Styles of Asteraceae:
A synopsis of their morphological and functional diversity

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ABSTRACT

The style of an individual flower of the Asteraceae (or Compositae) is one of the most important floral organs in two respects. 1. It is important for the systematics of the family: its characters have significantly contributed to its past and present classification. 2. The styles in their different forms are essential components in the mechanisms of secondary pollen presentation. The latter ensures an optimization of pollination by pollen portioning, a widespread phenomenon in angiosperms. After a detailed study of style morphology (by SEM and sectioning) of more than 580 species of 346 genera covering all (presently accepted) 44 tribes of the Asteraceae, a total of 49 style types are established. Bringing together both morphology and function, the style types represent eight possibilities of secondary pollen presentation, which can be subsumed into four main functional categories: deposition, brushing, pump, and pump and brushing combined. From style characters and the position of the style tip within the anther tube shortly before anthesis, it is now in most cases easy to predict the mechanism of secondary pollen presentation. Pollen protection and pollen portioning are the two advantages achieved by secondary pollen presentation. Pollen portioning, the economical use of pollen grains, is one of the most important factors in the context of optimization of pollination. Particularly the pump mechanism seems to be an optimised mechanism in terms of pollen protection and refined pollen portioning (small pollen portions exuding from the five slits between the connective appendages (when looking from above, the "five-pointed star" is observable).

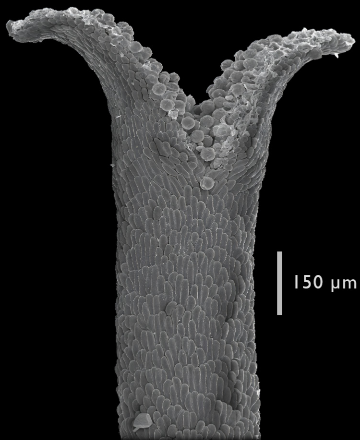
Keywords: Compositae, secondary pollen presentation

INTRODUCTION

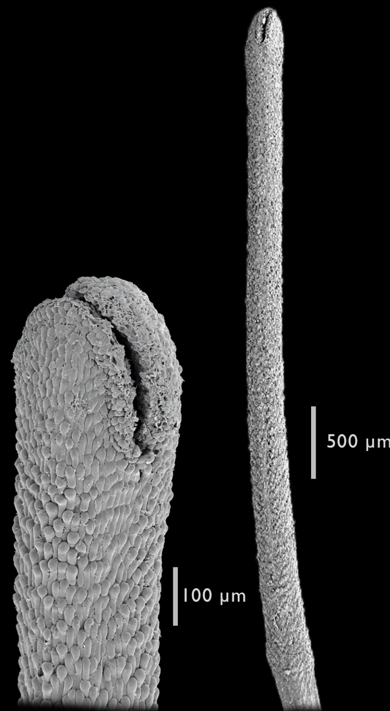
Organismal evolution is dominated by both chance events (e.g., mutation, recombination, migration, and isolation) as well as the principle of economy (optimization) through natural selection. For many years, this principle (giving rise to competition) was the focal point of interest in all our flower ecological and phylogenetic studies. We were particularly fascinated by the optimization of pollination. In this context, portioning of pollen is a widespread phenomenon. Pollen portioning is often achieved by successive opening of the anthers within a flower or by various mechanisms of secondary pollen presentation. The size of the pollen portion is connected with numerous different parameters within a highly complex, multifactorial network of correlations (see, e.g., Leins & Erbar, 2010). The most species-rich plant family, the Asteraceae, is characterized by particularly small pollen portions, which without exception are provided by secondary pollen presentation – most probably one of the key

The minimalist simplicity of the basal grade

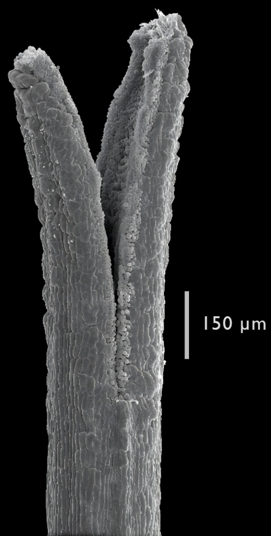
The roughness of the style surface or the presence of distinct bulges is associated with mechanisms of secondary pollen presentation in early-diverging lineages. These mechanisms include simple brushing after deposition or a pump-like action, where apical thickening of the style branches blocks the lower opening of the anther tube, preventing pollen grains from falling out downwards.



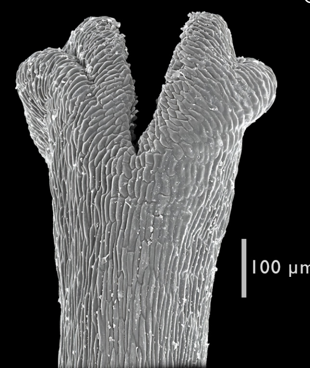
Arnaldoa macbrideana
BARNADESIEAE



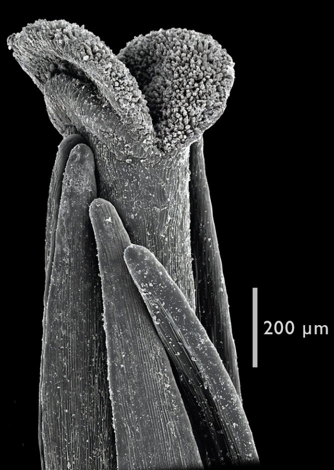
Schlechtendalia luzulifolia
BARNADESIEAE



Stifftia chrysantha
STIFFTIEAE

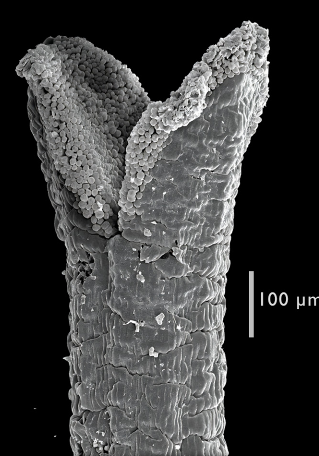


Barnadesia spinosa
BARNADESIEAE



Barnadesia arborea
BARNADESIEAE

Fulcaldea laurifolia
BARNADESIEAE



Famatinanthus decussatus
FAMATINANTHEAE

factors for the great evolutionary success of this family in terms of biodiversity (species richness) and distribution in different habitats nearly all over the world. The styles of the individual flowers play an important role within the different mechanisms of secondary pollen presentation.

Asteraceae show a great variety of styles, despite their very uniform flower structure. This diversity has fascinated synantherologists since the beginning of systematic studies of the family. Thus, style characteristics were an important feature for early classifications (into tribes) of this plant group. Most of the early studies on Compositae were illustrated by plates with details of inflorescences, floral parts, and in particular, styles. Starting with the small drawings of Tournefort (1700), Vaillant (1721), and Berkhey (1760), the illustrations became more informative when they were used for tribe characterisation by Cassini (e.g., 1826), Lessing (1832), Bentham (1873), and Hoffmann (1894). Of particular note are the six tables with excellent style drawings by Hildebrand (1870) that are often overlooked. Over time, further characteristics from different scientific disciplines (e.g., embryology, palynology, phytochemistry, and since the 1990s, molecular data) changed the taxonomic picture of the family, but style characteristics (still demonstrated by realistic illustrations) continued to play a role (e.g., Heywood et al., 1977; Bremer, 1994; Funk et al., 2009a).

We pursued two main objectives with our comprehensive study (Erbar & Leins, 2021). In a first step, we studied many styles throughout the whole family, reviving style morphology and anatomy. We considered, however, not only arrangement of the style trichomes, but also their shape and structure, the shape of the style branches and shaft as well as the location of the stigmatic tissue. We have done this with the understanding that the occurrence and position of style trichomes can be linked with the mode of secondary pollen presentation. The mechanism can often be inferred from the style characters (particularly in the pure brushing or pure pump mechanism). In a second step, we demonstrated modes of secondary pollen presentation in each of the 44 presently accepted tribes and 49 style types, the latter resulting from our comparative study. The possibilities of secondary pollen presentation found in Asteraceae can be categorized into eight mechanisms.

In this review, we focus on the most significant findings related to the structure, function, and diversity of styles in the Asteraceae family

STYLE CHARACTERS

In our comprehensive study of styles (Erbar & Leins, 2021) we presented the style morphology of more than 580 species of 346 genera covering all (presently accepted) 44 tribes of the Asteraceae, studied by SEM (each genus was at least presented by a SEM image) and, in selected examples, by histological sections. Our studies have shown that stylar features of the Asteraceae often cannot be discerned with a stereo microscope alone.

The comparison of style morphology results in the characterization of 49 style types (Fig. 1 in Erbar & Leins, 2021). We introduced a new possibility to visualize as many important style characters as possible in a diagrammatic presentation (Erbar & Leins, 2015). We use special side/front sketches of the style from shortly below the bifurcation up to the tips of the style branches (total length of the style is not recognized; see legend for Figure 2–Figure 5). These special diagrams are used in the compilations in Figure 2–Figure 5, where the style types are arranged according to the asignment to the different mechanisms of secondary pollen presentation.

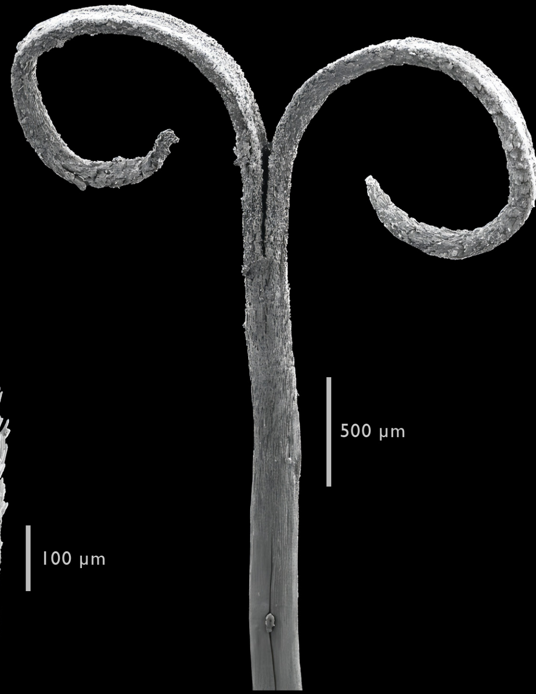
In this synopsis, we will not repeat the formalised description of the types detailed in Erbar & Leins (2021) but instead highlight essentials, sometimes in comparison to similar types although essentially maintaining the sequence of subfamilies and tribes.

Early-diverging subfamilies

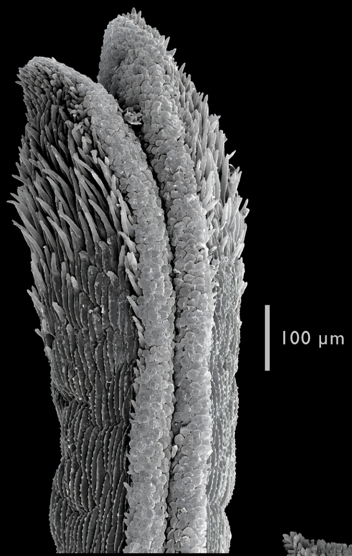
The ***Arnaldoa*-style type** (see page 2 and Figure 2) is characterized by a shortly bifid, papillate style and a continuous stigmatic tissue. The ***Schlechtendalia*-style type** (see page 2 and Figure 2) differs in that the lower end of the papillary zone is somewhat thickened. The shortly bifid styles of the ***Barnadesia* type** (see page 2 and Figure 4) are smooth. Each rounded style branch has a transverse bulge at its base and continuous stigmatic tissue. The glabrous styles of the ***Fulcaldea* type** (see page 2 and Figure 4) exhibit an ellipsoidal thickening distinctly

Trichome diversity in the basal grade

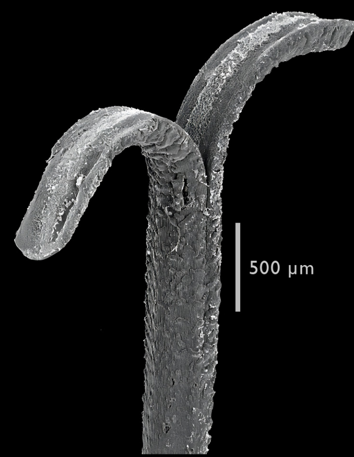
The shape and arrangement of style trichomes influence the mechanisms of secondary pollen presentation. In *Dinoseris* and *Wunderlichia*, bi- to multiseriate style trichomes permit only a deposition or simple brushing mechanism. In contrast, other styles participate in pump mechanisms.



Dinoseris salicifolia
STIFFTIEAE

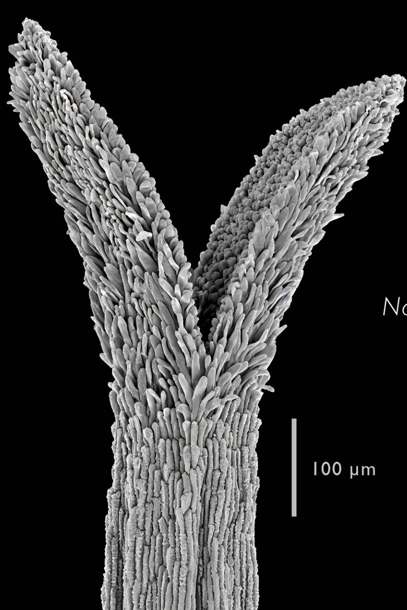


Onoseris odorata
ONOSERIDEAE

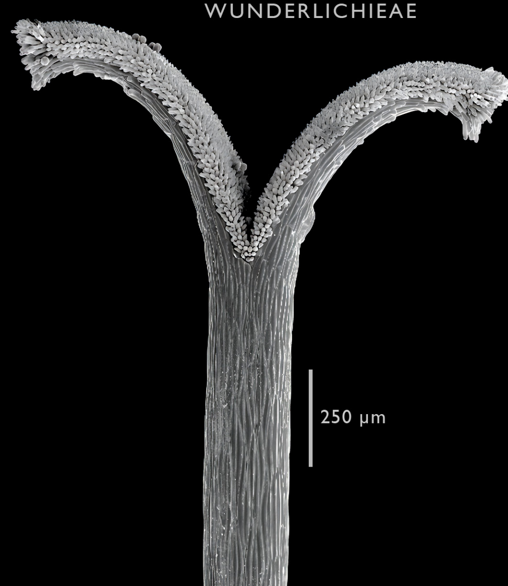


Wunderlichia mirabilis
WUNDERLICHIEAE

Nassauvia abbreviata
NASSAUVIEAE



Aphyllocladus spartioides
ONOSERIDEAE



Leucheria suaveolens
NASSAUVIEAE

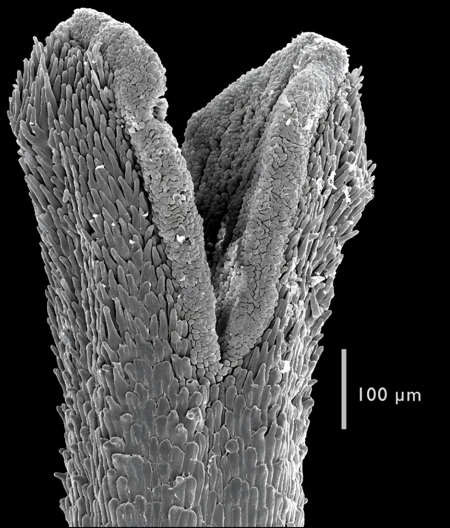
below its bifurcation. The ***Famatinanthus*-style type** (see page 2 and Figure 2) is unique with its indented epidermis (cobblestone-like epidermis units). The ***Stifftia*-style type** (see page 2 and Figure 4) is characterized by its rounded style branches somewhat thickened at their tips, rugulose due to protruding epidermal cell groups and a continuous stigmatic tissue. In the ***Dinoseris* type** (see page 4 and Figure 4), the rather long, slender styles are rugulose over their entire length (to somewhat below the bifurcation) by bi- to multiseriate style trichomes. Scale-like multi-seriate style trichomes occur in the ***Wunderlichia*-style type** (see page 4 and Figure 2), too. However, as a particular feature, the style branches adhere together by cell wall swelling of the median ventral tissue. Stigmatic tissue covers the entire **free** part of the inner surface of the style branches, i.e. except for the adhesion zone. *Aphyllocladus*- and *Onoseris*-style types do not differ so greatly in the style trichomes arrangement (in *Onoseris* type, the style trichomes are confined to the very tips of the style branches, whereas in *Aphyllocladus* type, style trichomes may cover almost the entire dorsal side of the style branches), but in the shape of the stigmatic area. In the ***Aphyllocladus*-style type** (see page 4 and Figure 5), the stigmatic tissue is continuous over the entire inner side of the style branches, whereas the ***Onoseris*-style type** (see page 4 and Figure 4) belongs to those style types, in which the stigmatic area becomes U-shaped. This happens by the median ventral tissues (i.e. a pollen tube transmitting tissue) of the style branches adhering proximally. Strictly speaking, the stigmatic tissue also covers the entire inner surface of the **free** part of the style branches. *Leucheria*- and *Nassauvia*-style types have distinctly bifid styles that are glabrous except for the very tips of their branches and stigmatic tissue covers their entire inner surface. In the ***Leucheria*-style type** (see page 4 and Figure 4), truncate style branches bear apically a tuft of trichomes, whereas in the ***Nassauvia*-style type** (see page 4 and Figure 4), acute style branches are apically provided with trichomes, which are longer at the base of the triangular apex than those ones at its dorsal surface. The styles of the ***Proustia* type** (see page 6 and Figure 5) differ in that the upper third of its style branches is covered with trichomes. In the ***Mutisia*-style type** (see page 6 and Figure 5), style trichomes cover the distal part of the (acute to subacute) style branches, too. Characteristic, however, is the stigmatic tissue arranged in two marginal bands that become confluent apically. Ventrally (between the receptive marginal bands), the inner stylar branch surface is covered with non-receptive (long) papillae (inversely U-shaped stigma on the inside of the style branches; see page 6). The ***Gochnatia*-style type** (see page 6 and Figure 4) is characterized by a stout, glabrous style that is dorsally, just beneath the apices of the short style branches, thickened. Stigmatic tissue covering the entire inner surface of the style branches projects apically and laterally forming a more or less prominent ridge.

