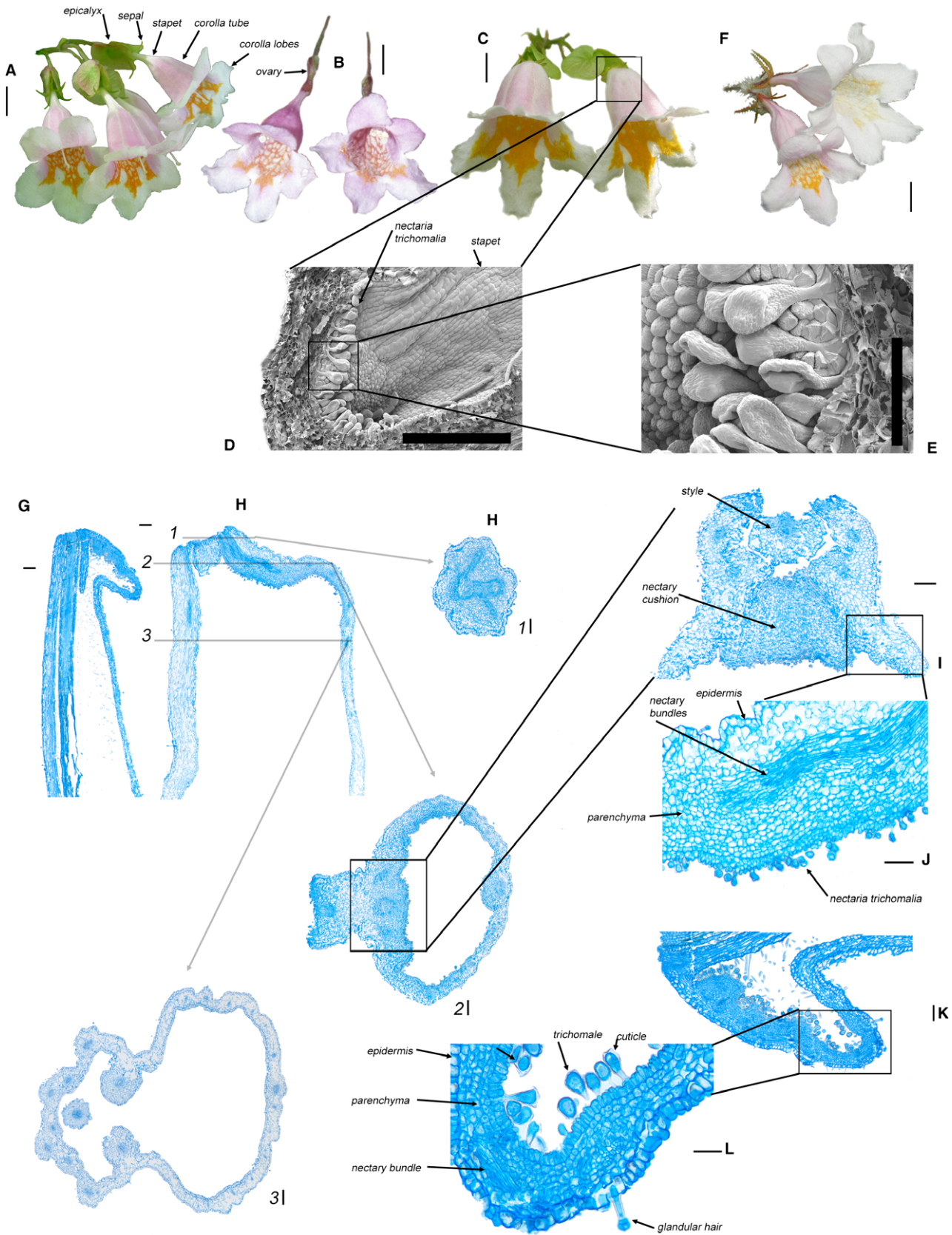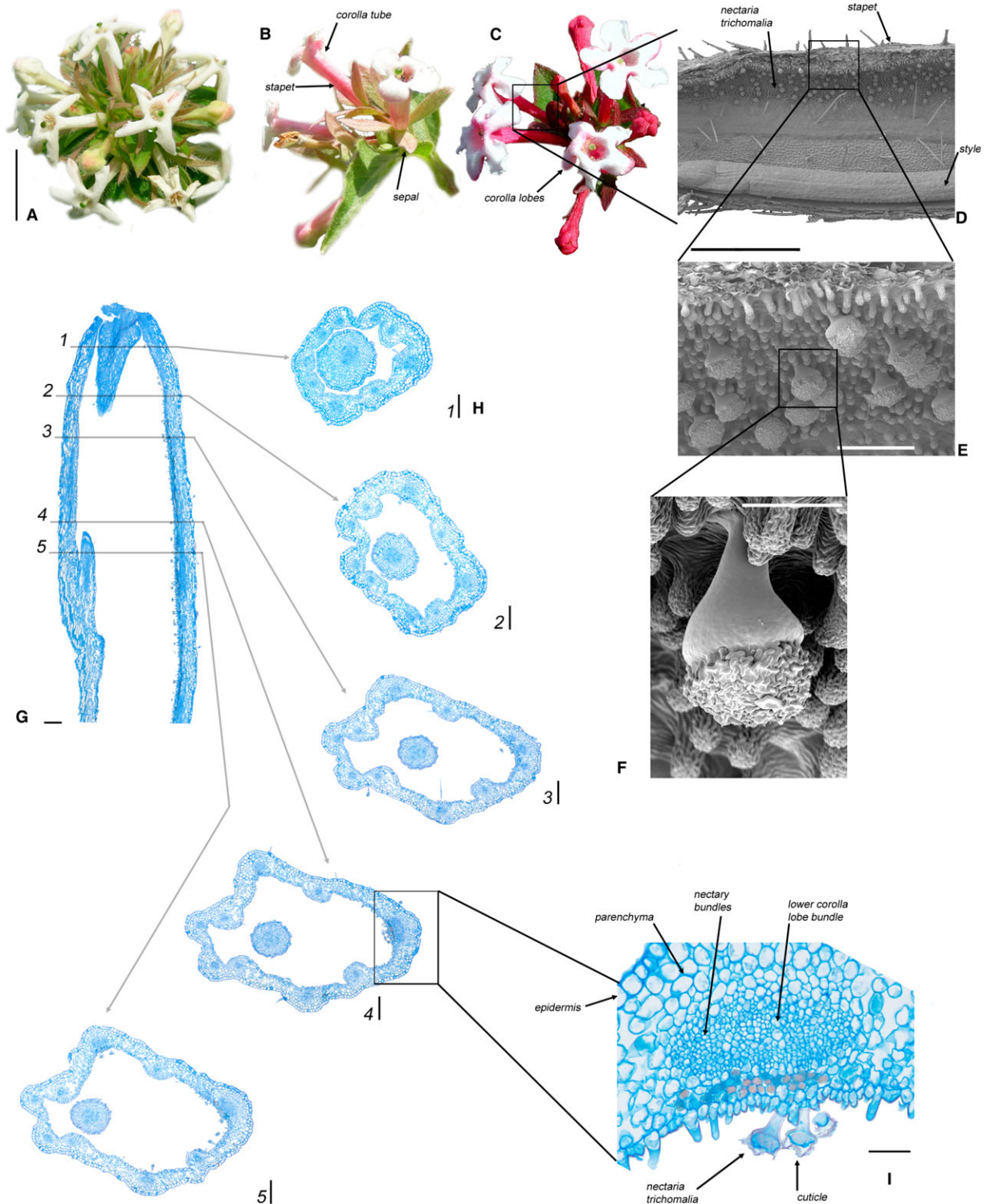
**Figure 14.** *Zabelia triflora* group, zonate nectary [scanning electron microscopy (SEM) and serial sections]. A, *Zabelia triflora*, flowers. B, *Zabelia corymbosa*, flowers. C–F, *Zabelia tyaihyoni*. C, Flowers. D, Longitidinal section of the stapet showing the ventral zone of the nectary. E, F, Detail of nectaria trichomalia. G–I, *Zabelia triflora*. G, Longitudinal section of the stapet. H, Five serial transverse sections through the base of the stapet, showing the occurrence of the nectary. I, Longitudinal section of the stapet through the nectary showing the vasculature. Scale bars: A–C, 1 cm; D, 1 mm; G, H, 200 μm; E, I, 100 μm; F, 30 μm.
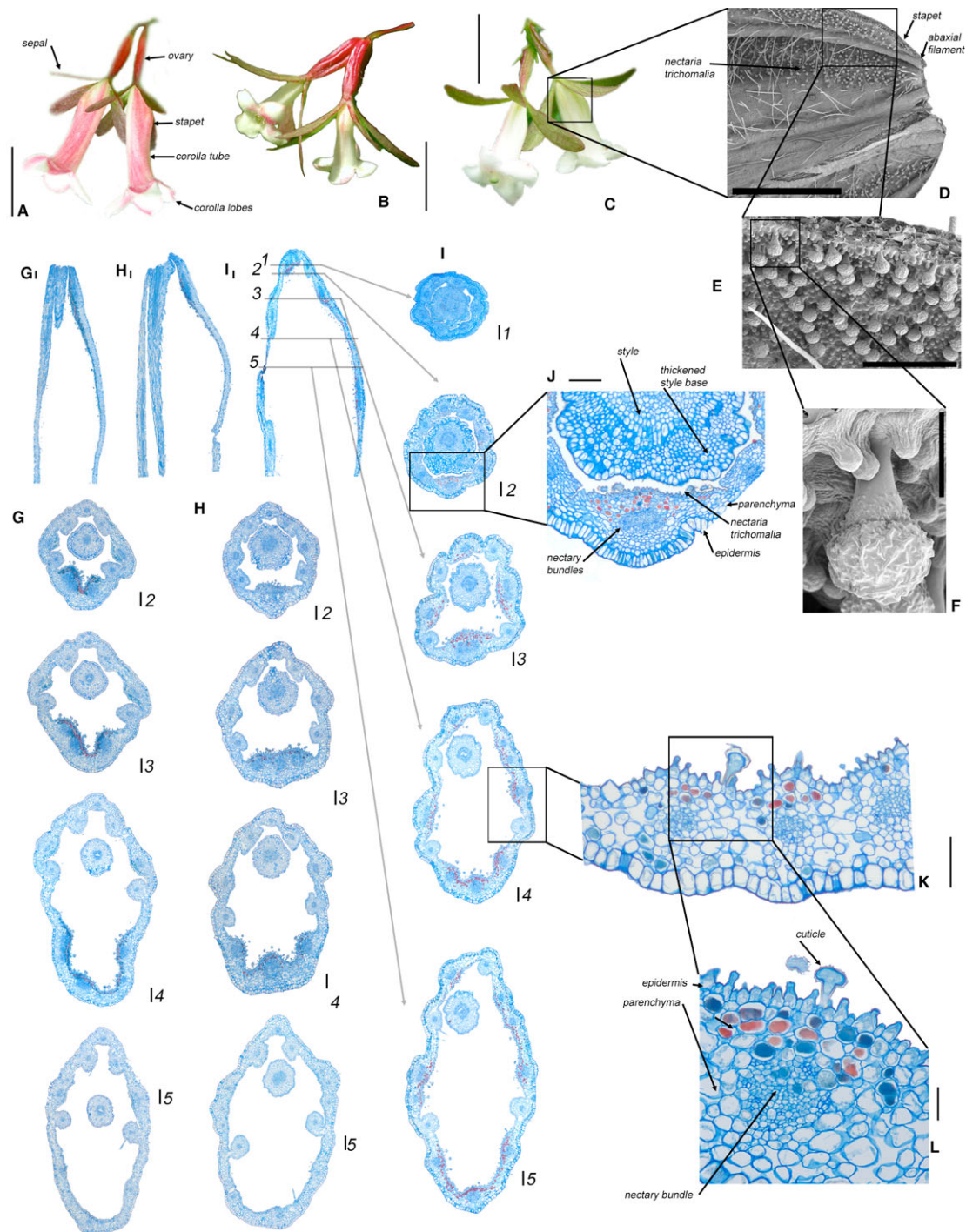
**Figure 15.** *Zabelia biflora* group of species, flowers and zonate nectary [scanning electron microscopy (SEM) and serial sections]. A, *Zabelia integrifolia*, flowers. B, *Zabelia coreana*, flowers. C–F, *Zabelia dielsii*. C, Flowers. D, Longitidinal section of the stapet showing the ventral, dorsal and lateral zones of the nectary. E, F, Detail of nectaria trichomalia. G, *Zabelia integrifolia*, longitudinal and five serial transverse sections through the base of the stapet, showing the position of the