Pertyoideae and Carduoideae

An inversely U-shaped stigma realized by the loss of receptivity of the papillate median tissue, as well found in the *Mutisia* type as described above (although unusual in the basal tribes), also characterises the ***Ainsliaea*-style type** (Figure 5). In *Ainsliaea* type, in contrast to *Mutisia* type, style trichomes extend from the apex of its rather short, rounded to truncate style branches to somewhat beneath the bifurcation. Styles of ***Oldenburgia* intermedia type** (Figure 4) and ***Oldenburgia papionum* type** (see page 6 and Figure 4) are rather thick with short, apically rounded style branches, thus resembling somewhat the *Gochnatia* type, but differ from it and from each other by the occurrence of trichomes. Common to all three types is a dorsal swelling beneath the apex of each stylar branch. In *Oldenburgia intermedia* type, the surface of the style branches is glabrous but with some papillae apically, in *O. papionum* type, the style is papillate-hairy from the apex of the style branches to somewhat below the bifurcation with even some longer, acute trichomes apically. Both *Oldenburgia*-style types differ from the *Gochnatia*-style type primarily by the arrangement of the stigma (two stigmatic marginal bands confluent apically in *Oldenburgia* type versus stigmatic tissue covering the entire inner surface of the style branches in *Gochnatia* type). In the stigma arrangement both *Oldenburgia* types resemble the *Ainsliaea* type. The style of the ***Dicoma* type** (Figure 4) is distinctly bifid, with a short-pilose distal part of its style branches and long style trichomes forming a subapical tuft beneath the pilose area. The median ventral tissues of the style branches adhere proximally, resulting in stigmatic tissue confined to the very distal area and the peripheral areas next to the adhesion

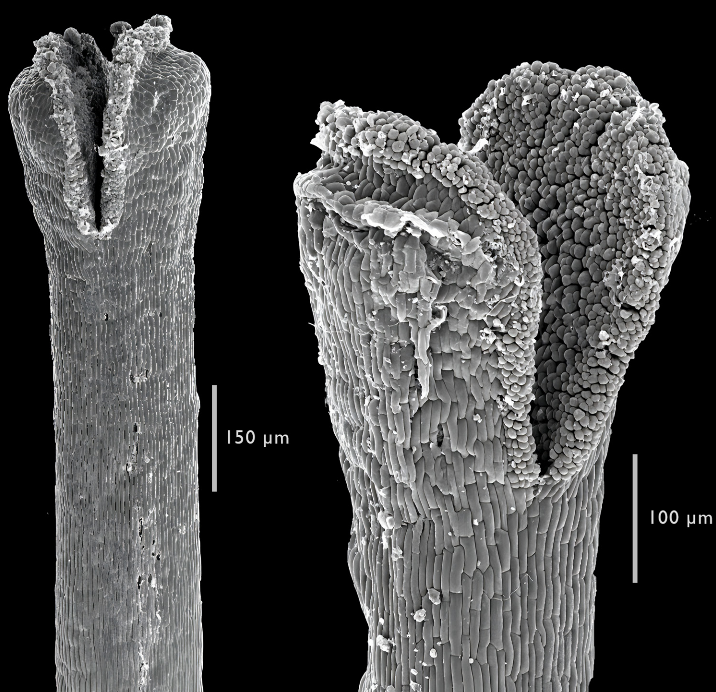
Variation in style surface and hair arrangement in early diverging clades: endless diversity

The substantial styles with apically rounded (*Oldenburgia*) or thickened (*Gochnatia*) style branches are well-suited for a pump mechanism in secondary pollen presentation. In *Mutisia* and *Proustia*, trichomes located on the upper part of the style branches facilitate the brushing out of pollen grains, enhancing the efficiency of the pump mechanism, which initially causes pollen to protrude from the anther tube.

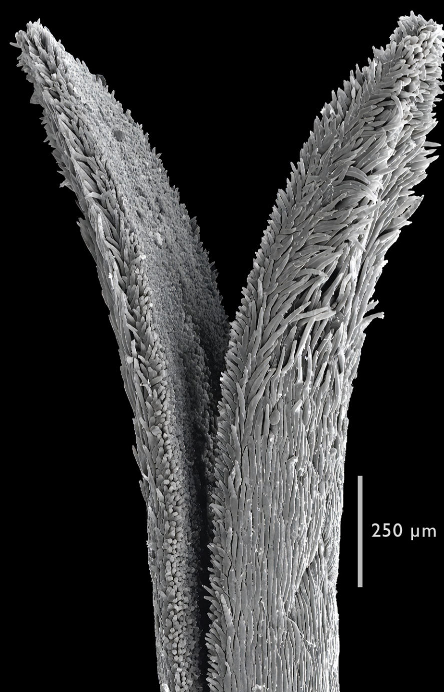


Oldenburgia papionum
OLDENBURGIEAE

Proustia pyrifolia
NASSAUVIEAE



Gochnatia foliolosa
GOCHNATIEAE



Mutisia acuminata
MUTISIEAE

zone. ***Macledium/Cyanus***, ***Arctium***, ***Centaurea***, ***Carduus***, and ***Staehelina* types** (see page 9 and Figure 5) are characterized by a distinct collar of trichomes – either directly beneath the style branches (as in *Macledium/Cyanus*-, *Centaurea*- and *Staehelina*-style types) or further down on the stylar shaft (as in *Arctium*- and *Carduus*-style types). *Macledium/Cyanus*- and *Arctium* types show a continuous stigmatic area, whereas in *Centaurea* and *Carduus* types the median ventral tissues (i.e., a pollen tube transmitting tissue) of the style branches adhere proximally so that stigmatic tissue is confined to the very distal area and the peripheral areas next to the adhesion zone; the stigmatic margins fold back at late anthesis when stigma is receptive. In the *Staehelina* type, the stigmatic tissue is arranged in two marginal stripes that are confluent apically, ventrally between the receptive marginal bands the inner stylar branch surface is covered with non-receptive papillae. In *Centaurea* L. species, style branches and adhesion zone differ in length (compare *C. scabiosa* L. and *C. sphaerocephala* L., see page 9). Styles of the ***Berardia*** and ***Cousinia* types** (Figure 5) share stigmatic tissue covering the entire inner surface of the style branches, but differ in size and arrangement of style trichomes. In the *Berardia*-style type, style trichomes extend from below the stylar tips distinctly beneath the bifurcation. In the *Cousinia*-style type the style trichomes on the branches and the shaft are disorderly scattered, some longer ones are observable in the region beneath the bifurcation, shorter ones extend well onto the stylar shaft.

Vernonioideae, Cichorioideae, and Corymbioideae

The ***Arctotis*-style type** (see page 9 and Figure 3) is easy to identify by its barrel-shape and the collar of long trichomes at the base of this thickened apical part. Stigmatic papillae cover the entire inner surface of style branches. ***Vernonia* type** (see page 9 and Figure 3) and ***Cichorium* type** (Figure 3) share distinctly bifid, slender styles that are evenly pilose along the style branches and a large part of the stylar shaft (hairy zone usually considerably extending beneath the bifurcation point). Whereas in the *Cichorium* style type stigmatic tissue covers the entire inner surface of the style branches from the stylar tip to the bifurcation point, the uppermost part of the style branches is free of stigmatic papillae (but covered with style trichomes). The **Corymbium-style type** (see page 11 and Figure 3) differs from the *Cichorium* type in that the hairy part of the stylar shaft is somewhat thickened.

Asteroideae

Stigmatic tissue arranged in two discrete lateral lines (sometimes broad stigmatic bands nearly touch each other, but at least one sterile cell line inbetween) characterizes the *Anthemis/Senecio*-, *Jacobaea*-, *Aster*-, *Madia*-, *Gynura*-, *Gaillardia*-, *Cosmos*-, and *Eupatorium*-style types. Differences exist in the shape of the stylar branch tips and in the arrangement of style trichomes. In the ***Anthemis/Senecio* type** (see page 11 and Figure 4), the truncate, obtuse or conical style branches are glabrous except for the very tips of its style branches: (sub)apically they are provided with a tuft of trichomes. Styles of the ***Jacobaea* type** (Figure 5) feature, in addition to the (sub)apical tuft of trichomes, shorter trichomes distributed dorsally along the branches. These trichomes extend from approximately one-third of the branch's length to its entire length, with their density and length gradually decreasing toward the proximal end. *Aster*-, *Madia*-, *Gynura*-, *Gaillardia*-, *Cosmos*-, and *Eupatorium*-style types are characterized by stylar branch appendages that may be very specialized. The glabrous style branches of the ***Aster* type** (see page 28 and Figure 5) generally have hairy acute appendages, triangular (or oblong) in shape (in their style, the North American *Symphyotrichum* species, formerly placed among the asters, and the European asters match). In the ***Madia* type** (Figure 5), the hairy style branches exhibit narrowly triangular appendages. Long, gradually tapering appendages provided with long, obtuse, sometimes clavate trichomes that are loosely scattered over the appendages are characteristic for styles of the ***Gynura* type** (see page 11 and Figure 3). In the ***Gaillardia* type** (Figure 5), the linear, tapering appendages of the style branches that are much longer than the stigmatic area and have a tuft of longer trichomes at the base of the hairy appendage are most distinctive. The ***Cosmos* type** (see page 11 and Figure 5) is characterized by a hairy triangular stylar branch appendage, whose tip is markedly defined. The ***Eupatorium* type** (see page 13 and Figure 3) is clearly recognisable by enlarged papillate-pilose stylar branch appendages (filiform, lanceolate or clavate, sometimes slightly broadened or flattened distally) above the stigma-bearing area that is mostly shorter than the appendages. The last eleven style types exhibit apically confluent stigmata (inversely U-shaped stigmata). ***Doronicum***, ***Dimorphotheca***, and ***Ligularia* types** (see page 13, Figure 4, and Figure 5) share truncate to sligthly obtuse style branches with an apically (or subapically) tuft of trichomes. In *Doronicum* and *Dimorphotheca* types, the styles are glabrous except for the very tips of their style branches, whereas in the *Ligularia* type, additionally to the tuft of trichomes, shorter trichomes are found dorsally over the entire stylar branch length. In the *Doronicum* and *Ligularia* types, stigmatic tissue is continuous over a major portion of the inner surface (apart from a small proximal area), in the *Dimorphotheca* style the stigmatic tissue is arranged in two discrete lateral lines confluent apically. The ***Osmitopsis* type** (see page 13 and Figure 4) shares with the *Doronicum* and *Ligularia* types the arrangement of the stigmatic tissue, but the style branches are truncate with an apical small tuft of trichomes. A distinctive feature is the presence of large-headed glands on the style. ***Adenostyles***, ***Garuleum***, ***Inula***, ***Blumea*** and ***Pluchea* style types** (see page 13, page 14, Figure 3, and Figure 5) share subacute to rounded stylar branch tips and style trichomes that extend beyond the tips. In *Adenostyles*, *Garuleum*, *Blumea*, and *Pluchea* types, style trichomes even cover the entire length of the style branches, extending to their base or even just beneath the bifurcation (in *Blumea* and *Pluchea* style types trichomes rounded to blistered and more or less densely packed). The styles of the *Inula* type are glabrous except for the distal part of its rounded, sometimes apically somewhat broadened style branches (style trichomes not reaching the bifurcation). The *Inula* type shares with the *Adenostyles* and *Blumea* types that the stigmatic bands run parallel to each other before they are confluent apically (near the stylar branch tip). In the *Garuleum* and *Pluchea* types, the stigmatic bands are separated only over a short distance; the stigma is continuous over the largest part of the stylar branch. Triangular to oblong hairy stylar branch appendages and hairy zones extending halfway down their style branches characterize the ***Calea*** and ***Helianthus* type** (see page 14, and Figure 5). In the *Calea* type, the stigmatic tissue is arranged in two distinct lateral lines that converge subapically but remain confined to a narrow band. In the *Helianthus* type, the "confluent zone" is so extensive that the stigmatic tissue covers most of the inner surface, leaving only a more or less wedge-shaped proximal area devoid of receptive papillae. Notably, in *Helianthus* species, these non-receptive areas vary in size. Unlike all other style types with apically confluent stigmatic areas, the outermost tip of the inner surface of the style branches in both the *Calea* and *Helianthus* types is free of stigmatic papillae.

The value of style characters for more detailed phylogenetic considerations – just a few remarks

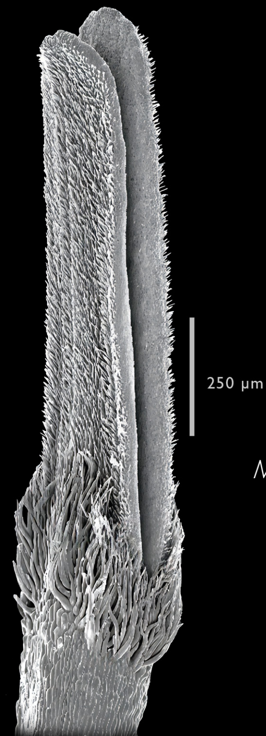
Plotting our style types onto a modern phylogenetic tree as reference (Figure 1), we can find astonishing correspondences in several clades, but also deviations in others; the latter showing e.g., parallelisms. Nevertheless, the whole family can be divided into four groups with regard to style types (for a detailed discussion and references see Erbar & Leins, 2021).

The "basal" (i.e., early-diverging) subfamilies Barnadesioideae, Famatinanthoideae, Stifftioideae, Mutisioideae, Wunderlichioideae, Gochnatioideae, and Hecastocleidoideae: Apart from the *Mutisia*-style type (Figure 5), the other 14 style types established for this group are confined to these subfamilies (yellow squares in Figure 1). In addition, the *Adenostyles*-style type (Figure 5)occurs in the African members of the Mutisieae; this style type is otherwise only found in Asteroideae. The high diversity of stylar shapes within the seven subfamilies, which represent only about 3% of the species of Asteraceae, is remarkable. Styles are completely smooth or sub-papillate or hairy. The *Gochnatia*-style type, which is the most frequent one, occurs in several clades. Two tribes (Barnadesieae and Nassauvieae) are characterized by several style types, which, however, do not occur in the other tribes. *Onoseris*- and *Wunderlichia*-style type are characterized by an adhesion zone of the median ventral tissues of the inner surface of the style branches. Apart from the basal subfamilies, adhesion zones are only found in three style types within the Carduoideae (*Dicoma*-, *Centaurea*-, *Carduus*-style type). The monotypic genus *Famatinanthus* exhibits with its cobblestone-like surface a unique style type, supporting the elevation to tribe and subfamily rank due to *cpDNA* data (Panero et al., 2014).

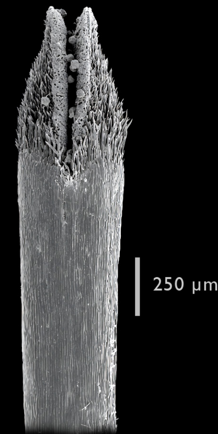
Pertyoideae and Carduoideae: The eleven style types established for these two subfamilies

Distinctive features: collar versus long hairy branches and shaft

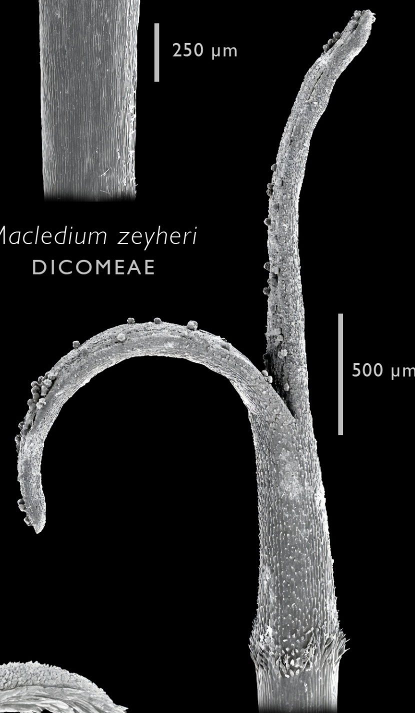
The five carduoid style types are distinguished by a prominent collar of trichomes located at various positions along the style shaft, facilitating a specialized pump mechanism for secondary pollen presentation. In *Arctotis*, the entire pollen output is displayed on the pilose, barrel-shaped stylar region, exemplifying a unique brushing mechanism. Long, filiform style branches with sweeping trichomes extending below the bifurcation point characterize the styles of Cichorieae and Vernonieae. The distinction lies in the arrangement of the stigmatic papillae: in the *Vernonia* type, the stigmatic papillae do not extend to the uppermost part of the style branches.



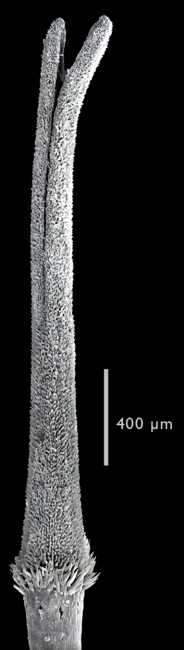
Centaurea sphaerocephala
CARDUEAE



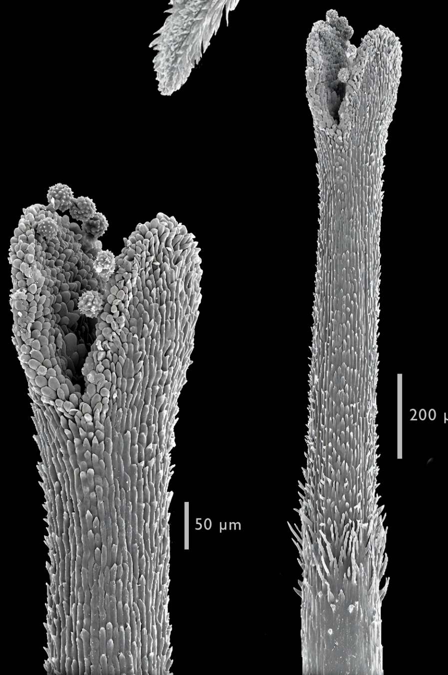
Macledium zeyheri
DICOMEAE



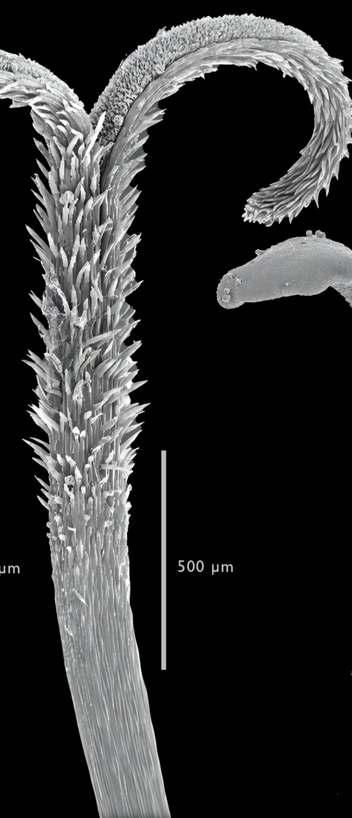
Arctium tomentosum
CARDUEAE



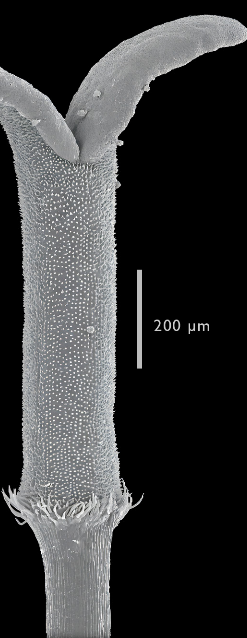
Carduus defloratus
CARDUEAE



Staehelina dubia
CARDUEAE



Vernonia noveboracencis
VERNONIEAE



Arctotis venusta
ARCTOTIDEAE

(purple squares in Figure 1) are not found in other subfamilies. Five of them are characterized by a collar of long style trichomes, either at or distinctly beneath the bifurcation point.

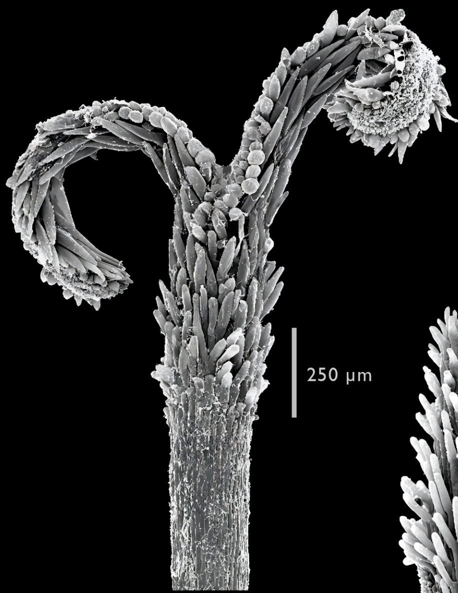
The adhering of the style branches can be used to distinguish two related genera, namely *Centaurea* L. and *Cyanus* Mill. In *Cyanus*, the style branches are separate and divergent, whereas they adhere to each other for most of their length (mediated by their median ventral tissues) in *Centaurea* (see page 9). *Cousinia* Cass. (about 600 species) is the largest genus of the tribe Cardueae and one of the largest of the whole Compositae. We cannot confirm that the *Cousinia* style resembles the vernonioid style type sensu Bremer (1987, 1994), in which the sweeping trichomes are all of equal length and extend well below the bifurcation as stated in the literature. We observed that long, slender trichomes are disorderly scattered along the style branches and shorter trichomes extend well onto the stylar shaft; some longer trichomes are found in the region beneath the bifurcation, thus suggesting a not well-developed collar of trichomes. Thus we established a style type of its own for *Cousinia* (Figure 5).

Vernonioideae, Cichorioideae, and Corymbioideae: The four style types (red squares in Figure 1) occurring in these subfamilies are confined to them. The prevailing style types (*Vernonia* and *Cichorium* type, see page 9) differ only by the fact that in the *Vernonia* type the continuous stigmatic tissue does not reach the uppermost part of the inner surface of the style branches; instead, this uppermost part is covered with style trichomes. Although the difference in the extent of the stigmatic tissue is small, the occurrence of the two style types matches the new delimitation of subfamilies Vernonioideae and Cichorioideae (Mandel et al., 2019). Only the narrowly defined Cichorioideae. exhibit the long, filiform styles with the stylar branch inner surfaces completely covered by stigmatic papillae (Figure 3).

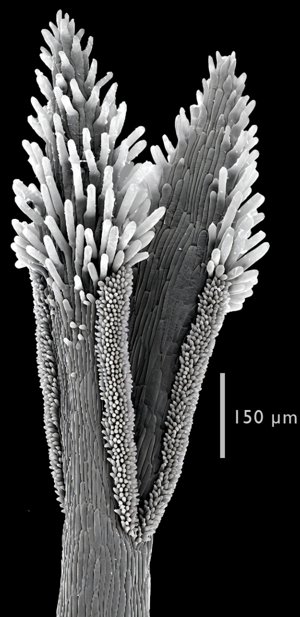
Asteroideae: The subfamily is a well-defined group (both morphologically and molecularly) and contains about 62% of the species in the family (about 15,500 species in a total of about 1,230 genera; Pelser & Watson, 2009). The largest and most widely distributed tribes are the cosmopolitan Astereae and Senecioneae. The 20 tribes presently accepted in the large subfamily Asteroideae are categorized in three main lineages recognized as supertribes Senecionodae, Asterodae, and Helianthodae. Of the 20 style types occurring in this largest subfamily, 18 of them are confined to this group (orange squares in Figure 1). Most of the styles have either truncate to slightly obtuse style branches with a (sub)apical tuft of trichomes or style branches with hairy, triangular or oblong appendages. The arrangement of the stigmatic tissue in two small ventro-marginal bands is common in the Asteroideae and, important to emphasize, restricted to this subfamily (see page 24). However, further arrangements can be found: broad stigmatic bands, nearly touching each other, or separate lines become apically confluent. In a few instances, the stigmatic tissue seems to cover the whole inner surface. Detailed investigations, mainly by histological cross sections, reveal that there is at least one sterile cell line in the middle of the inner stylar branch surface or a small wedge-shaped sterile area at the proximal end of the stylar branch (Erbar & Leins, 2016).

Senecionodae (with only tribe Senecioneae) exhibit six style types, five of which are restricted to the Asteroideae and one (*Gynura* type) is only found in this tribe. Only the *Adenostyles* type is found in a basal group (*Gerbera* L., *Perdicium* L., Mutisieae). *Senecio* sect. *Jacobaea* and *Senecio* s.str. form two clades that are only distantly related (DNA sequence data, Pelser et al. 2002). As regards style characters, we distinguish two style types. The typical senecioid style, as usually referred to in the literature, is two-branched with two parallel stigmatic bands on the inside, and the glabrous style branches are truncate with (sub)apical sweeping trichomes. This corresponds to our *Anthemis*/*Senecio*-style type (Figure 4). More often we found the *Jacobaea*-style type (Figure 5), which differs by having trichomes not only in the (sub)apical tuft of trichomes, but shorter trichomes extending dorsally along the branches (about third of the branch up to its entire length, density and length of the trichomes decreasing proximally). Both style types occur outside the Senecioneae (see red squares with numerals 32 and 33 in Figure 1).

The four tribes of Asterodae exhibit nine different style types. Three of them are restricted to a single genus (*Dimorphotheca* type #38, *Garuleum* type #37, *Osmitopsis* type #40). The large tribe Astereae is moderately diverse in style morphology.

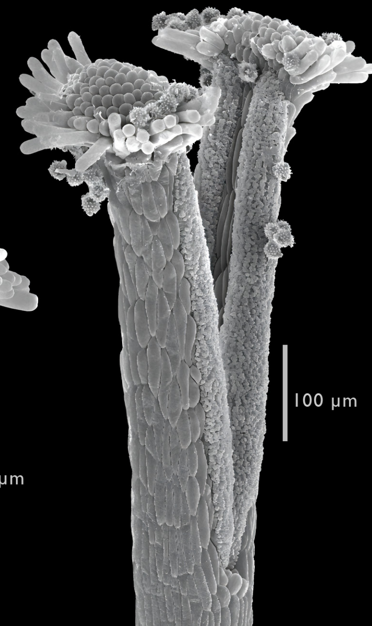


Corymbium africanum
CORYMBIEAE



Symphyotrichum dumosum
ASTEREAE

Anthemis maritima
ANTHEMIDEAE

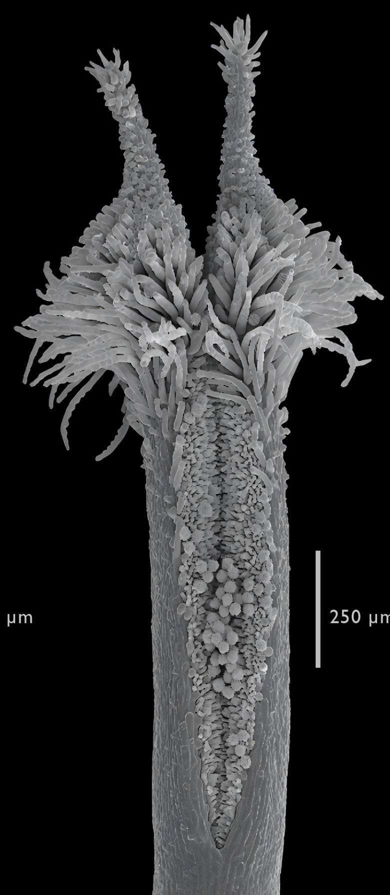


Senecio vernalis
SENECIONEAE

Style branch appendages in Asteroideae: to be, or not to be

Style branch tips exhibit various morphologies across different genera. In *Corymbium*, they taper to a point, while in *Anthemis* and *Senecio*, they are truncate and feature an apical tuft of trichomes. Some species possess distinct appendages: *Symphyotrichum* displays acute triangular, hairy appendages; *Cosmos* has triangular appendages with a well-defined tip; and *Gynura* presents long, gradually tapering appendages with sparsely distributed trichomes.

Gynura splendens
SENECIONEAE



Cosmos bipinnatus
COREOPSIDEAE

Most members, however, are characterized by one type, namely the *Aster*-style type. Anthemideae, in contrast, are uniform throughout (apart from *Osmitopsis*).

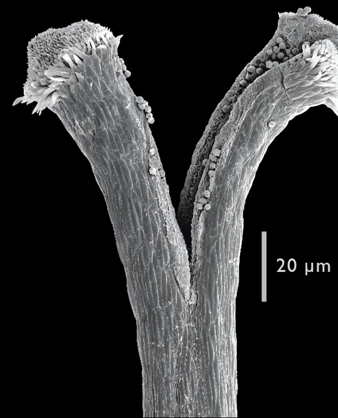
Supertribe Helianthodae (15 tribes exhibit 16 different style types), the largest of the three supertribes of Asteroideae, is dominated by the so-called "Heliantheae alliance" (12 tribes exhibit 11 different style types) sensu Panero (2007) that contains about 5500 species or about 20%–25% of the species recognized in Asteraceae, including sunflowers (*Helianthus* L. , sneezeweeds (*Helenium* L.), tarweeds (*Madia* Molina), and eupatoriums (*Eupatorium* L.). Any obvious systematic pattern in the distribution of these style types is absent. In addition, variability in style types does not correlate with the tribe size. Five different style types are found in the moderately large tribe Inuleae as well as in the large tribe Heliantheae. In the largest tribe within Helianthodae and one of the largest tribes in Asteraceae, namely Eupatorieae, only one style type occurs that is moreover rather distinct from all other types.

Concerning Asteroideae as a whole, again, no obvious systematic pattern in the distribution of style types becomes obvious. Twenty style types are found in the twenty tribes (Figure 1). From outside the Asteroideae, only the *Mutisia* type is found (in Helenieae). Apart from the *Adenostyles* type, all other style types are confined to the Asteroideae. Only three style types occur in all three supertribes: the *Anthemis*/*Senecio* type (in six tribes), the very similar *Jacobaea* type (in three tribes) and the *Aster* type (in four tribes). Style types with separate parallel stigmatic lines characterize seven types, but style types with apically confluent stigmata (inversely U-shaped stigmata) as well as almost continuous stigmata (only proximally a small, wedge-shaped area of sterile tissue) are found.

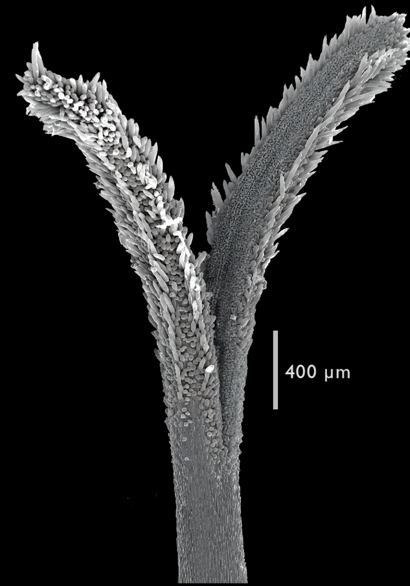
Concerning Asteraceae as a whole (Figure 1), there are only two "outliers", i.e., style types occurring in two of the four groups mentioned, namely the *Mutisia* and *Adenostyles* types (see page 6, page 13, and Figure 5). The *Mutisia* type occurs in the Mutisieae investigated (except for *Mutisia coccinea* A.St.-Hil.), in two genera of the Inuleae (i.e., *Calostephane* Benth., *Pegolettia* Cass.) and in one genus of the Helenieae (i.e., *Pelucha* S.Watson). Within the Asteroideae, the *Adenostyles* type occurs in Senecioneae, Astereae, and in the Inuleae. Thus, Mutisieae and Inuleae are the two tribes, where these style types occur side by side. Both style types are relatively similar; they match the stigma arrangement, but differ in the stylar hair arrangement, namely style trichomes positioned in the distal part of the style branches (*Mutisia* L.f.) versus hairy zone extending to the base of the branches or just beneath the bifurcation (*Adenostyles* Cass.). In addition, they differ in the shape of the style trichomes. They are obtuse in the *Adenostyles* type (as common in the Asteroideae) and more or less acute in the *Mutisia* type (in the early-diverging groups rounded as well as acute style trichomes are found). The different hair arrangement is the key factor in determing the relative involvement of pumping and brushing in secondary pollen presentation (see chapter on secondary pollen presentation).

STYLE TYPES AND SECONDARY POLLEN PRESENTATION

We were interested in the styles especially with regard to their function in secondary pollen presentation (e.g., Leins & Erbar, 1990, 2006, 2010). In secondary pollen presentation, covering different portioning mechanisms, pollen is relocated from the anthers (the place of their origin) onto another floral structure, as the flower is still unopened. In all Asteraceae, it is the style that is involved in secondary pollen presentation. In bud stage, the introrse anthers open inwards, releasing the pollen grains into the anther tube (in addition to the capitulum inflorescence, the anthers adhering in a tube characterizes the family throughout). An elongating style pushes and/or brushes the pollen out of the anther tube. It is mainly the arrangement of the style trichomes that is correlated to a function in the process of secondary pollen presentation. After detailed studies, we assigned the 49 style types to one of the mechanism of secondary pollen presentations (see Figure 2–Figure 5); for a more detailed discussion see Erbar & Leins (2021). The mechanisms differ in the absence or presence of style trichomes, in the length of a hairy zone and in the position of the stylar tip at anther dehiscence. Pure pump and brushing mechanisms are easily

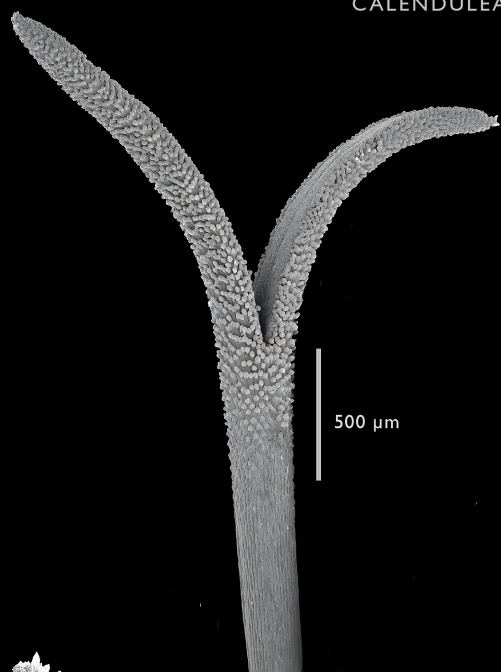


Dimorphotheca cuneata
CALENDULEAE

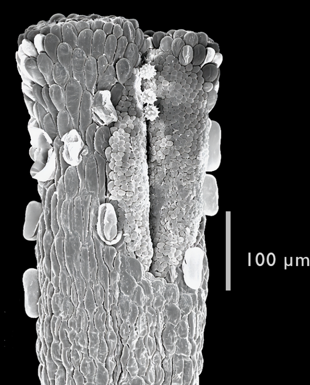


Ligularia dentata
SENECIONEAE

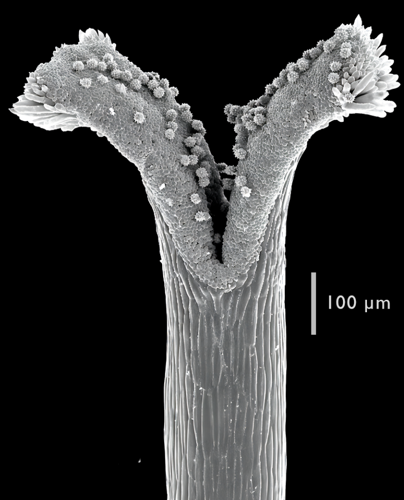
Eupatorium cannabinum
EUPATORIEAE



Adenostyles alliariae
SENECIONEAE



Osmitopsis asteriscoides
ANTHEMIDEAE



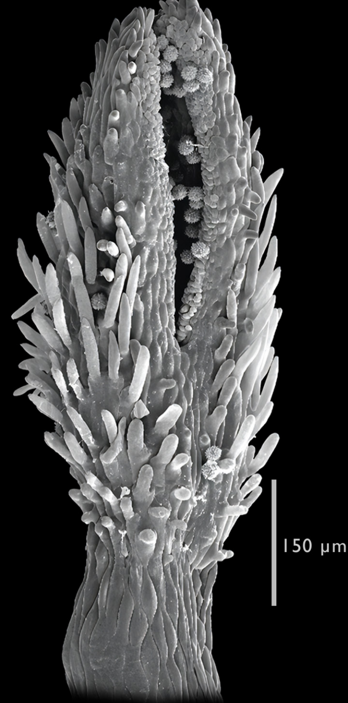
Doronicum hungaricum
SENECIONEAE

Unique styles in Asteroideae

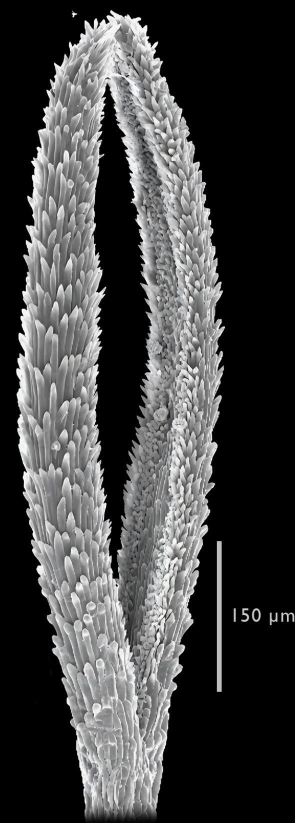
The *Doronicum* and *Ligularia* style types are present in two tribes, while the Eupatorium type is exclusive to the Eupatorieae. The *Dimorphotheca* and *Osmitopsis* types are characteristic of specific genera; notably, *Osmitopsis* is distinguished by its prominent glandular structures. The *Adenostyles* type exhibits an unusual distribution, occurring in both the Mutisieae and certain tribes within the Asteroideae.