nectary. H, *Zabelia coreana*, longitudinal and five serial transverse sections through the base of the stapet, showing the position of the nectary. I, *Zabelia dielsii,* longitudinal and five serial transverse sections through the base of the stapet, showing the position of the nectary. J–L, *Zabelia dielsii.* J, Transverse section of the ventral side of the stapet through the nectary. K, Transverse section of the lateral side of the stapet through the nectary. L, Detail of the nectary. Scale bars: A–C, 1 cm; D, 2 mm; E, 300 μm; G–I, 200 μm; J, K, 100 μm; L, 50 μm; F, 40 μm.
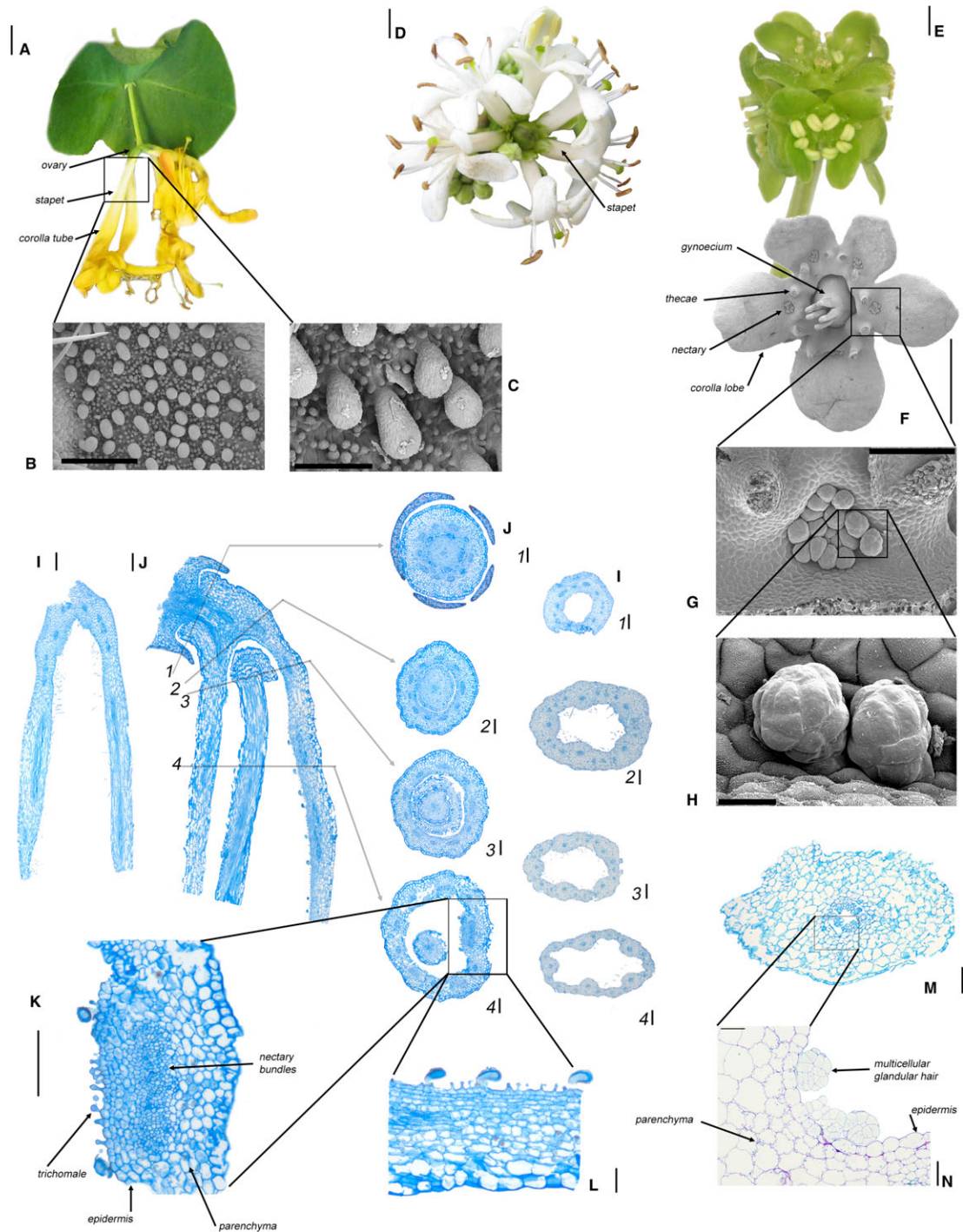
**Figure 16.** *Lonicera*, *Heptacodium* and *Adoxa*, flowers and zonate nectary [scanning electron microscopy (SEM) and serial sections]. A–C, *Lonicera tragophylla*. A, Flowers. B, Longitudinal section of the corolla showing the nectary. C, Detail of the nectary. D, *Heptacodium miconioides*, flowers. E–H, *Adoxa moschatellina*. E, Inflorescence and flowers. F, Flower. G, Nectary. H, Multicellular glandular hairs. I, *Heptacodium miconioides*, longitudinal and four serial transverse sections through the stapet showing the position of the nectary. J, *Lonicera tragophylla*, longitudinal and four serial transverse sections through the stapet showing the position of the