A frequently overlooked trait in Asteroideae: apically confluent stigmata

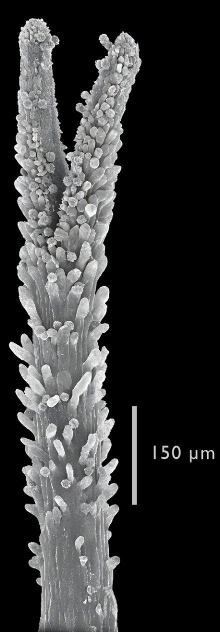
Within the Asteroideae, 11 distinct types exhibit variation in the size of the confluent zone. In the *Inula* and *Blumea* types, the confluent zone is restricted to the upper portion of the style branch, characterized by a long furrow of non-receptive cells separating discrete lateral stigmatic lines. In the *Garuleum* and *Pluchea* types, the lateral lines converge apically into a broad band, leaving only a small, wedge-shaped area proximally free of stigmatic papillae. In the *Calea* and *Helianthus* types, the uppermost tip, located above the confluent band, remains devoid of stigmatic papillae.



Garuleum schinzii
CALENDULEAE

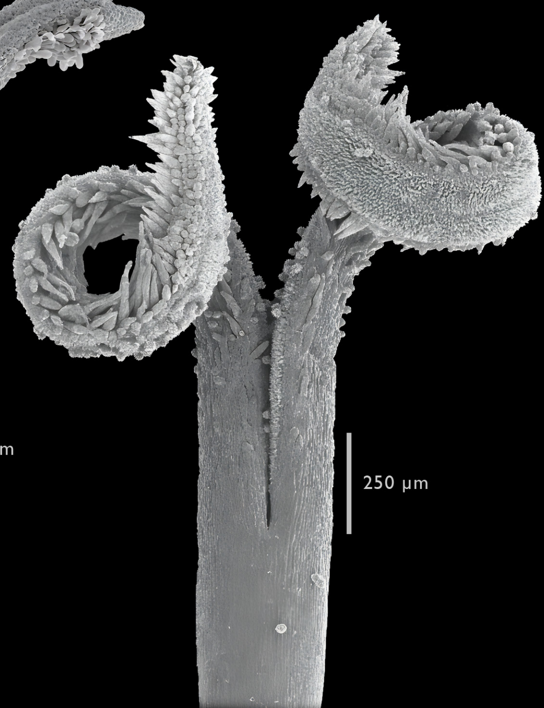


Blumea gariepina
INULEAE



Pluchea chingoyo
INULEAE

Calea cymosa
NEUROLAENEAE



Helianthus annuus
HELIANTHEAE

Inlua helenium
INULEAE

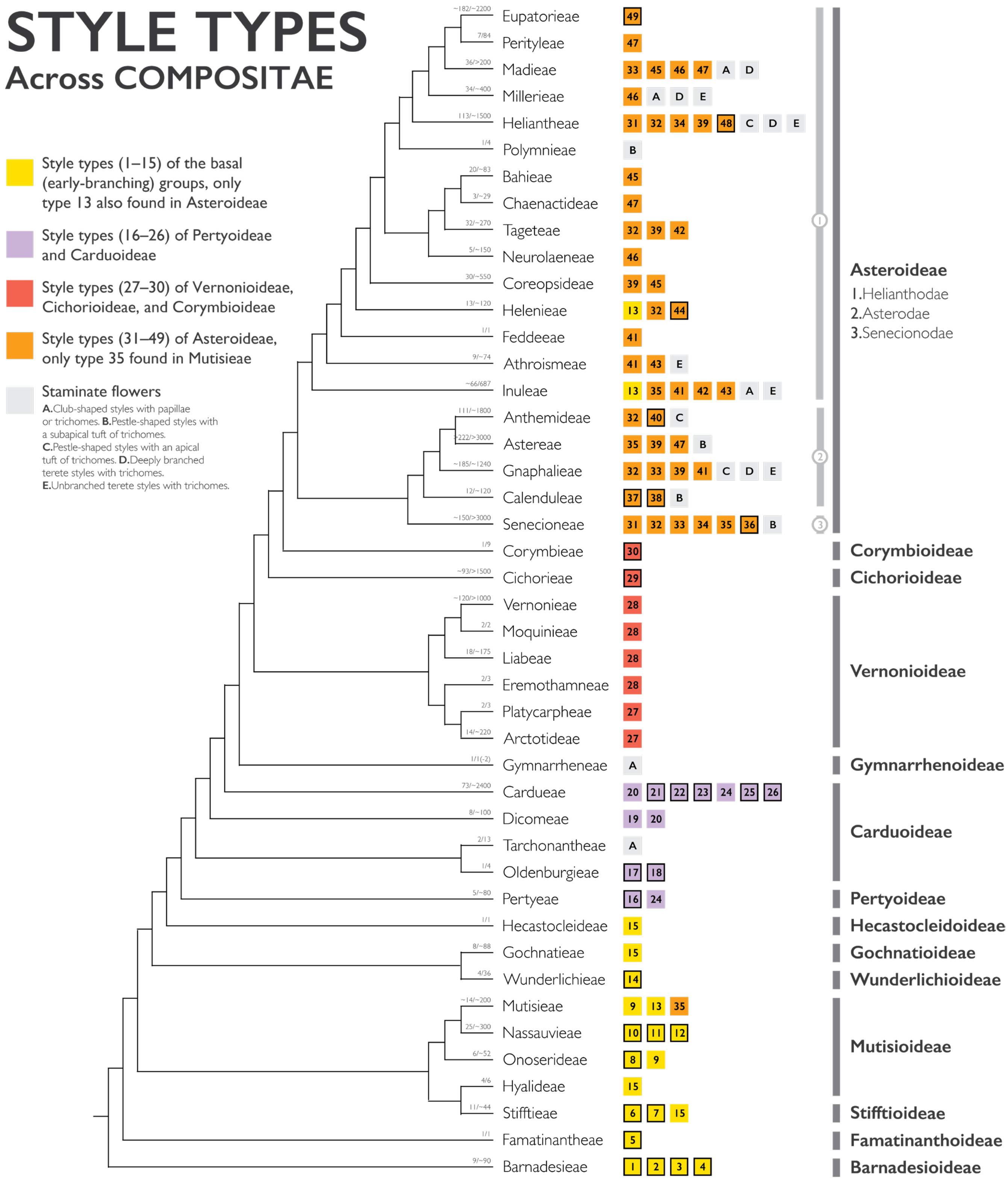


Figure 1. A generalized phylogenetic tree of Asteraceae (based on Funk et al., 2009b; Panero et al., 2014; Mandel et al., 2019) onto which the different style types are plotted. Only two style types occur in a basal tribe and in Asteroideae: type 13 (*Mutisia* type) in Mutisieae as well as in Inuleae and Helenieae, type 35 (*Adenostyles* type) in Senecioneae, Astereae, and Inuleae as well as Mutisieae; black outlined symbols: style type confined to the corresponding tribe.

distinguishable by the arrangement of the style trichomes: style trichomes only at the very tips of the style branches versus styles with a long hairy part. There are, however, more possibilities of pollen presentation in the Asteraceae. A pump and a brushing mechanism can be combined, a deposition mechanism as well as combinations of deposition and brushing occur. In addition, pump and brushing mechanisms can be modified to become different from the common type.

Deposition, brushing, pump and their combinations and variations are categorized into eight mechanisms (Figure 6–Figure 13): deposition/simple brushing mechanism, brushing mechanism, pump mechanism (with blocking trichomes), pump mechanism (with apical thickening), special pump mechanism, special brushing mechanism, combination of pump and brushing mechanism, combination of pump and slightly brushing mechanism. The most important preconditions and preadaptations, respectively, for all mechanisms include: radial symmetry of the flower bud, existence of a complete androecial whorl in a flower (i.e., five stamens), more or less long introrse anthers united by the coherence of their cuticles into a tube, protandry of the flower the release of pollen occurs prior to the receptivity of the stigmatic surface). All mechanisms share three sequential processes before and/or during anthesis: 1. elongation of the filaments or stamen-corolla tube or both before the anthers open to bring these into the right position; 2. opening of the anthers, discharge of the pollen grains into the anther tube; 3. elongation of the style, by this presenting the pollen grains to the pollinating agent (see, e.g., Leins & Erbar, 2006, 2010).

In the **deposition/simple brushing mechanism** (Figure 6), the anther tube surrounds the style in bud stage. Just before the opening of the anthers, it only needs a moderate elongation of the filaments or stamen-corolla tube so that the long anther tube is level with the long papillate part of the style. After the deposition of the pollen grains onto the style, these are then carried out of the anther tube by stylar elongation and presented to the pollinators on the outside of the style. As with all other mechanisms, the style branches later on separate to expose the stigmatic surface. The rounded flat papillae or scale-like bi- to tri-seriate style trichomes (Figure 2) are not suitable for efficient sweeping. But the capturing of pollen grains may be assisted by abundant pollenkitt, by which the pollen grains adhere to the style Figure 6C). *Arnaldoa macbrideana* Ferreyra (Barnadesieae) is an example of the mechanism.

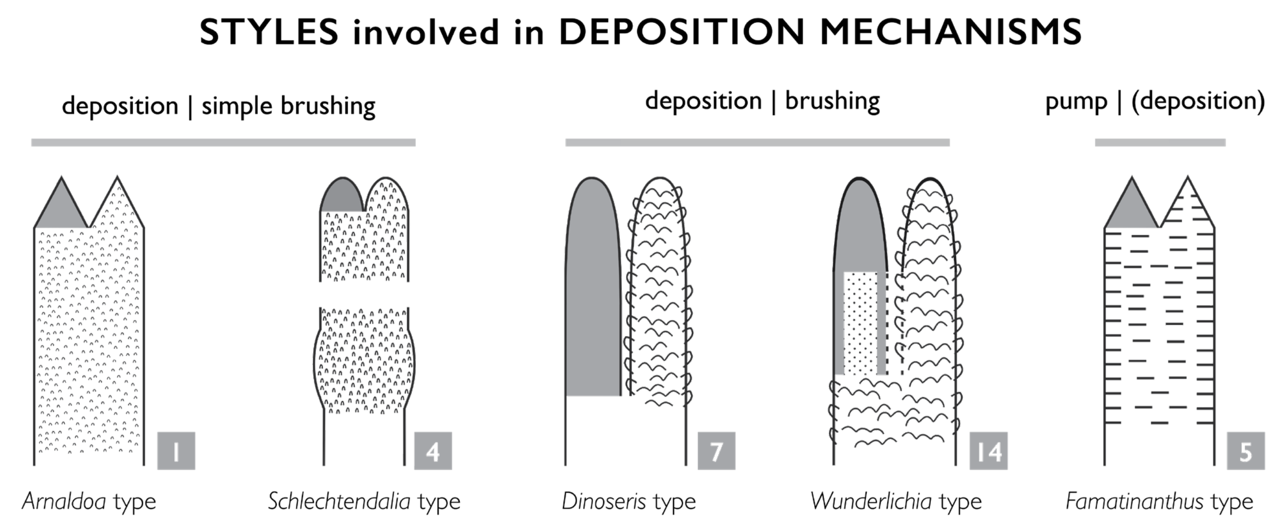
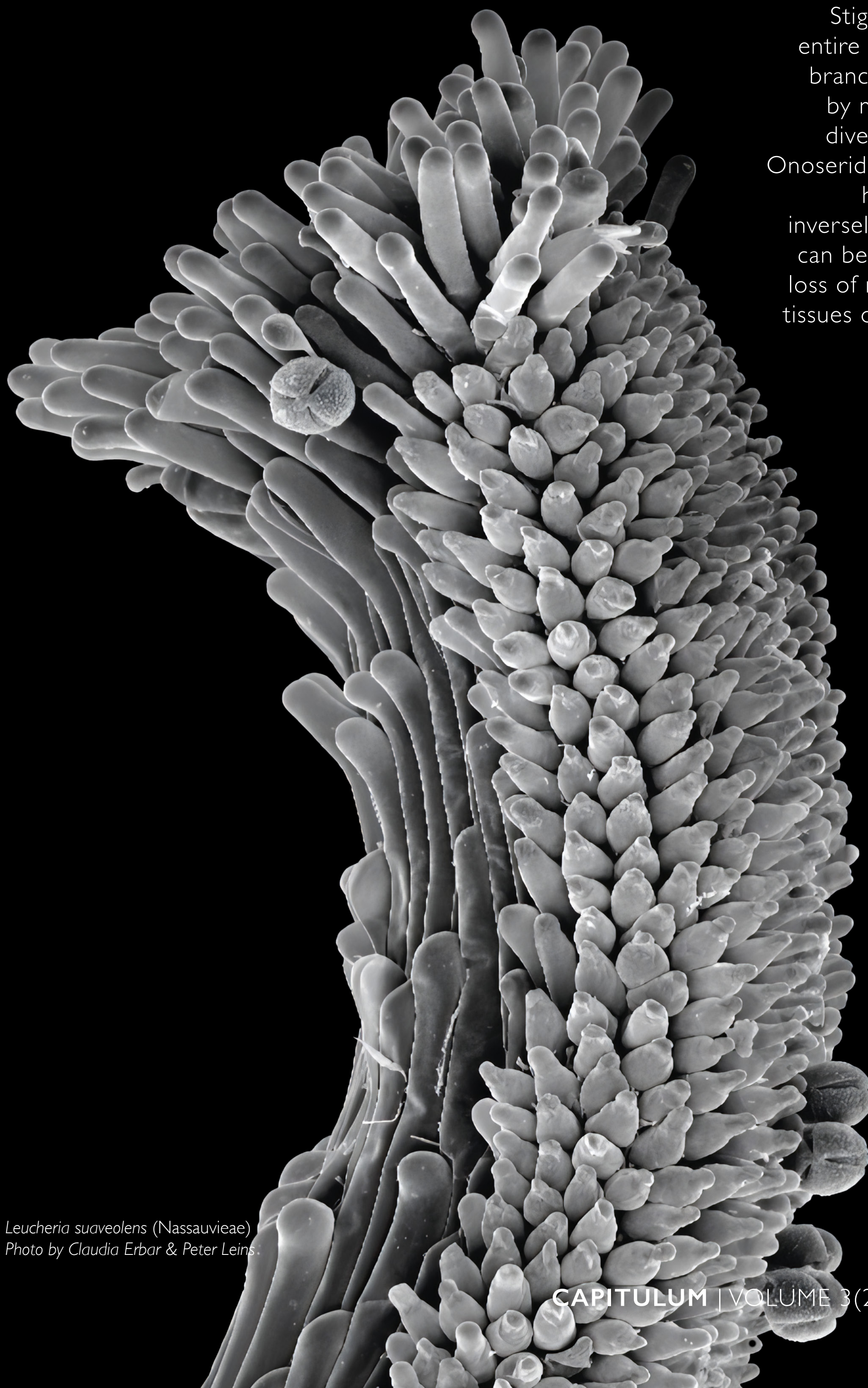


Figure 2. Compilation of style types involved in deposition mechanisms. The numerals correspond to the style type numbering in Erbar & Leins (2021). Illustrations feature single-plane diagrams rather than perspective drawings. The style branches are rotated 90° and flipped open, allowing both the adaxial (ventral, left) and abaxial (dorsal, right) views to be displayed in the same diagram. Variations in the length of the style branches are only approximated in the illustrations, and additional stylar parts are not depicted. The stigmatic tissue is shaded in grey, and any adhesion zone, if present, is indicated with dotted markings. Dorsal protrusions, as observed in the *Barnadesia*, *Gochnatia*, and both *Oldenburgia* style types, are shifted into a transverse plane, positioned on the left and right sides of the style branches.

A continuous field of stigmatic papillae

Stigmatic tissue covering the entire inner surface of the style branches is a character shared by most species in the early-diverging tribes. In Mutisieae, Onoserideae, and Wunderlichieae, however, a quite different inversely U-shaped arrangement can be found, realized either by loss of receptivity of the median tissues or by median adhesion of the style branches.