nectary. K–L, *Lonicera tragophylla*. K, Transverse section of the ventral side of the stapet through the nectary. L, Longitudinal section of the stapet through the nectary. M, N, *Adoxa moschatellina*. M, Transverse section through a petal and the nectary. N, Longitudinal section through a petal and the nectary. Scale bars: A, 6 mm; D, 5 mm; E, F, 2 mm; B, G, 300 μm; I, J, M, 200 μm; C, N, K, L, 100 μm; H, 50 μm.
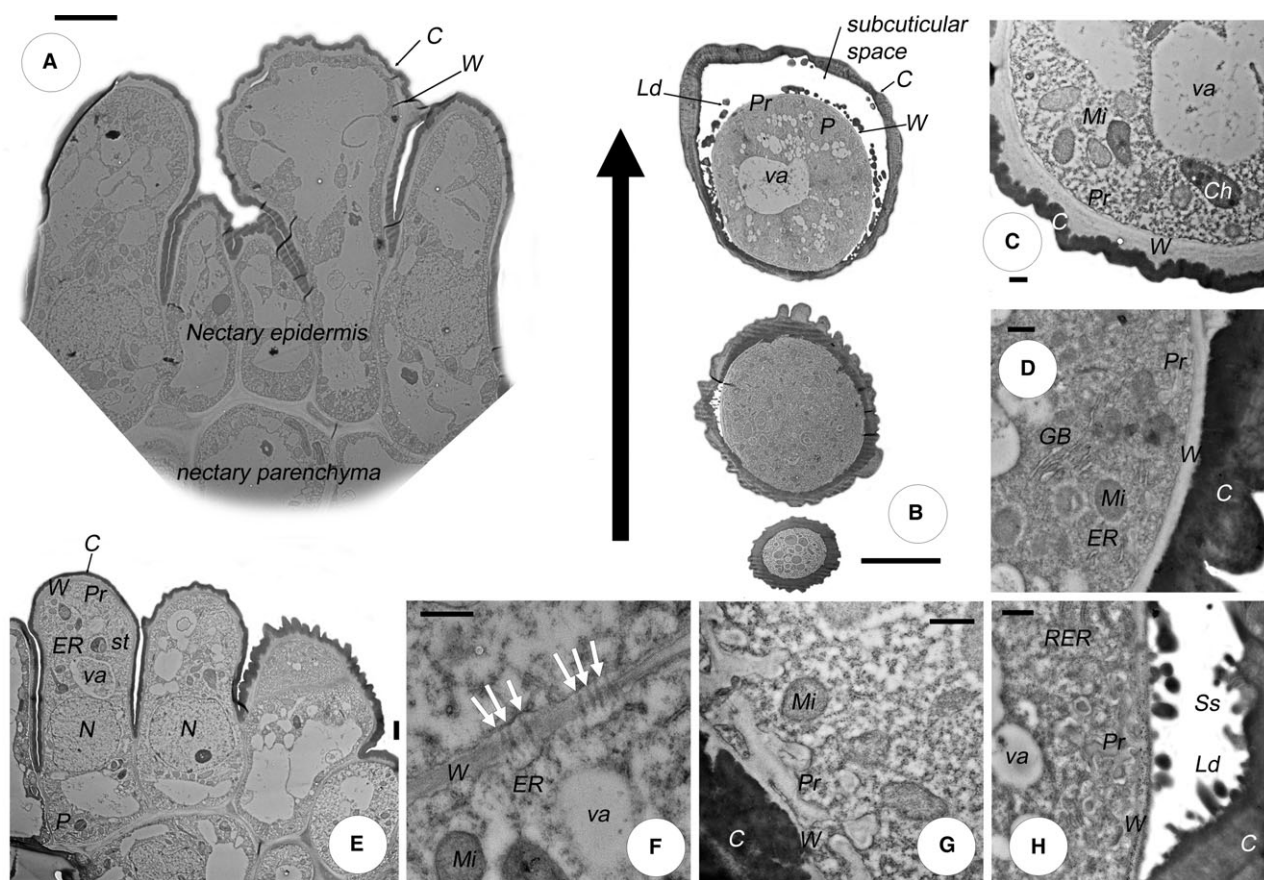
**Figure 17.** Ultrastructure of the nectary in *Linnaea borealis*. A, Overview of glandular hairs, epidermis and nectary parenchyma. B, Transverse serial section through a glandular hair; note the conspicuous wall protuberance at the apex. C, Detail of the cell periphery with wall protuberances. D, Wall protuberances, secretion mechanism and cuticle. E, Details of glandular hairs. F, Nectary parenchyma cells connected via plasmodesmata. G, Wall protuberances. H, Wall protuberance and subcuticular space. Scale bars: A, B, 10 μm; E, 2 μm; C, D, F–H, 500 nm. C, cuticle; Ch, chloroplast; ER, endoplasmic reticulum; GB, golgi body; Ld, lipid droplet; Mi, mitochondria; N, nucleus; P, plastid; Pr, wall protuberance; RER, rough endoplasmic reticulum; Ss, subcuticular space; st, starch grain; va, vacuole; W, wall; arrows indicate plasmodesmata.

the stapet. This is also associated with a more localized zonation of the glandular hairs. Typically found in *D. spathulata*, the morphology and variation have been studied by Kurosawa & Hara (1955), who discussed the variation in clavate nectary sizes and its occurrence in hybrids and intermediate forms of *D. spathulata*. For example, in *D. sanguinea*, the nectary is much smaller, fused to the stapet and not clavate. This variety has reddish flowers, uniquely in Linnaeeae, and this could affect its pollinators, as shown in the convergent *Weigela middendorffiana* (Carrière) K.Koch (Takashi & Kudo, 2003; Inoue, Ushijima & Yokoyama, 2007). *Weigela* Thunb. also has clavate nectaries, and there is strong parallelism between the flowers in *Weigela* and *Diabelia* in Japan. In this study, we also observed a rudimentary clavate cushion in the flowers of *Abelia macrotera*

var. *deutziaefolia* (Fig. 10). Like other floral characters, there is strong parallelism between *Abelia* and *Diabelia*.

CALYX AND NECTARY BULGE

In Linnaeeae, the calyx and nectary are linked morphological characters. The bulging nectary is located at the base of the stapet, opposite the abaxial petal and in between the two abaxial sepals. When the abaxial sepals are fused, as in *Abelia uniflora* and *Diabelia serrata*, the nectary bulge is opposite the fused sepals, and both have to be accommodated in the same space. In *Dipelta elegans*, the calyx is completely fused and cupular, and the nectary bulge is inserted in the tube (Fig. 13). As a possible consequence, the nectary bulge is also the smallest in the genus, whereas it is
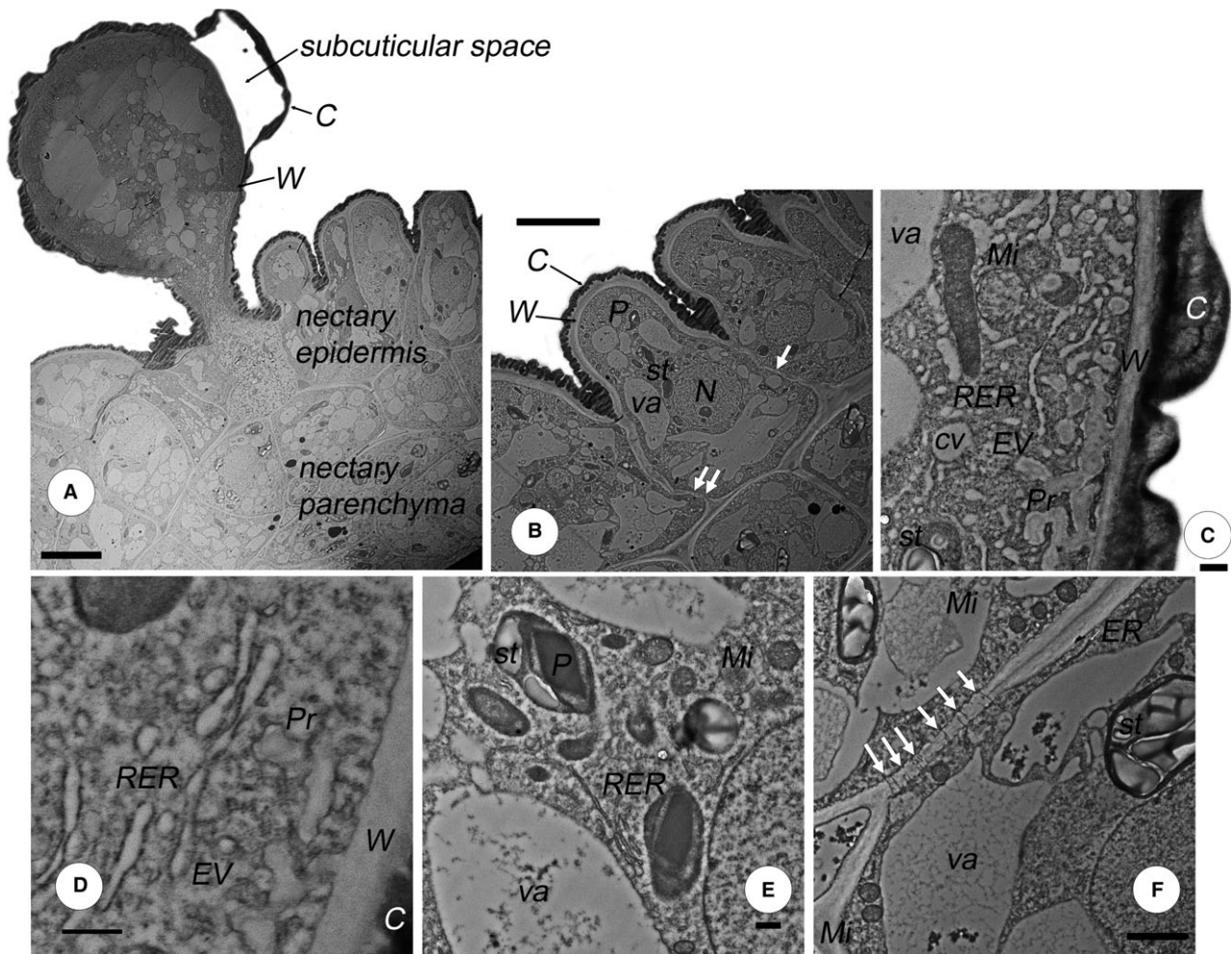
**Figure 18.** Ultrastructure of the nectary in *Abelia macrotera* var. *macrotera*. A, Overview of the glandular hair, epidermis and nectary parenchyma. Note the subcuticular space. B, Nectary parenchyma and epidermis. C, Detail of the cell periphery with wall protuberances. D, Wall protuberances and secretion mechanism. E, Dense cytoplasm with plastids and starch grains. F, Nectary parenchyma cell connected via plasmodesmata. Scale bars: A, B, 10 μm; F, 2 μm; C–E, 500 nm. C, cuticle; cv, ribosome coated vesicle; ER, endoplasmic reticulum; EV, vesicles budding off from the ER cisternae; Mi, mitochondria; N, nucleus; P, plastid; Pr, wall protuberance; RER, rough endoplasmic reticulum; st, starch grain; va, vacuole; W, wall; arrows indicate plasmodesmata.

much larger in *D. floribunda*, in which the sepals are free. This could represent parallel evolution of the calyx and nectary, and could explain why the natural range of *D. elegans* is restricted and why it is almost extinct.

NECTARY DISC, DICHOGAMY AND GREEN MUCILAGINOUS STIGMAS

A conspicuous nectary disc is present in *Lonicera* section *Periclymenum*, but was lost in the rest of the genus. However, we found a thickening of the stylar base, which could be a remnant of the nectary disc, in *Zabelia* (Figs 14–17). This has not been documented

or studied previously, and it demonstrates how some characters can remain and become less conspicuous, but still indicative of evolutionary histories.

Green mucilaginous stigmas (wet papillate *sensu* Heslop-Harrison, 1992) can be observed in *Lonicera*, *Heptacodium* and *Zabelia*, and this seems to be associated with dichogamy and protandry, although this has not been studied in detail. Wet stigmas are correlated with gametophytic self-incompatibility, whereas sporophytic self-incompatibility systems are associated with dry, papillate stigmas (Brewbaker, 1967; Heslop-Harrison & Shivanna, 1977). Homogamous species are restricted to Linnaeeae, and their stigmas are dry and papillate; they have
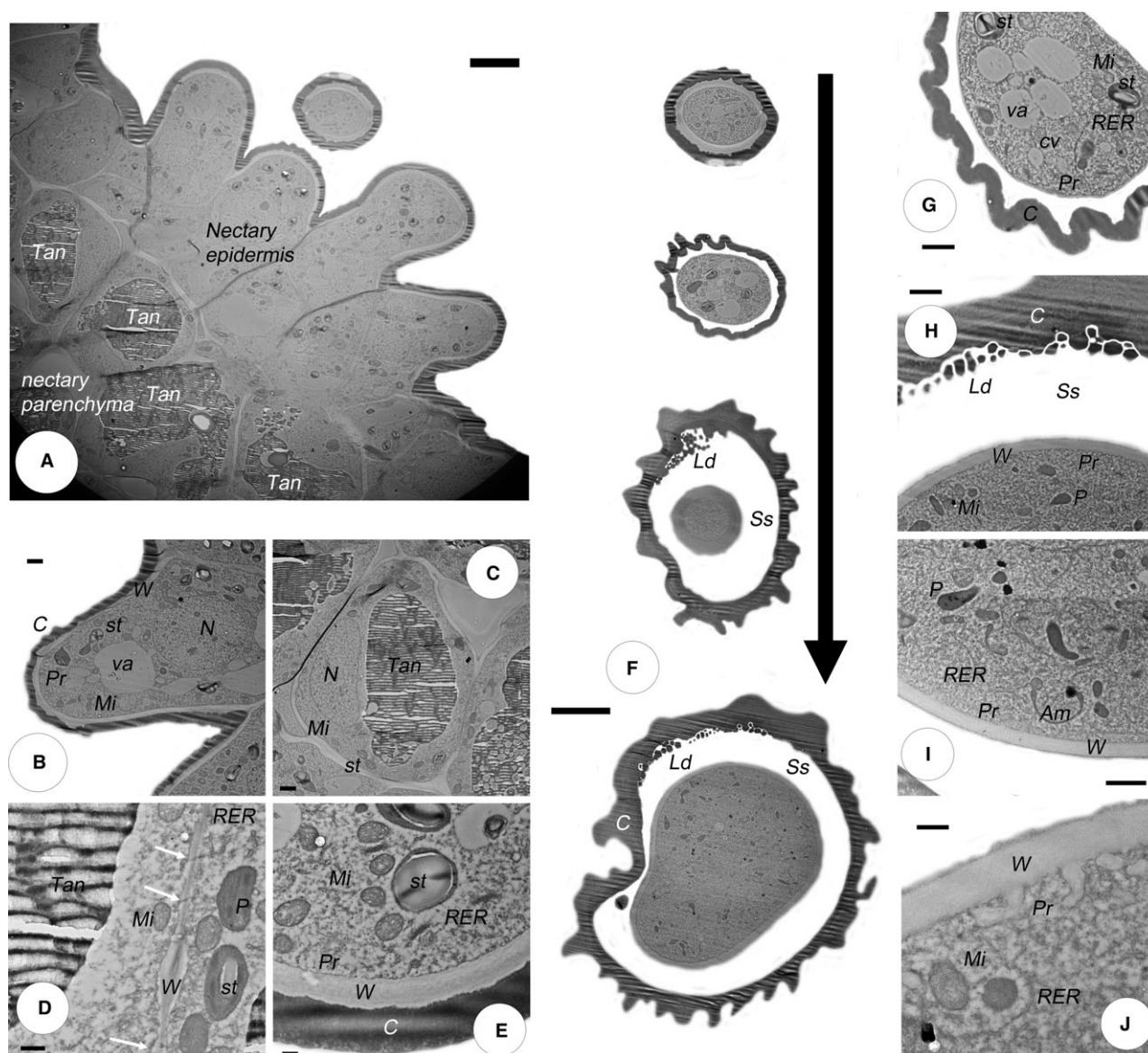
**Figure 19.** Ultrastructure of the nectary in *Zabelia coreana*. A, Overview of the glandular hair, epidermis and nectary parenchyma; note the subcuticular space. B, Glandular hair at an early developmental stage. C, Prenectary parenchyma cells with large vacuole filled with tannins. D, Prenectary parenchyma cell connection with plasmodesmata. E, Detail of the cell periphery with wall protuberances. F, Transverse serial section through the glandular hair; note the conspicuous cuticle protuberance at the apex. G–J, Detail of the cell periphery with wall protuberances. Scale bars: A, F, 10 μm; B, C, G–I, 2 μm; D, E, J, 500 nm. Am, Amyloplast; C, cuticle; cv, ribosome coated vesicle; Ld, lipid droplet; Mi, mitochondria; N, nucleus; P, plastid; Pr, wall protuberance; RER, rough endoplasmic reticulum; Ss, subcuticular space; st, starch grain; Tan, vacuole filled with tannins; va, vacuole; W, wall; arrows indicate plasmodesmata.