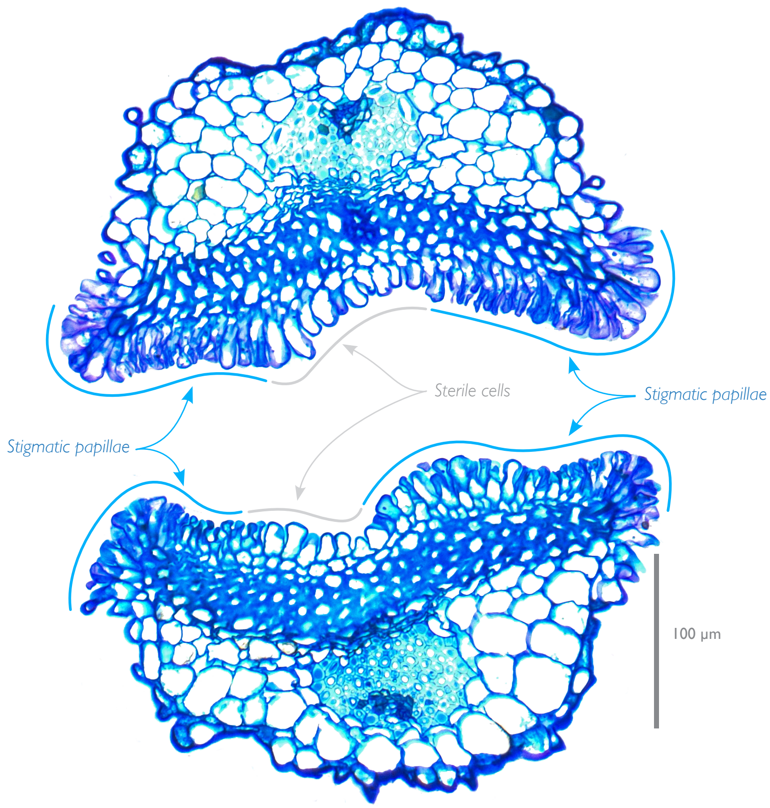


Leucheria suaveolens (Nassauvieae)
Photo by Claudia Erbar & Peter Leins

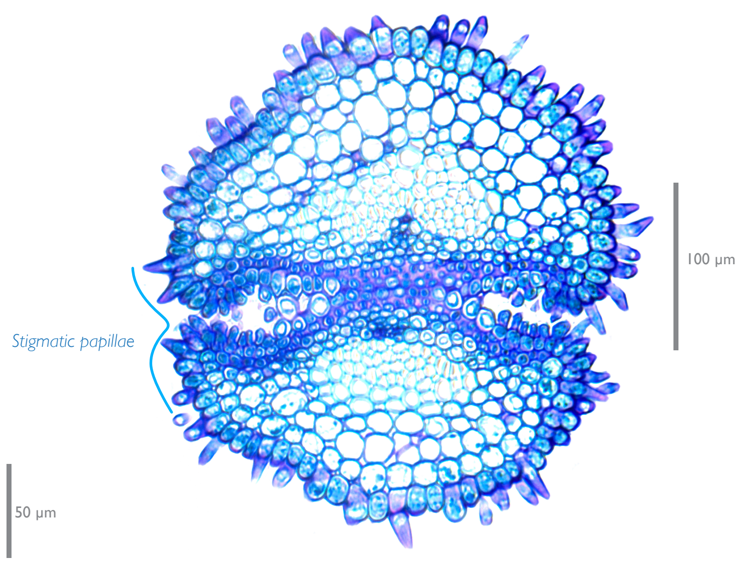
Into the LIGHT

An overlooked feature came to light by histological sections, revealing that stigmatic tissue, in addition to covering the entire inner surface of the style branch or being arranged in two discrete lines, can also take an inversely U-shaped configuration, realized in various ways.

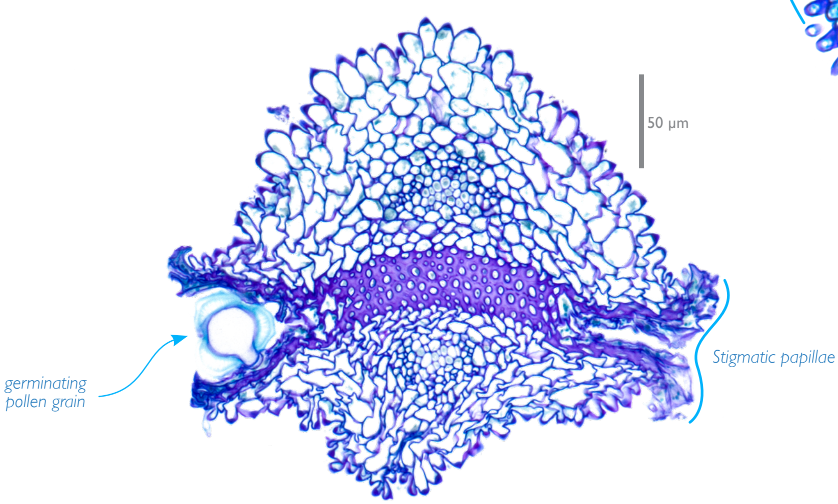
In *Oldenburgia*, two marginal stigmatic bands converge apically. In *Onoseris* and *Carduus*, the U-shaped stigma results from the adhesion of the style branches to each other, facilitated by their median ventral tissues.



Oldenburgia papionum
OLDENBURGIEAE



Carduus defloratus
CARDUEAE



Onoseris odorata
ONOSERIDEAE

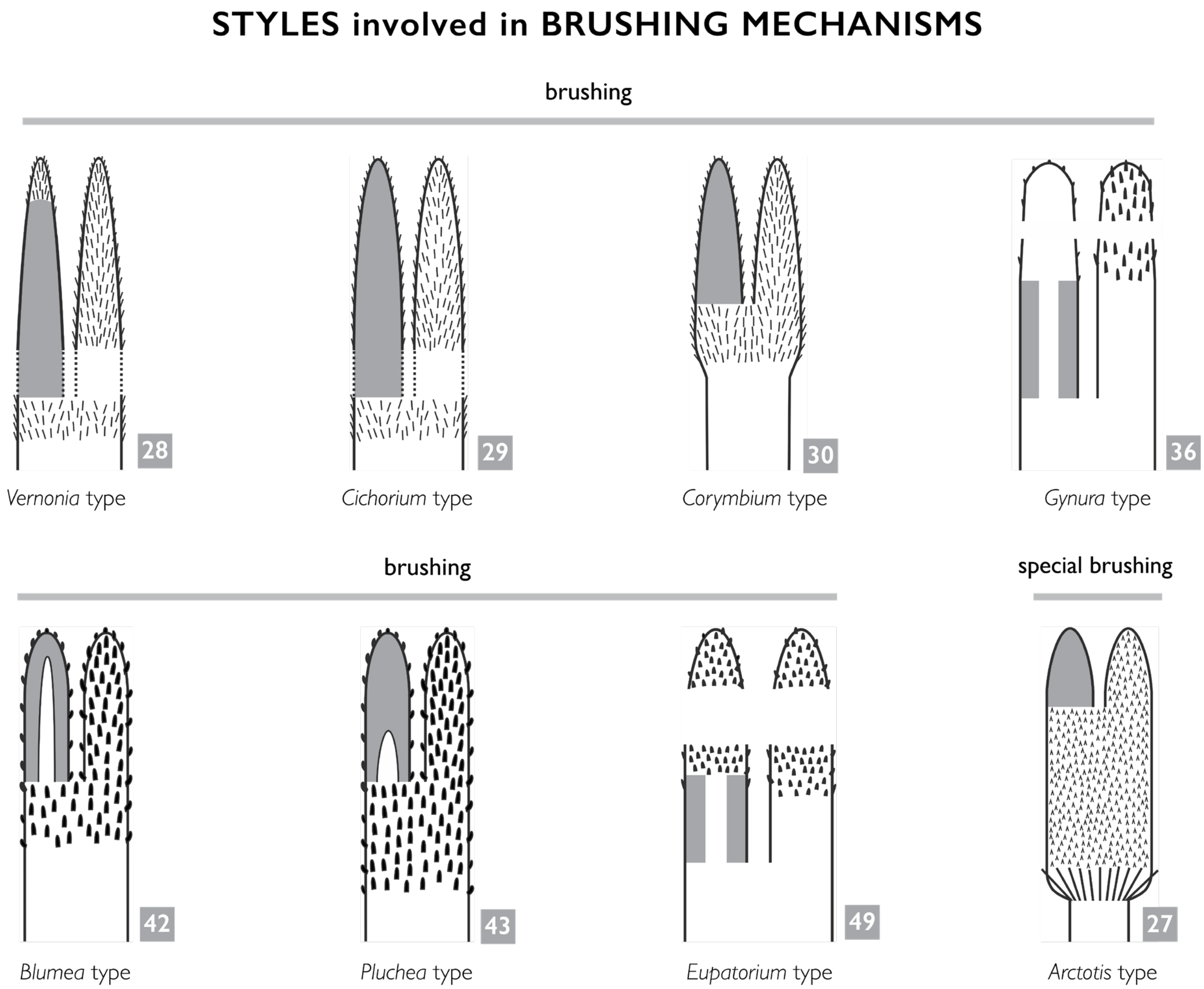


Figure 3. Compilation of style types involved in brushing mechanism The numerals correspond to the style type numbering in Erbar & Leins (2021). Illustrations feature single-plane diagrams rather than perspective drawings. The style branches are rotated 90° and flipped open, allowing both the adaxial (ventral, left) and abaxial (dorsal, right) views to be displayed in the same diagram. Variations in the length of the style branches are only approximated in the illustrations, and additional stylar parts are not depicted. The stigmatic tissue is shaded in grey, and any adhesion zone, if present, is indicated with dotted markings.

In the **brushing mechanism** (Figure 7), the back of the style branches and the upper part of the stylar shaft bear short acute trichomes. In old flower buds, the tip of the style is above the top of the anther tube. Shortly before anthesis, growth of the filaments and the stamen-corolla tube brings the anthers up to the same level as the hairy part of the style so that the style nearly fills the anther tube. The hairy part of the style is as long as the anther tube. The anthers open and the pollen is partly loaded onto the style trichomes. By elongation of the style, the pollen grains are little by little completely swept out of the anthers by the brushing (sweeping) trichomes, which now are somewhat spreading. Thus, once the pollen is released, the hairy part of the style emerges out of the anther tube and gradually presents the pollen on its outside surface (Figure 7B). *Cichorium intybus* L. serves as a representative example of this mechanism, characteristic of the entire tribe Cichorieae.

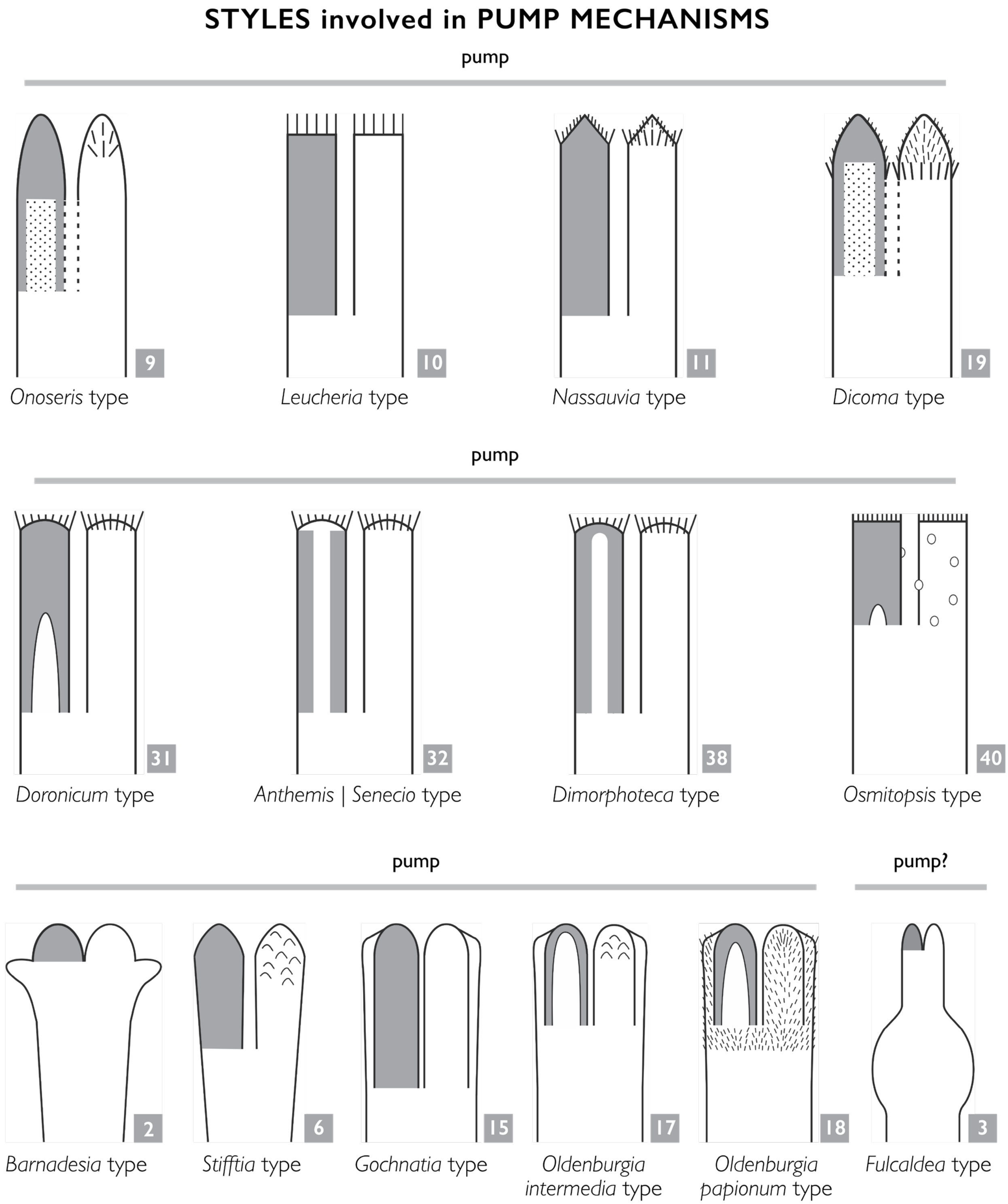


Figure 4. Compilation of style types involved in pump mechanisms. The numerals correspond to the style type numbering in Erbar & Leins (2021). Illustrations feature single-plane diagrams rather than perspective drawings. The style branches are rotated 90° and flipped open, allowing both the adaxial (ventral, left) and abaxial (dorsal, right) views to be displayed in the same diagram. Variations in the length of the style branches are only approximated in the illustrations, and additional stylar parts are not depicted. The stigmatic tissue is shaded in grey, and any adhesion zone, if present, is indicated with dotted markings.

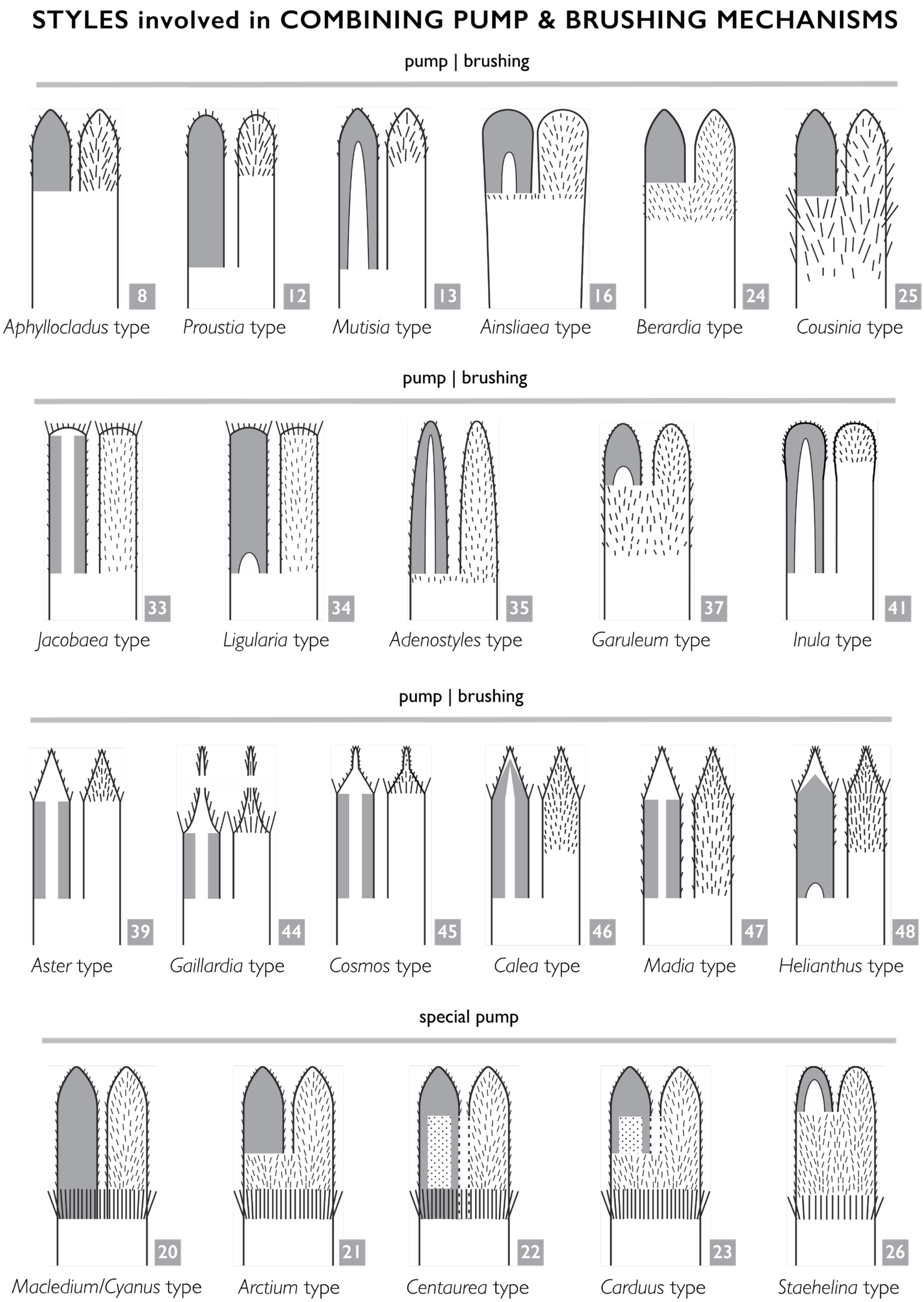


Figure 5. Compilation of style types involved in mechanisms combining pumping and brushing. The numerals correspond to the style type numbering in Erbar & Leins (2021). Illustrations feature single-plane diagrams rather than perspective drawings. The style branches are rotated 90° and flipped open, allowing both the adaxial (ventral, left) and abaxial (dorsal, right) views to be displayed in the same diagram. Variations in the length of the style branches are only approximated in the illustrations, and additional stylar parts are not depicted. The stigmatic tissue is shaded in grey, and any adhesion zone, if present, is indicated with dotted markings.