no nectary disc remnant. *Zabelia* and *Heptacodium* show again how they differ from Linnaeeae (Jacobs *et al.*, 2011).

## POLLINATION

Based on our observations of the nectary and flower morphology, we propose that there are three distinct pollination syndromes in Linnaeeae: entomophily (bees, flies, hawkmoths); ornithophily (hummingbirds); and anemophily (wind). Entomophily is the plesiomorphic condition, associated with both nectary types (zonate and gibbous), and with bilabiate, hypocrateriform or infundibuliform corollas.

As discussed by Fukuoka (1968), the nectary of *Vesalea floribunda* is formed of three bands of
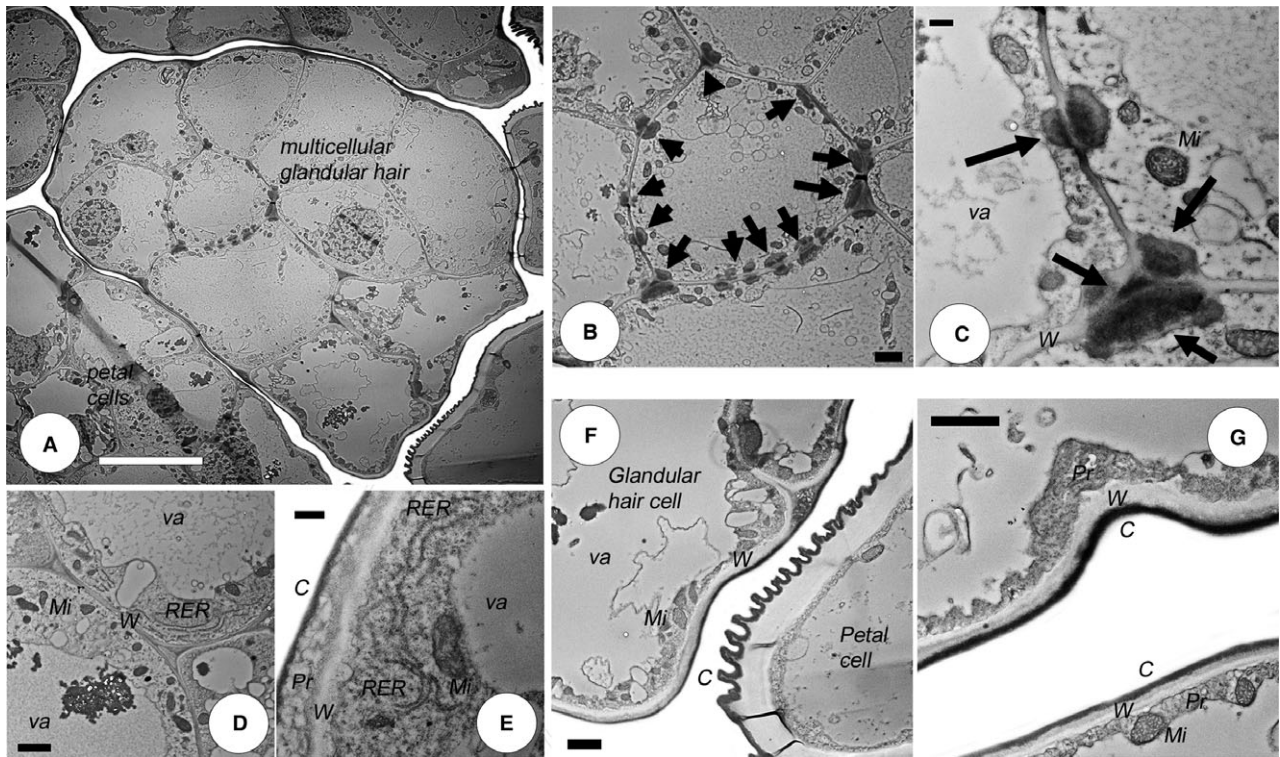
**Figure 20.** Ultrastructure of the nectary in *Adoxa moschatellina*. A, Transverse section of the multicellular glandular hair and petal cells; B, Central cell of the hair with conspicuous plasmodesmata and RER. C, Cell communication and nectar transport. D, Cell periphery with wall protuberance. E, Hair periphery with thin cuticle and wall protuberance. F, Glandular and petal cell dimorphism and cuticle. G, Hair periphery with thin cuticle and wall protuberance. Scale bars: A, 10 μm; B, D, F, G, 2 μm; C, E, 500 nm. C, cuticle; Mi, mitochondria; Pr, wall protuberance; RER, rough endoplasmic reticulum; va, vacuole; W, wall; arrows indicate plasmodesmata.

glandular hairs in ventral and lateral positions (Fig. 7). These glandular hairs are particularly dense and cover one-third of the length of the reddish and unscented corollas. This could be linked to potential pollination by birds. Hummingbirds require larger amounts of nectar; therefore, a single small zone of hairs would not produce sufficient quantities. Fukuoka (1968) did not study material of other *Vesalea* spp., which differ significantly. In *V. coriacea*, *V. mexicana*, *V. occidentalis* and *V. subcoriacea*, the nectary is only formed by a single zone of hairs in between the abaxial filaments and on the ventral side of the stapet. The lateral zone between the abaxial and adaxial filament is not as wide and the stapet is more cylindrical (Fig. 8). The corollas are pink to white, infundibuliform-bilabiate and fragrant, and this could be linked to entomophily.

In *Abelia chinensis* (Fig. 11), the infundibuliform to campanulate corolla with exserted versatile anthers, scented corolla and reduced nectary would indicate that wind and insect pollination are both effective. Field observations are necessary to verify our hypothesis and to provide more detailed statements on the range of species that pollinate these taxa.

### INFRASPECIFIC VARIATION

The nectary is a useful morphological character for studying the classification of Caprifoliaceae and for distinction between genera. It is also a source of useful characters for studying taxa and infraspecific variation, or even variation among populations. Kurosawa & Hara (1955) identified several species, forms and varieties of *Diabelia* on the basis of the morphology of the nectary. In this study, we also identified varieties and species in which the nectary represents a diagnostic character: In *Abelia macrotera* var. *deutziaefolia*, the bulge expands outwards and forces the abaxial calyx lobe to spread further apart. This variety has a more conspicuous clavate cushion that protrudes in between the abaxial filaments (Fig. 10). In *Zabelia*, the nectary hairs are present in one to four zones in between the abaxial filaments, the adaxial filaments as well as on the upper corolla lobe. Lateral and adaxial nectaries are
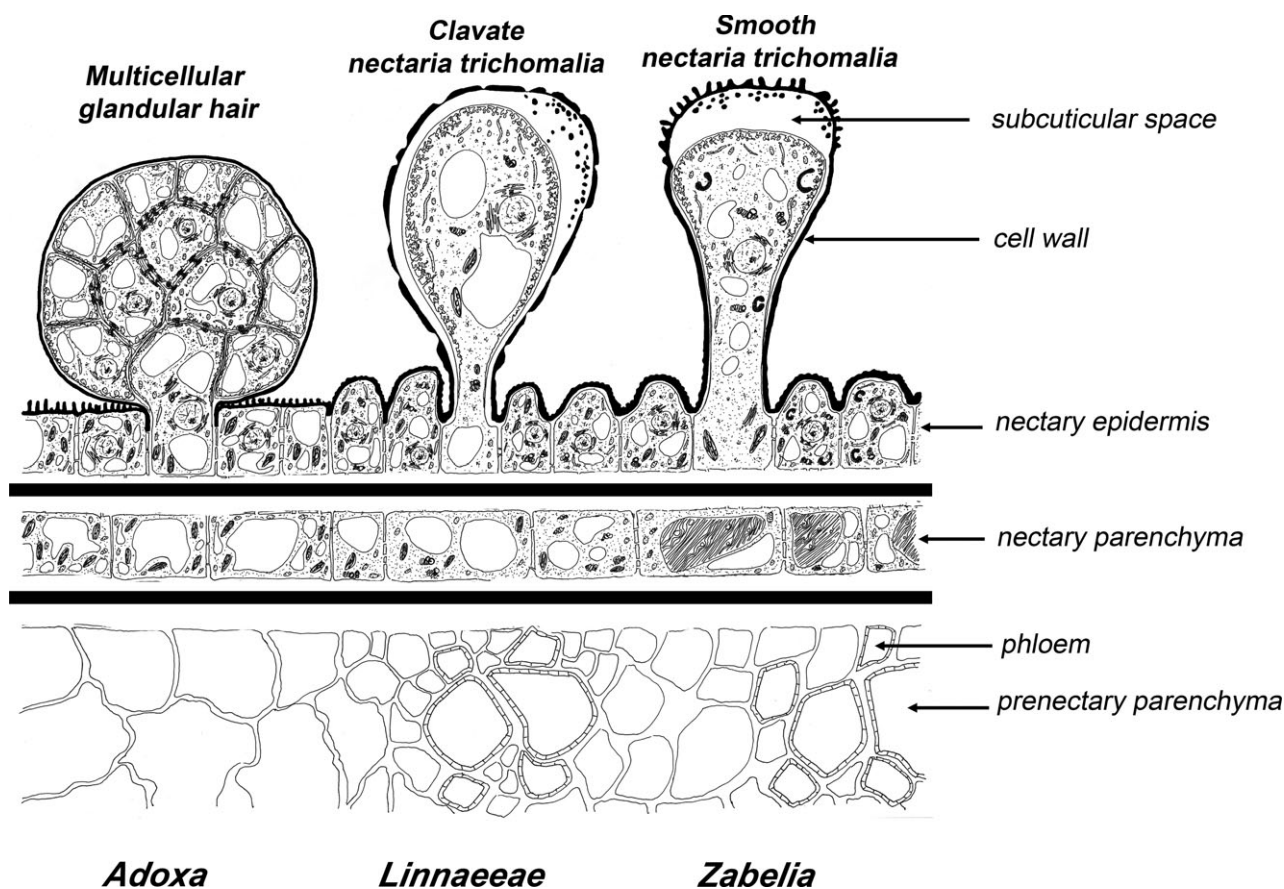
**Figure 21.** Schematic drawing of the nectaria trichomalia ultrastructure and granulocrine secretion in *Adoxa*, Linnaeeae and *Zabelia*.

missing in *Z. integrifolia* and *Z. coreana*, but present in *Z. dielsii* (Fig. 15).

### SECRETION

Secretion is typically granulocrine, with the transport of sugar solution into vesicles derived from the dilated cisternae of ER or from dictyosomes that fuse with the plasmalemma, releasing nectar.

In *Zabelia*, *Heptacodium* and Linnaeeae, the cuticle on the hair becomes detached from the cell wall and large subcuticular spaces filled with nectar are formed. Nectar is probably released in areas with a thin cuticle and microchannels, as observed by Weryszko-Chmielewska & Bożek (2008).

The cuticle of the secretory cells is ribbed (with thick ribs and thin areas). Our study shows that the ribs are not randomly distributed. In *Zabelia*, the basal part of the hair is smooth and of even thickness, whereas the apical part is thick and has large ribs. The function of the smooth basal part can only be hypothesized if the thinner part helps in the release of nectar. If the nectar is only released in the apical part, the basal part may help to build up the hydrostatic pressure.

In *A. moschatellina*, the central cell has a larger number of plasmodesmata, implying a role in the collection and conveyance of prenectar from the nectary parenchyma to the distal secreting cells (Sawidis, Eleftheriou & Tsekos, 1986; Robards & Stark, 1988; Rocha & Machado, 2009). The cuticle of the tip cells is thin, even though the petal epidermis has a ribbed cuticle. Wall protuberances, mitochondria and ER are well developed, but no accumulation can be seen in the subcuticular spaces between the wall and the cuticle. Secretion in *Adoxa* is likely to be diffuse through a permeable cuticle or microchannels. The formation of droplets as described by Wagenitz & Laing (1984) could not be verified and seems unlikely (Fig. 21).

### TANNINS AND LIPID DROPLETS

In this study, we also observed lipid droplets (Figs 18–20) in the subcuticular space of *Linnaea*

**Table 1.** Morphological characters of the flowers and the nectary in Linnaeeae and related taxa

| Genera | Sex | Filament curtain | Nectary bundles | Cushion | Corolla shape | Pollination | Pollinators |
|---|---|---|---|---|---|---|---|
| *Linnaea* | Homogamous | No | 4 | No | Infundibuliform-campanulate | Entomophilous | Bees/flies |
| *Vesalea* | Homogamous | Yes/no | 4–9 | No | Tubular to infundibuliform | Ornithophilous/entomophilous | Humming birds/bees |
| *Diabelia* | Homogamous | No | 3–5 | Clavate " | Bilabiate | Entomophilous | Bees/butterflies |
| *Dipelta* | Homogamous | No | 9–15 | Yes % | Bilabiate with inflate tube | Entomophilous | Bees |
| *Kokwitzia* | Homogamous | No | 9 | Yes % | Bilabiate with inflate tube | Entomophilous | Bees |
| *Abelia* | Homogamous | Yes/no | 6–13 | Yes £ | Bilabiate | Entomophilous/anemophilous | Bees/wind |
| *Zabelia* | Dichogamous | No | 3–6 | No | Hypocrateriform | Entomophilous | |
| *Heptacodium* | Dichogamous | No | 3 | No | Hypocrateriform | Entomophilous | |
| *Lonicera* | Dichogamous | No | 1–5 | No | Tubular-bilabiate | Entomophilous | Bees/hawkmoth |
| *Adoxa maschatellina* | | No | 1 | No | Rotate-regular () | Entomophilous | Flies |
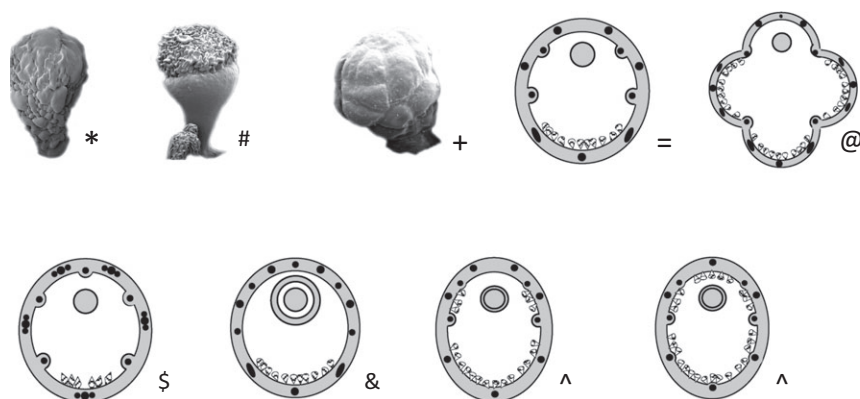
*borealis*, *Abelia macrotera* and *Zabelia biflora*. This was not observed in the previously studied examples of *Lonicera* (Fahn & Rachmilevitz, 1970; Weryszko-Chmielewska & Bożek, 2008).