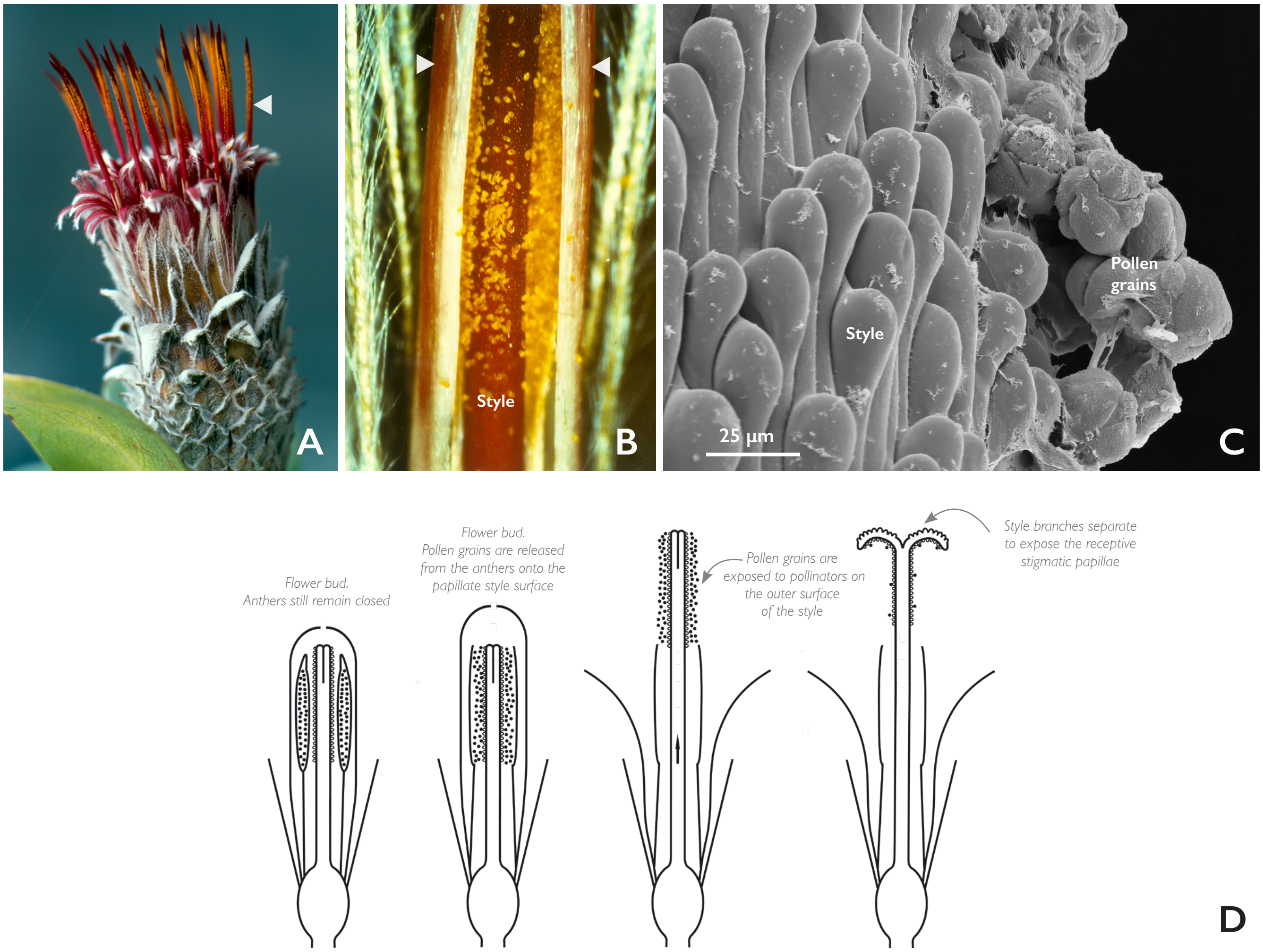


Figure 6. Deposition/simple brushing mechanism, exemplarily shown in *Arnaldoa macbrideana*. **A.** Lateral view of capitulum with styles exposed; arrow indicates pollen grains on outer surface of the style. **B.** Close up of anther tube, indicated with arrows, part of the pollen already deposited onto the style. **C.** SEM image of style with papillate surface and pollen grains attached by abundant pollenkitt. **D.**The deposition/simple brushing mechanism (see Figures 7–13) is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

A pure pump mechanism comes in two variations (Figure 8 and Figure 9). In the **pump mechanism with apical style trichomes** (Figure 8), the style branches mostly are truncate. In older flower buds, the tip of the style is more or less on the same level with the top of the anther tube. Shortly before anthesis, growth of the filaments – supported or even substituted by growth of a stamen-corolla tube – raises the anther tube higher than the stylar tip (Figure 8A). Thus, the tips of the style branches block the lower opening of the anther tube (Figure 8B) and prevent pollen grains falling out when the anthers open and the pollen grains are released into the cavity of the anther tube as a temporary receptacle. After the pollen is released within the anther tube, the elongating style functions as a piston, gradually expelling the pollen from the tube.

Since the connective appendages arch over the anther tube, the pollen exudes from the five slits between them, thus forming a five-pointed star when viewed from above (Figure 8C). As a typical example *Senecio vernalis* Waldst. & Kit. is shown. In general, the **pump mechanism with apical thickening of the style branches** (Figure 9) corresponds to the common pump mechanism (Figure 8). The difference is that the blocking of the lower opening of the anther tube is achieved by apical (or subapical) thickenings of the style branches. Typical examples are *Barnadesia* Mutis ex L.f. species (Figure 9).

In the **special pump mechanism** of Cardueae and Dicomeae (Figure 10), the tip of the style and its proximal collar of trichomes can be found within the anther tube prior to the opening of the anthers. When the corolla opens, growth of stamen-corolla tube and filaments lifts the anther tube to a position in which the style tip remains within the anther tube but the collar of trichomes beneath the hairy stylar part is on the same level as the lower end of the anther tube (Figure 10A). Since the ring of trichomes creates a zone effectively thicker than the distal part of the style, it well blocks the lower opening of the anther tube. The anthers open and the pollen is released into the cavity of the anther tube. The pollen-containing box is extended by considerable apical connective appendages. The growing style then pushes the pollen gradually out (Figure 10B). Pollen grains first protrude from five slits between the connivent anther appendages (Figure 10C) and then pollen heaps are observable above the anther tube's upper end (Figure 10D, yellow arrow). During this early phase of anthesis, small pollen portions

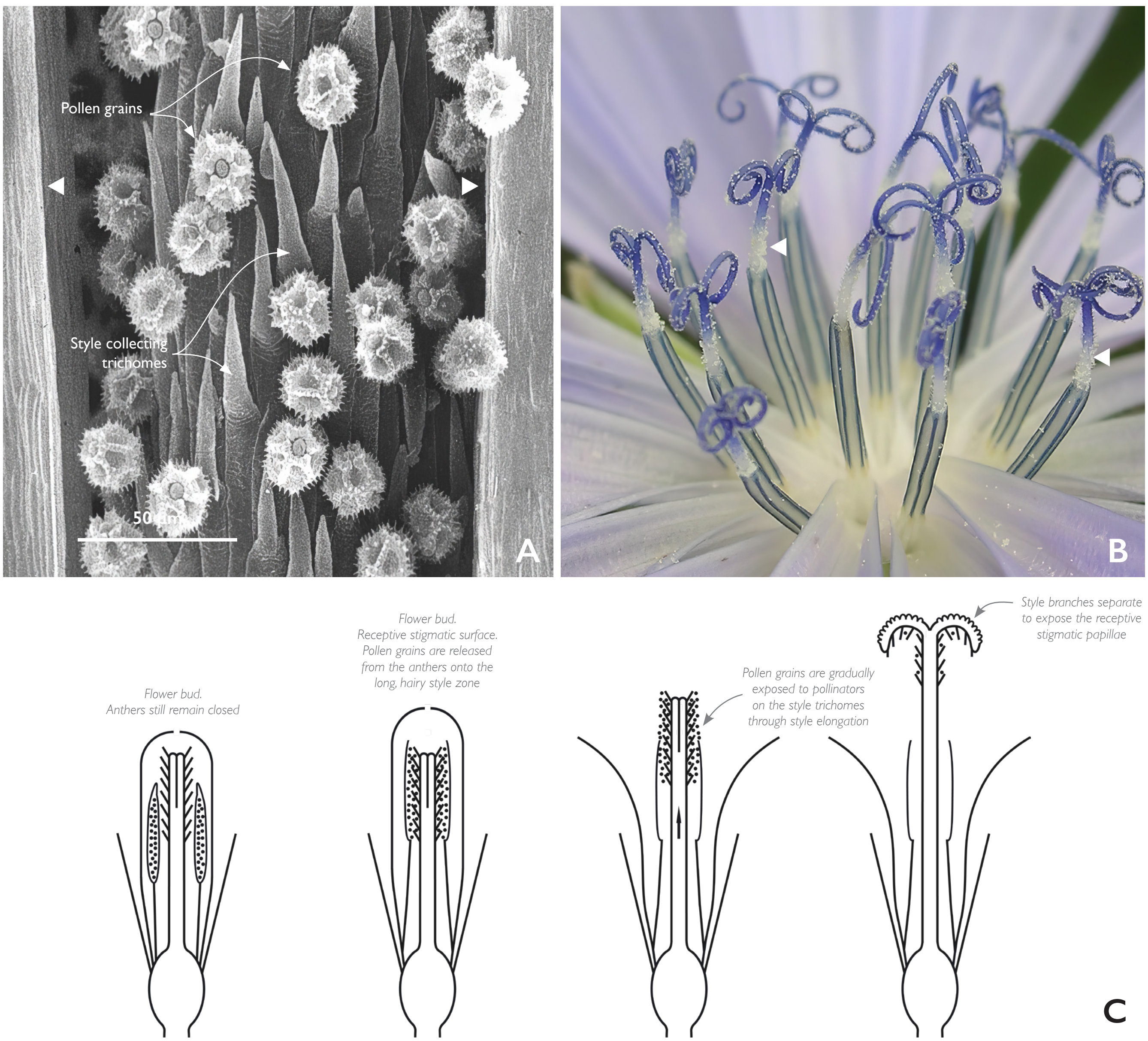
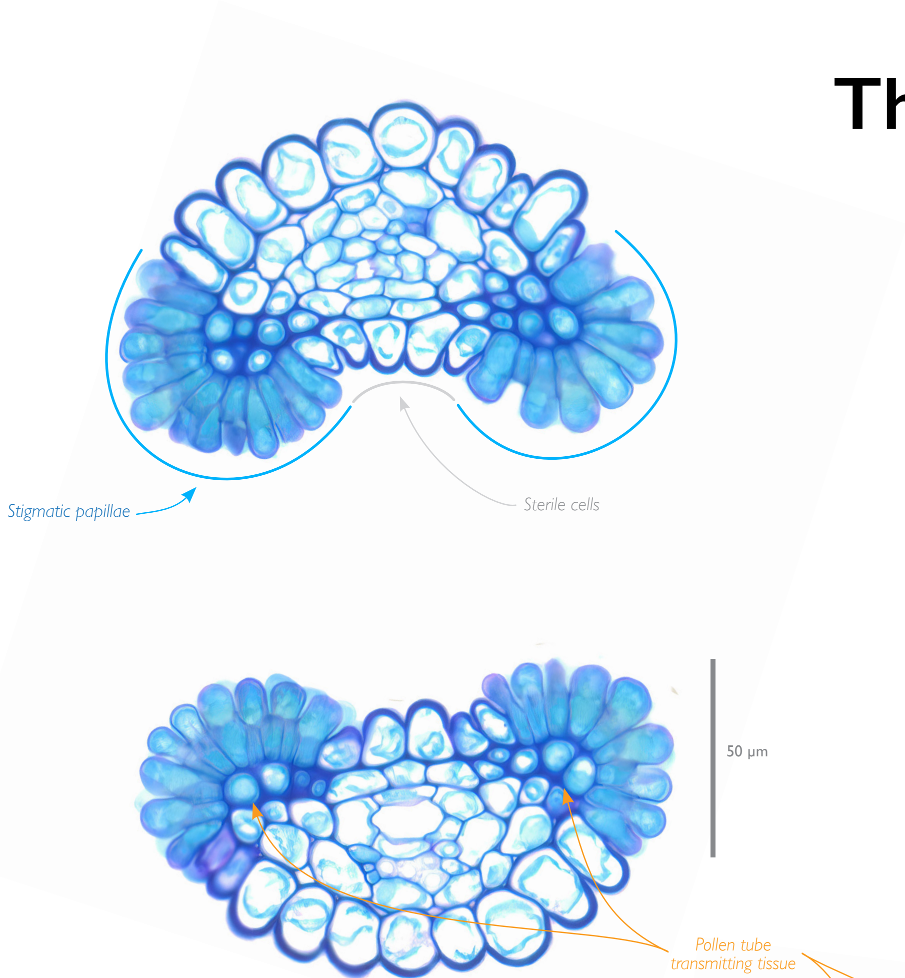


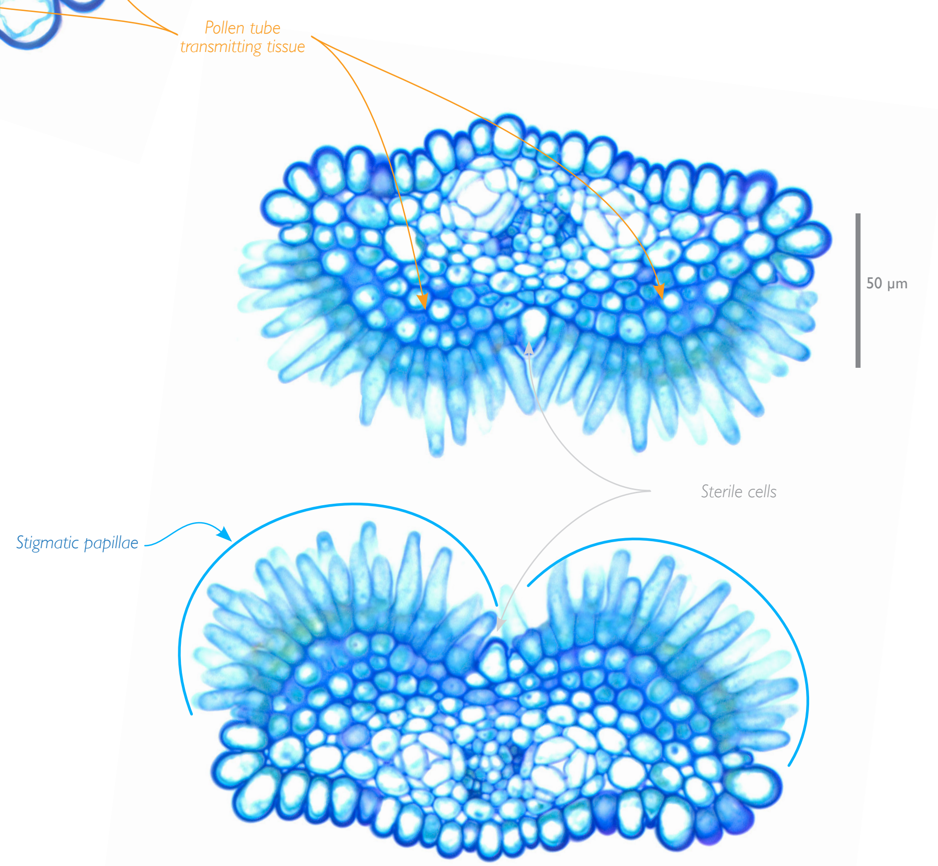
Figure 7. Brushing mechanism, exemplarily shown in *Cichorium intybus*. **A.** SEM image of style shaft still inside anther tube (marked with arrows). **B.** Oblique view of center of capitulum; white arrows indicate pollen grains on style shaft **C.** The brushing mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

The quintessential stigmatic marginal bands

In most members of the Asteroideae, the stigmatic tissue is organized into two relatively small ventro-marginal bands, as seen in *Anthemis*. However, a recurring trend toward an increased stigmatic surface is evident. For instance, in *Bidens*, the broad stigmatic bands nearly meet but are separated by at least one sterile cell line.