In *Zabelia biflora*, the nectary parenchyma cells around the vascular bundles are heavily stained (Fig. 19). In TEM, we observed large vacuoles occupying most of the cells which were not penetrated by the fixative. This could be caused by tannins, some recent examples of which have also been observed in nectary secretory cells (Paiva, 2012). Nectar contains water, sugars and amino acids to attract pollinators, and nectar is protected from nectar robbers and microorganisms by secondary compounds and antimicrobial proteins (Heil, 2011).

## CONCLUSIONS

We have shown that the detailed (re)investigation of characters provides important insights into the evolution of taxa, and fosters our understanding of overlooked morphological structures, such as the nectary, in the twinflower clade Linnaeeae. The unique nectary hair in *Zabelia* provides additional support for the segregation of this genus from Linnaeeae.

The zonate nectary is a plesiomorphic character shared by *Linnaea* and *Vesalea*. This is in accordance with recent phylogenetic results, showing that *Linnaea* and *Vesalea* form a clade. Biogeographical links between the two genera in North America, rather than with Chinese *Abelia*, are supported here and further adaptation to ornithophily in Mexico is shown.

| Fragrant | Calyx | Colour | Nectaria trichomalia | Nectary disc | Stigmas | Type |
|---|---|---|---|---|---|---|
| Yes | 5 | White–pink with markings and hairs | Unicellular clavate* | No | Dry papillate > | Zonate 1 = |
| Yes/no | 5 | Red–purple–pink–white | Unicellular clavate | No | Dry papillate > | Zonate 1 to 3 @ |
| No | 2–5 | White–yellow–purple–red with markings and hairs | Unicellular clavate | No | Dry papillate > | Gibbous " |
| Yes | 5 free / fused | White–pink–purple with markings | Unicellular clavate | No | Dry papillate > | Gibbous % |
| Yes | 5 | White–pink–purple with markings | Unicellular clavate | No | Dry papillate > | Gibbous % |
| Yes/no | 2–5 | White–pink–purple with or without markings | Unicellular clavate | No | Dry papillate > | Gibbous £ |
| Yes | 4–5 | White-reddish | Unicellular smooth # | Thickening ^ | Wet papillate < | Zonate 1–4 ^ |
| Yes | 5 | White | Unicellular clavate | | Wet papillate < | Zonate 1 $ |
| Yes | 5 | Yellow without markings | Unicellular clavate | Nectary disc & | Wet papillate < | Zonate 1 & |
| Yes musky | 2–4 | Green | Multicellular + | No | Dry papillate > | Zonate 5 () |



The nectary hair of *Zabelia* represents a model to study nectar secretion, because of the differentiation in the cuticle structure, and could lead to discoveries relating to secretion mechanisms either through permeable membranes or microchannels. Interaction with pollinators and coevolutionary models would need to be studied further in the field, and this would help us to understand the different adaptation in their morphology observed here.

## REFERENCES

**Bentley B, Elias T. 1983.** *The biology of nectaries*. New York: Columbia University Press.

**Brewbaker JL. 1967.** The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. *American Journal of Botany* **54:** 1069–1083.

**Christenhusz M. 2013.** Twins are not alone: a recircumscription of *Linnaea* (Caprifoliaceae). *Phytotaxa* **125:** 25–32.

**Davis AR, Peterson RL, Shuel RW. 1988.** Vasculature and ultrastructure of the floral and stipular nectaries of *Vicia faba* (Leguminosae). *Canadian Journal of Botany* **66:** 1435–1448.

**Endress PK. 1994.** *Diversity and evolutionary biology of tropical flowers*. Cambridge: Cambridge University Press.

**Endress PK, Matthews ML. 2012.** Progress and problems in the assessment of flower morphology in higher-level systematics. *Plant Systematics and Evolution* **298:** 257–276.

**Erbar C. 2014.** Nectar secretion and nectaries in basal angiosperms, magnoliids and non-core eudicots and a comparison with core eudicots. *Plant Diversity and Evolution* **131:** 63–143.

**Fahn A, Rachmilevitz T. 1970.** Ultrastructure and nectar secretion in *Lonicera japonica*. *Botanical Journal of the Linnean Society* **63:** 51–56.

**Fukuoka N. 1968.** Phylogeny of the tribe Linnaeeae. *Acta Phytotaxonomica et Geobotanica* **23:** 82–94.

**Giger E. 1912.** *Linnaea borealis* L., eine monographische Studie. *Beihefte zum Botanischen Centralblatt* **30:** 1–78.

**Graebner P. 1901.** Die Gattung *Linnaea* (einschließlich *Abelia*). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **29:** 120–145.

**Heil M. 2011.** Nectar: generation, regulation and ecological functions. *Trends in Plant Science* **16:** 191–200.

**Heslop-Harrison J. 1992.** The angiosperm stigma. In: Tiessi MCA, ed. *Sexual plant reproduction*. Berlin: Springer-Verlag, 59–68.

**Heslop-Harrison JS, Shivanna KR. 1977.** The receptive surface of the angiosperm stigma. *Annals of Botany* **41:** 1233–1258.

**Hilliard O. 1994.** *The Manuleae. A tribe of Scrophulariaceae*. Edinburgh: Edinburgh University Press.

**Inoue MN, Ushijima J, Yokoyama J. 2007.** Effect of *Weigela hortensis* (Caprifoliaceae) floral morphology on pollinator behavior. *Plant Species Biology* **22:** 77–86.

**Jacobs B, Geuten K, Pyck N, Huysmans S, Jansen S, Smets E. 2011.** Unraveling the phylogeny of *Heptacodium* and *Zabelia* (Caprifoliaceae): an interdisciplinary approach. *Systematic Botany* **36:** 231–252.

**Jacobs B, Pyck N, Smets E. 2010.** Phylogeny of the *Linnaea* clade: are *Abelia* and *Zabelia* closely related? *Molecular Phylogenetics and Evolution* **57:** 741–752.

**Karnovsky M. 1965.** A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27:** 137–138.

**Kurosawa S, Hara H. 1955.** Variations in *Abelia spathulata* Sieb. et Zucc., with special reference to its floral gland. *Journal of Japanese Sciences* **30:** 289–297.

**Landrein S. 2010.** *Diabelia*, a new genus of tribe Linnaeeae subtribe Linnaeinae (Caprifoliaceae). *Phytotaxa* **3:** 34–38.

**Landrein S, Prenner G. 2013.** Unequal twins? Inflorescence evolution in the twinflower tribe *Linnaeeae* (Caprifoliaceae *s.l.*). *International Journal of Plant Sciences* **174:** 200–233.

**Landrein S, Prenner G, Chase M, Clarkson JJ. 2012.** *Abelia* and relatives: phylogenetics of Linnaeeae (Dipsacales–Caprifoliaceae *s.l.*) and a new interpretation of their inflorescence morphology. *Botanical Journal of the Linnean Society* **169:** 692–713.

**Manktelow M. 1999.** The filament curtain: a structure important to systematics and pollination biology in the Acanthaceae. *Botanical Journal of the Linnean Society* **133:** 129–160.

**Nicolson S, Nepi M, Pacini E. 2007.** *Nectaries and nectar*. Dordrecht: Springer.

**Paiva EAS. 2012.** Anatomy, ultrastructure, and secretory activity of the floral nectaries in *Swietenia macrophylla* (Meliaceae). *American Journal of Botany* **99:** 1910–1917.

**Pyck N, Smets E. 2000.** A search for the phylogenetic position of the seven-son flower (*Heptacodium*, Dispacales): combining molecular and morphological evidence. *Plant Systematics and Evolution* **225:** 185–199.

**Rehder A. 1911.** Wilson Expedition to China. In: Sargent CS, ed. *Plantae Wilsonianae*. Cambridge: The University Press, 118–128.

**Ritterbusch A. 1991.** Morphologisches Beschreibungsmodell Tubiflorer Kronen, ein Beitrag zur Terminologie und Morphologie der Asteriden-blüte. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **112:** 329–345.

**Robards AW, Stark M. 1988.** Nectar secretion in *Abutilon*: a new model. *Protoplasma* **142:** 79–91.

**Rocha JF, Machado SR. 2009.** Anatomy, ultrastructure and secretion of *Hibiscus pernambucensis* Arruda (Malvaceae) extrafloral nectary. *Revista Brasileira de Botanica* **32:** 489–498.

**Roels P, Smets E. 1996.** A floral ontogenetic study in the Dipsacales. *International Journal of Plant Sciences* **157:** 203–218.

**Roland A. 1978.** General preparations and staining of thin sections. In: Hall JL, ed. *Electron microscopy and cytochemistry of plant cells*. New York: Elsevier.

**Sawidis T, Eleftheriou EP, Tsekos I. 1986.** The floral nectaries of *Hibiscus rosa-sinensis*. *Annals of Botany* **59:** 643–652.

**Scobie AR, Wilcock CC. 2009.** Limited mate availability decreases reproductive success of fragmented populations of *Linnaea borealis*, a rare, clonal self-incompatible plant. *Annals of Botany* **103:** 835–846.

**Smets E. 1986.** Localization and systematic importance of the floral nectaries in the Magnoliatae (dicotyledons). *Bulletin du Jardin Botanique National de Belgique* **56:** 51–76.

**Smets E, Cresens E. 1988.** Types of floral nectaries and the concepts 'character' and 'character-state' – a reconsideration. *Acta Botanica Neerlandica* **37:** 121–128.

**Sturm K. 1910.** Monographische Studien über *Adoxa moschatellina* l. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* **55:** 391–462.

**Takashi YI, Kudo G. 2003.** Floral color change in *Weigela middendorffiana* (Caprifoliaceae): reduction of geitonogamous pollination by bumble bees. *American Journal of Botany* **90:** 1751–1757.

**Wagenitz G, Laing B. 1984.** Nectaries of the Dipsacales and their systematic significance. *Botanische Jahrbücher*

*für Systematik Pflanzengeschichte und Pflanzengeographie* **104:** 483–507.

**Wang HF, Landrein S, Dong WP, Nie ZL, Kondo K, Funamoto T, Wen J, Zhou SL. 2015.** Molecular phylogeny and biogeographic diversification of Linnaeoideae (Caprifoliaceae *s.l.*) disjunctly distributed in Eurasia, North America and Mexico. *PLoS ONE* **10:** 1–26.

**Weberling F. 1977.** Comparative and ontogenetic studies on the hair forms of the Dipsacales. *Beiträge zur Biologie der Pflanzen* **53:** 61–90.

**Weryszko-Chmielewska E, Bożek M. 2008.** Structure of trichomatous nectaries in flowers of *Lonicera kamtschatica* (Sevast.) Pojark. *Acta Agrobotanica* **61:** 13–26.

**Wilkinson AM. 1948.** Floral anatomy and morphology of some species of the tribes Linnaeeae and Sambuceae of the Caprifoliaceae. *American Journal of Botany* **35:** 365–371.

**Wilkinson AM. 1949.** Floral anatomy and morphology of *Triosteum* and of the Caprifoliaceae in general. *American Journal of Botany* **36:** 481–489.

**Zhang ZY, Zhou ZK, Gu ZJ. 2002.** Karyomorphology of *Heptacodium* (Caprifoliaceae *s.str.*) and its phylogenetic implications. *Taxon* **51:** 499–505.

## Appendix 1

Source and voucher information of the material used (taxon; accession details; voucher details; provenance)

*Abelia chinensis* R.Br.; RBG Kew Living Collection 1978-2338; (K); Donated by Coblands nurseries, cultivated.

*Abelia forrestii* (Diels) J.Q.Hu; non-cultivated; (K) Kew-KIB 2010 #10CS2051; China, Yunnan, Lushui Co., Lao Wo, altitude 1539 m, 24.vii.2010.

*Abelia macrotera* (Graebn. & Buchw.) Rehder var. *deutziaefolia* (H.Lév.) Landrein; Kew Living Collection 1985-4595; (K); China, Guizhou near Guaxi Hotel Guiyang.

*Abelia macrotera* (Graebn. & Buchw.) Rehder var. *macrotera*; RBG Kew Living Collection 2002-410; (K) T. Kirkham, Cole, Flanagan & McNamara #SICH2029; China, Sichuan, Nanjiang Co., Ji Gong Shan, altitude 1670 m, 30.ix.1999.

*Abelia macrotera* (Graebn. & Buchw.) Rehder var. *mairei* (H.Lév.) Landrein; Landrein, S. #300; (K); Yunnan Kunming above Xiao Shao.

*Abelia schumannii* (Graebn.) Rehder; Kew Living Collection 1958-80619; (K); A. Vogt; *Adoxa moschatellina* L.; Kew Living Collection 1977-1046; (K); UK, Devon, Lyn river.

*Diabelia serrata* (Siebold et Zucc.) Landrein; RBG Kew Living Collection 1998-1337; (K) Yuji Kurashiga #0665; Japan, Shikoku, Mt Yokohura, altitude 520 m, x.1993.

*Diabelia spathulata* (Siebold & Zucc.) Landrein; RBG Kew Living Collection 1996-4676; (K) Townsend & Bridger #80; Japan, Honshu, Kanagawa pref., Hakone, altitude 970 m, 1.xi.1996.

*Dipelta floribunda* Maxim.; (K); Cultivated at Cambridge Botanical; *Dipelta yunnanensis* Franch.; RBG Kew Living Collection 1992-3236; (K) Fliegner #1064; China, Sichuan, Muli Co., Nongsha Shan., altitude 3140 m, x.1992.

*Heptacodium miconioides* Rehder; RBG Kew Living Collection 1987-4041; (K) Sino-American Botanical Expedition; China, Zhejiang, south of Hangzhou, 1981.

*Kolkwitzia amabilis* Graebn.; RBG Kew Living Collection 1996-1609; (K) North American China Plants Exploration Consortium s.n.; China, Hubei, Near Yan Chi He, altitude 400 m.

*Lonicera tragophylla* Hemsley; Kew Living Collection 2009-1112; (K); cultivated.

*Vesalea coriacea* (Hemsl.) T.Kim & B.Sun ex Landrein; Nova, J.A. #406; (K); Mexico, San Luis Potosi.

*Vesalea coriacea* Hemsl. var. *subcoriacea* (Villarreal) H.F.Wang & Landrein; Fairey, J. # s.n.; (K); Mexico, Nuevo Leon, 'San Isidro Canyon'.

*Vesalea floribunda* M.Martens & Galeotti; Cultivated, Royal Horticultural Society at Wisley; (K).

*Linnaea borealis* L; Cultivated, White Cottage Nurseries; (K).

*Vesalea mexicana* (Villarreal) H.F.Wang & Landrein var. *grandifolia* (Villarreal) H.F.Wang & Landrein; Nova, J.A. #471; (K); Mexico, San Luis Potosi.

*Vesalea occidentalis* (Villarreal) H.F.Wang & Landrein; Gonzales, S. #500; (K); Mexico, Durango.

*Zabelia coreana* (Nakai) Hisauti & Hara; RBG Kew Living Collection 1993-372; (K) US National Arboretum: USNA # 61752; South Korea, Chungchong Pukto, 10.vi.1989.

*Zabelia corymbosa* (Regel & Schmalh.) Makino; Landrein, S. #121; (K); Kyrgyzstan, Talas Oblast, Chychkan Valley.

*Zabelia dielsii* (Graebn.) Makino; RBG Kew Living Collection 1995-1638; (K) Simmons *et al.* #SICH 525; China, Sichuan, above Kangding, altitude 2580 m, 20.ix.1991.

*Zabelia integrifolia* (Koidz.) Makino ex Ikuse & Kurosawa; RBG Kew Living Collection 1999-4173; (K); Japan, Shikoku, Makiyama.

*Zabelia triflora* (R. Br. ex Wall.) Makino; RBG Kew Living Collection 1999-3330; (K) Sinnott, Burnell & Jackson #182; Pakistan, Palas, altitude 1970 m, 9.ix.1995.

*Zabelia tyaihyoni* (Nakai) Hisauchi & H. Hara; (K); Cultivated from Royal Horticultural Society Wisley.