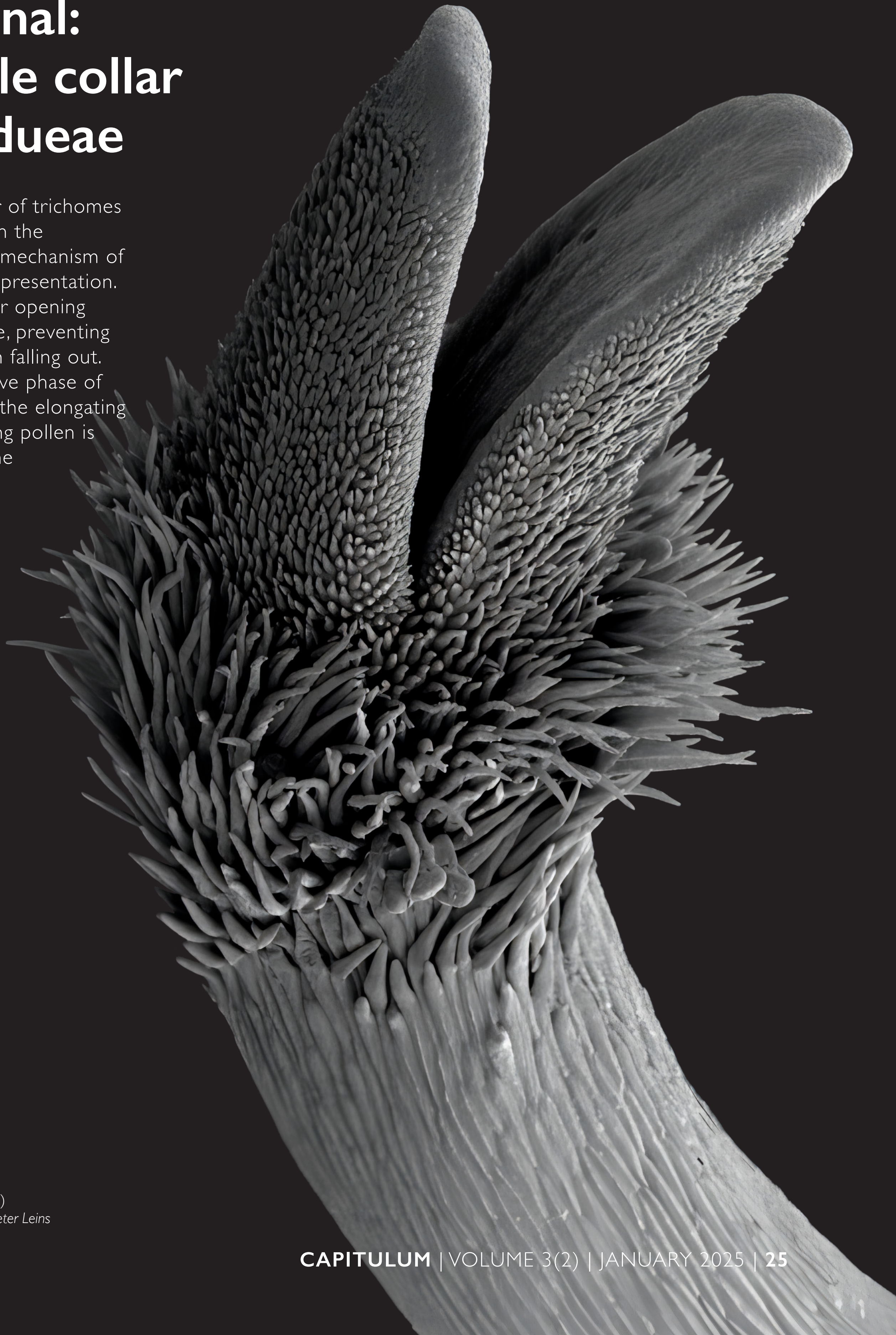
Anthemis maritima
ANTHEMIDEAE



Bidens ferulifolium
COREOPSIDEAE

Showy and functional: the style collar of Cardueae

The distinct collar of trichomes plays a vital role in the specialized pump mechanism of secondary pollen presentation. It blocks the lower opening of the anther tube, preventing pollen grains from falling out. Following the active phase of pollen release by the elongating style, the remaining pollen is brushed out by the collar trichomes.



Cyanus segetum (Cardueae)
Photo by Claudia Erbar & Peter Leins

Figure 8. Pump mechanism with blocking trichomes, exemplarily shown in *Senecio vernalis*. **A-B.** SEM images show the position of the style with apical trichomes located at the base of the anther tube (indicated by white arrows) during anther opening. **C.** Top view of capitulum; white arrow indicated pollen grains pushed from anther tube through the five slits between the connective appendages. **D.** The pump mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (1995). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

are presented. Immediately after, the style emerges from the anther tube with only a moderate or a considerable quantity of pollen on it. To some extent, the short-pilose distal stylar part may act as pollen presenter. The rest of pollen is brushed out by the collar of long trichomes (Figure 10D, white arrow) and pollen grains may be found in this collar even once the stigmatic surface becomes receptive, whereas the distal stylar part is then more or less free of pollen grains. As typical examples *Centaurea nemoralis* Jord. and *Notobasis syriaca* (L.) Cass. are presented.

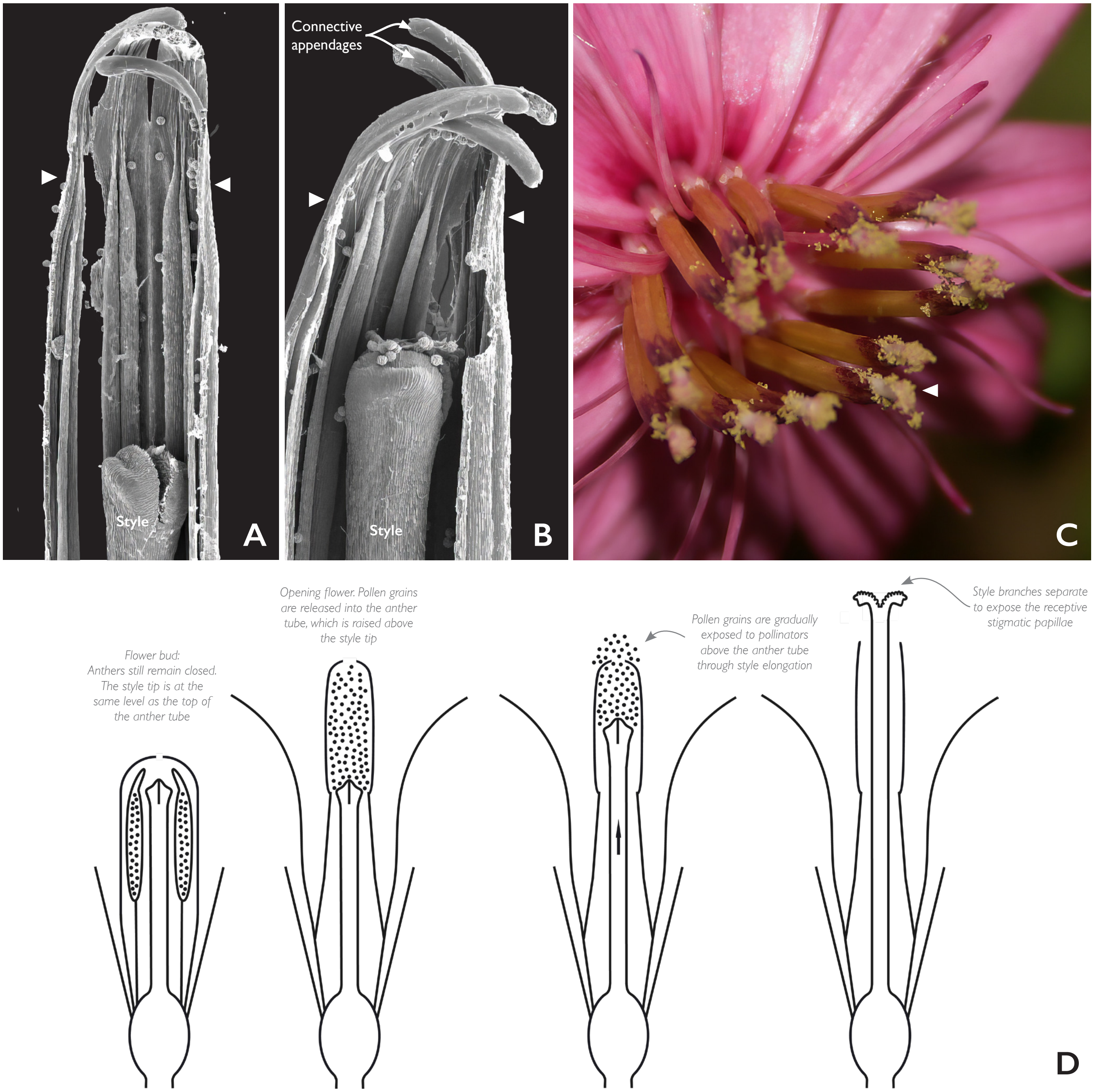
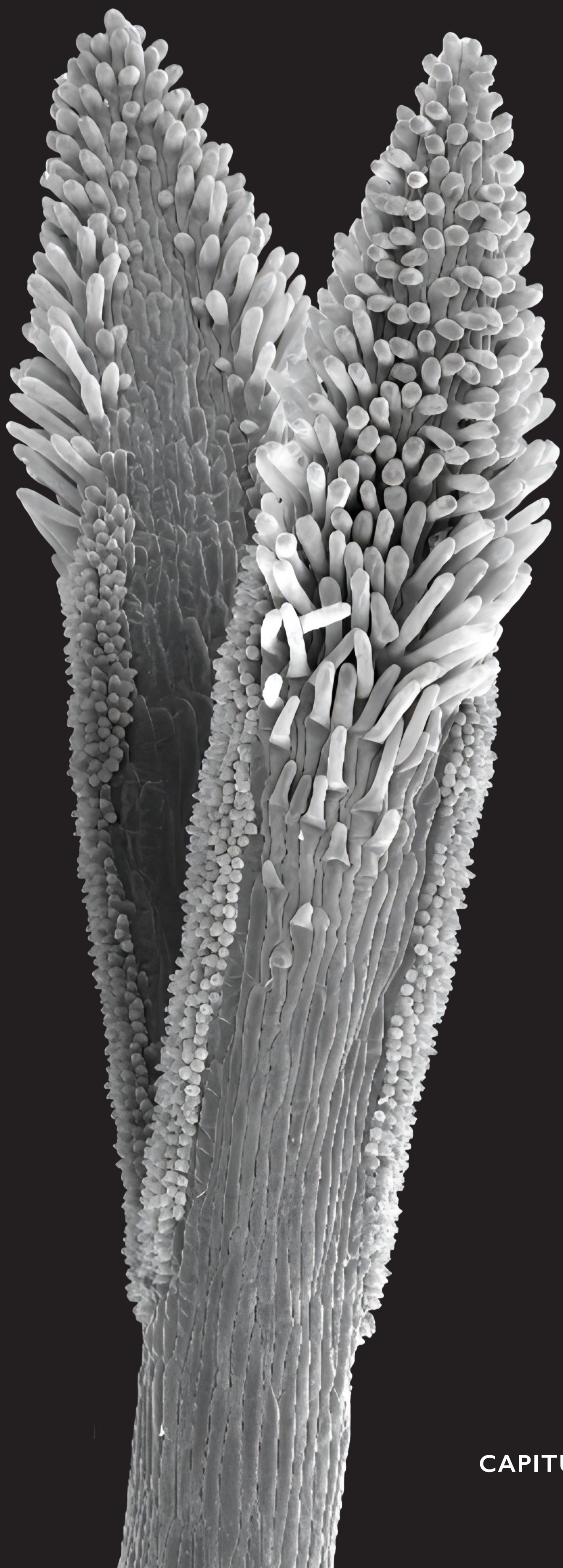


Figure 9. Pump mechanism with apical thickening, exemplarily shown in *Barnadesia*. **A.** SEM image of *B. spinosa* showing the position of the style with apical thickening located at the base of the anther tube; white arrows indicate anther tube. **B.** SEM image of *B. spinosa*, showing the style tip extending close to the upper end of the anther tube; white arrows indicate the anther tube. **C.** Top view of center of capitulum of *B. arborea*; white arrow indicates pollen being pushed out of anther tube. **D.** The pump mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis. *Photo credit, image C: D. Brandes, Braunschweig, Germany.*

In the **special brushing mechanism** of Arctotideae and Platycarpheae (Figure 11), the barrel-shaped swelling is proximally delimited by a collar of longer trichomes, distally the barrel and the short style branches are short-pilose. When the anthers open, the upper part of the style, which is abruptly and cylindrically thickened, is equal to the length of the anther tube. The proximal collar of trichomes blocks the lower opening of the anther tube. By growth of the stylar shaft, the barrel-shaped style part emerges



The unmistakable beauty of the Astereae style

The *Aster*-style type is characterized by acute, hairy style branch appendages and clearly separated, discrete lateral stigmatic lines. While this style type is most common in the Astereae, it is also present in the Gnaphalieae, Coreopsideae, Tageteae, and Heliantheae.

Aster alpinus (Astereae)
Photo by Claudia Erbar & Peter Leins

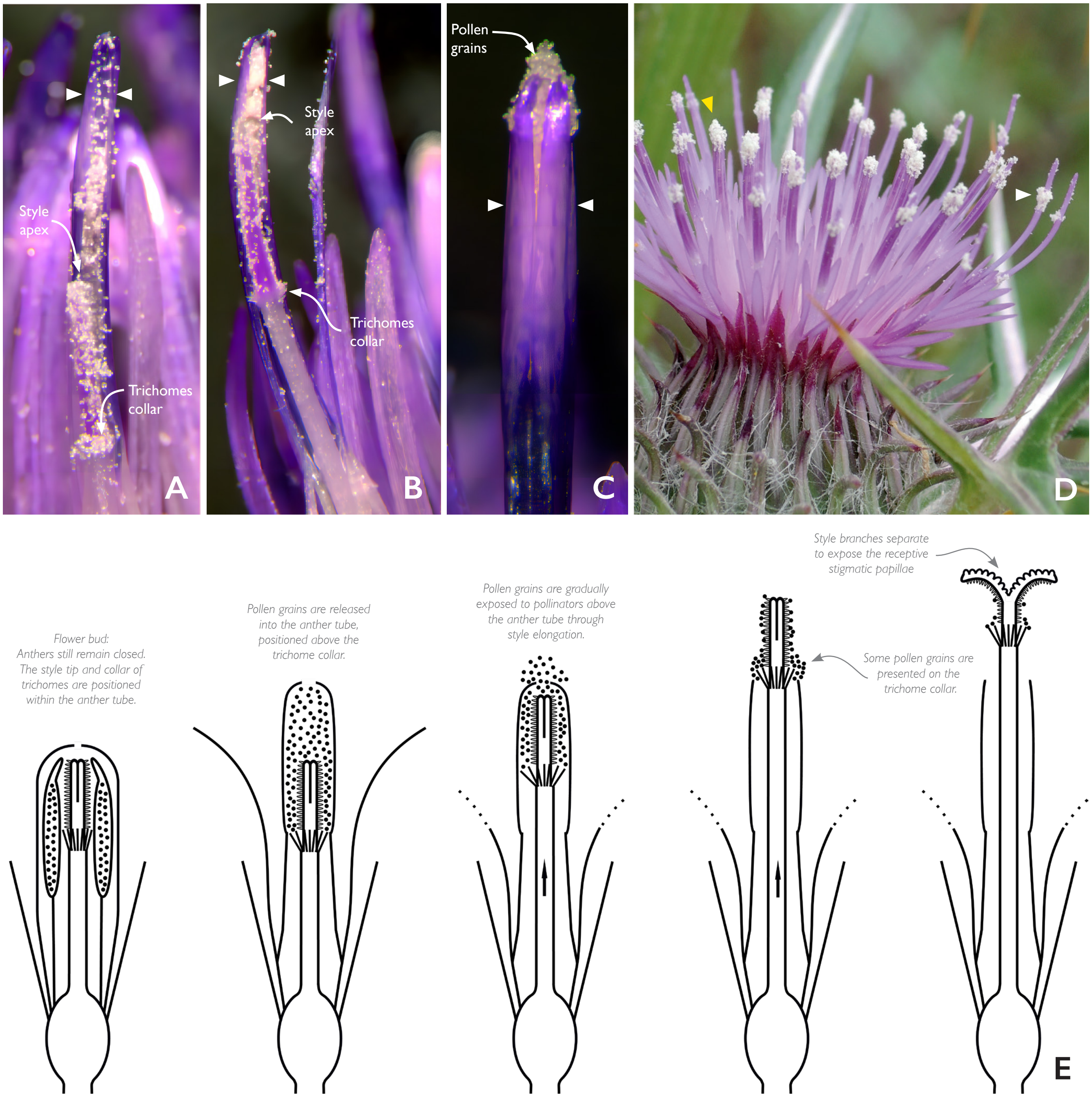


Figure 10. Special pump mechanism. **A–B.** Variations in the position of the style tip and the collar of trichomes within the artificially opened anther tube, caused by style elongation; white arrows indicate anther tube. **C.** Pollen grains emerging between the connivent anther appendages; white arrows indicate anther tube. **D.** Lateral view of capitulum of *Notobasis syriaca;* white arrow indicates collar of trichomes presenting left-over pollen grains; yellow arrow indicates pollen heap at the opening of the anther tube. **E.** The pump mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

from the anther tube with all the pollen on it (Figure 11A-B), presenting the pollen grains to the visitors (Figure 11C). The mechanism is shown in *Berkheya purpurea* (DC.) Mast.

Several arrangements of style trichomes on the style branches are found in the **combination of pump and brushing mechanism** (Figure 12): long style branches are hairy only in their distal part or

The exclusive and distinctive Asteroideae style

Two distinct ventro-lateral stigmatic bands represent the most common stigma arrangement in the subfamily Asteroideae and are exclusive to it. The *Cosmos*-style type, as shown here, is easily identified by its triangular style branch appendage with a prominently defined tip.

Cosmos bipinnatus (Coreopsideae)
Photo by Claudia Erbar & Peter Leins

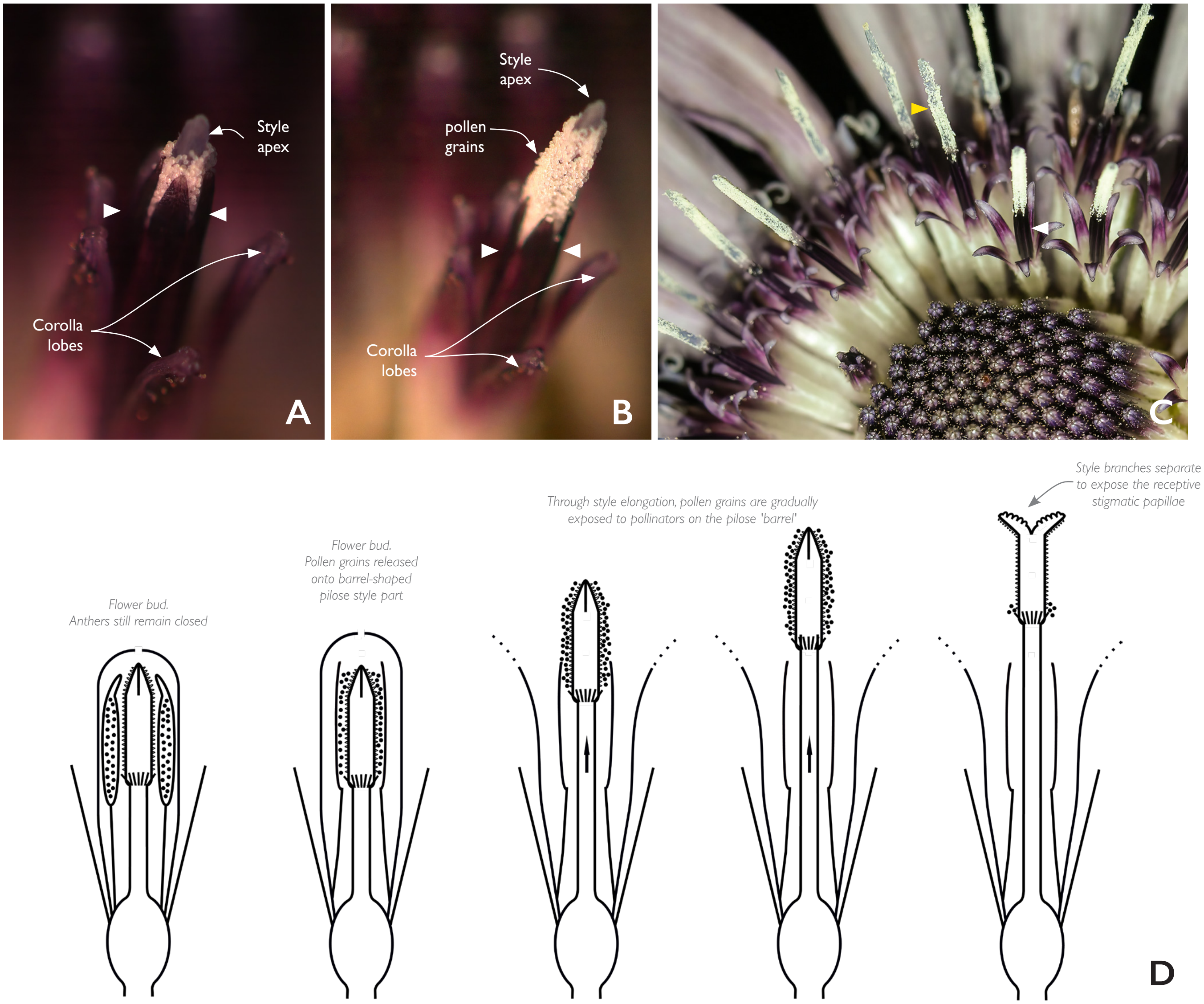


Figure 11. Special brushing mechanism, exemplarily shown in *Berkheya purpurea*. **A-B.** Lateral view of flowers showing the style emerging from the anther tube; white arrows indicate anther tube. **C.** Top view of capitulum; white arrow indicates anther tube, yellow arrow the short-pilose style acting as pollen presenter. **D.** The special brushing mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

rather short style branches are hairy over their entire length (and even beneath the bifurcation). In the case of short style branches, these are shorter than the anther tube. In the case of truncate style branches, a tuft of trichomes is present (sub) apically, with shorter trichomes extending dorsally along a portion of the stylar branch length. At anther dehiscence, the position of the stylar tips is correlated with the distribution of style trichomes. It is only the hairy part of the style that is embedded within the anther tube (Figure 12A). Some pollen is shed into the anther tube, some onto the stylar hair zone. Thus, by style elongation, some pollen is pushed out, and some is brushed out. At first, in the pumping phase, the pollen exudes from the slits between the connective appendages (note the "five-pointed star" when viewed from above, Figure 12B). Later on, the hairy part of the style emerges out of the anther tube and presents the pollen on its outside (Figure 12C). As examples *Bellis annua* L. and *Galatella linosyris* (L.) Rchb.f. in the Astereae tribe are chosen. A variation is the last mechanism, a **combination of pump and slightly brushing mechanism with apical pollen presentation** (Figure 13), in which the style branches possess long sterile appendages. These are triangular or

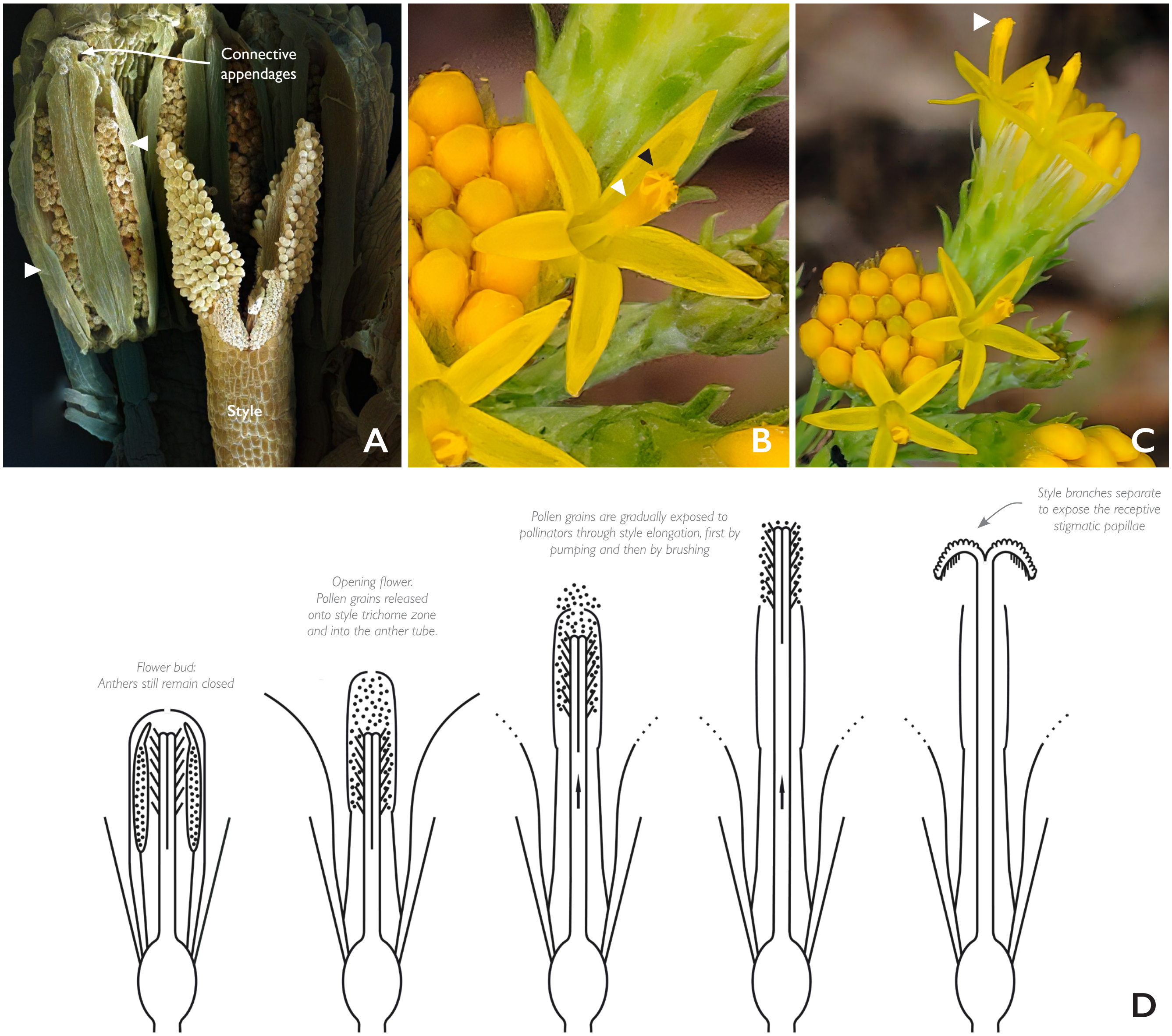


Figure 12. Combination of pump and brushing mechanism. **A.** SEM image showing a lateral view of a monoclinous disc flower of Bellis annua (artificially opened). At the time of anther dehiscence, only the hairy portion of the style remains embedded within the anther tube (indicated by white arrows). **B.** Top view of flower of *Galatella linosyris*; white arrow indicates anther tube; black arrow point to pollen grains protruding from five slits between the connivent anther appendages (pumping phase). **C.** Lateral view of capitulum of *Galatella linosyris*; white arrows indicate brushed-out pollen grains located on the outer surface of the style branches. **D**. The combination of pump and brushing mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

narrow and long or tapering or even with a markedly defined tip. At anther dehiscence, the hairy area of the style branches or some longer style trichomes at the base of the appendages block the lower opening of the anther tube (Figure 13A). Pollen is shed into the anther tube, in the middle of which to a small or (mostly) large extent the stylar appendages are located. The growing style pushes some pollen out (Figure 13B), the remaining pollen grains are hold by the stylar appendages during the style elongation and the pollen presenting phase of anthesis (Figure 13C). The mechanism is exemplarily shown in *Bidens ferulifolia* (Jacq.) Sweet (Coreopsideae).

In a simplistic and comprehensive way, we can further subsume these eight mechanisms into four main functional categories: deposition, brushing,

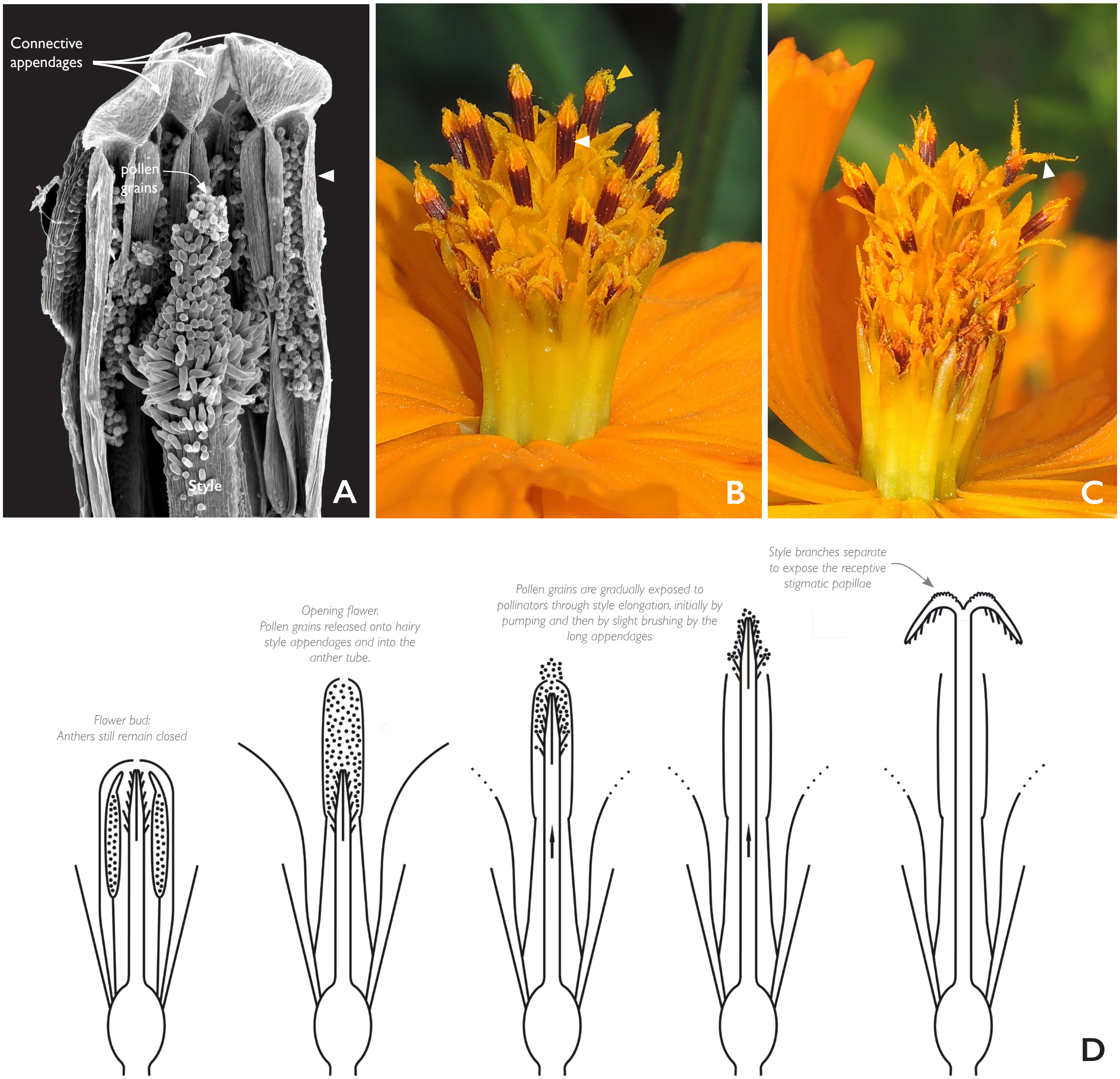
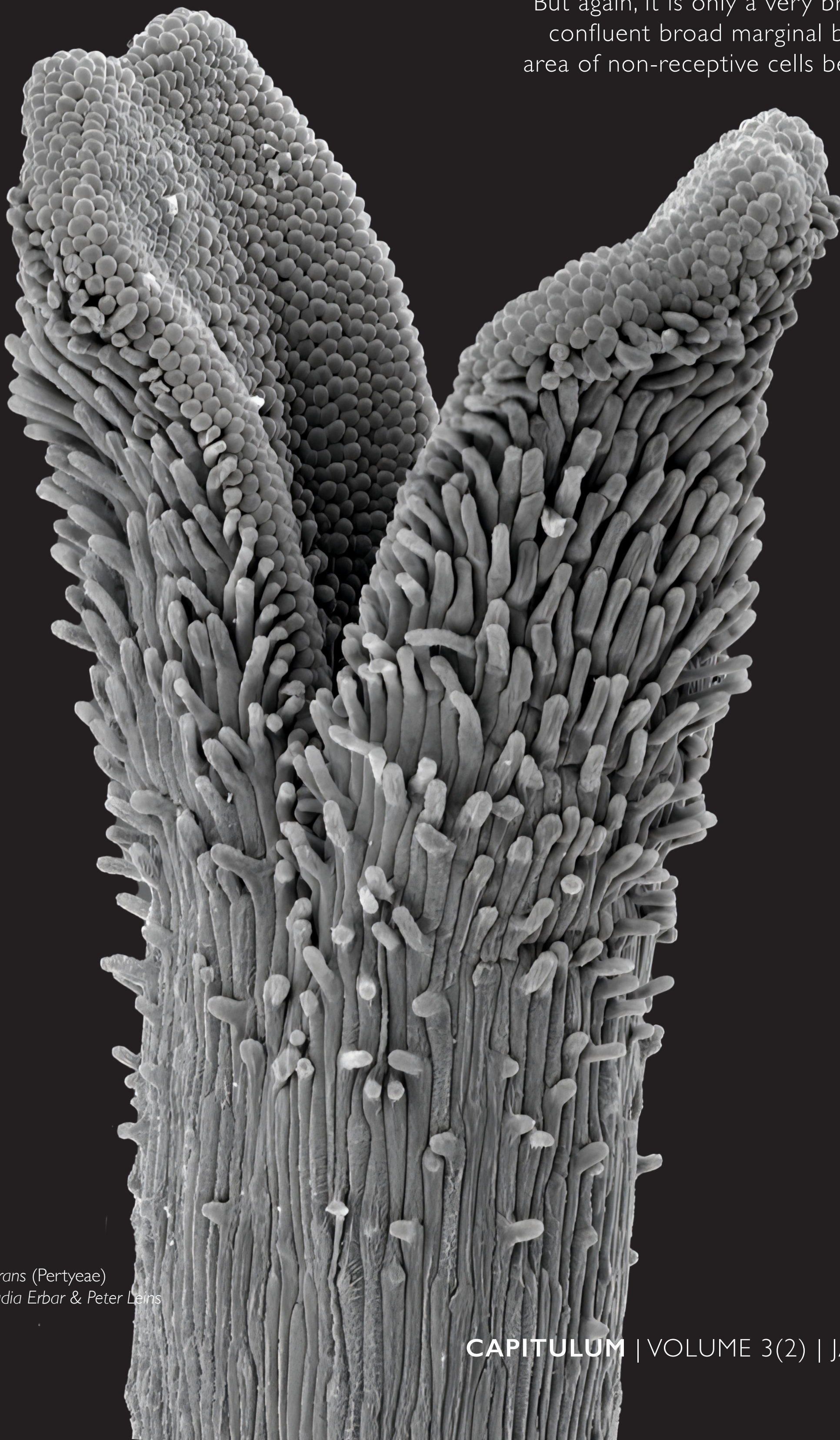


Figure 13. Combination of pump and slightly brushing mechanism with apical pollen presentation, exemplarily shown in *Bidens ferulifolium*. **A.** SEM image of the anther tube (indicated by a white arrow), showing the style with long appendages beginning to elongate shortly after anther opening. **B.** Lateral view of center of capitulum; white arrow indicates anther tube; yellow arrow points to pollen grains protruding from five slits between the connivent anther appendages. **C.** Lateral view of capitulum; white arrows mark pollen grains at the outside of the style branches. **D**. The combination of pump and slightly brushing mechanism is illustrated through highly schematic longitudinal sections, adapted from Erbar & Leins (2021). Each sequence, from left to right, depicts different stages, transitioning from a closed flower bud to the exposure of the receptive stigmatic surface at late anthesis.

pump, and pump and brushing combined. These simplifications allow for easier comparison when plotting the different mechanisms onto a phylogenetic tree (Figure 14). Five style types are involved in deposition mechanisms (Figure 2). These types as well as the mechanisms are confined to basal groups of Asteraceae (Figure 14). The rounded flat papillae or scale-like bi- to tri-seriate style trichomes in these styles are not suitable for efficient sweeping, but nevertheless contribute to

The great pretender

Superficially, stigmatic tissue appears to cover the entire inner surface of the style branches. But again, it is only a very broad zone of apically confluent broad marginal bands, leaving a small area of non-receptive cells between them basally.



Ainsliaea fragrans (Pertyeae)
Photo by Claudia Erbar & Peter Leins

some brushing in the secondary pollen presentation. In contrast, acute style trichomes characterize the pure brushing mechanism (three style types, #28–30 in Figure 3), which is the only mechanism in Cichorieae, Corymbieae, Eremothamneae, Liabeae, Moquinieae, and Vernonieae (Figure 14). In the special brushing mechanism of Arctotideae and Platycarpheae also acute style trichomes are present (#27 in Figure 3). Outside the Vernonioideae-Cichorioideae-Corymbioideae relationship, a pollen brushing accomplished by acute style trichomes is not found. The other four style types involved in pollen brushing (#36, 42, 43, 49; Figure 3) are characterized by obtuse or rounded style trichomes (in *Gynura* [Senecioneae], Inuleae, Athroismeae, Tageteae, and Eupatorieae). Pure pump mechanisms need styles that efficiently block the lower end of the anther tube (Figure 4). The blocking is achieved either by more or less prominent dorsal thickenings or by transverse bulges, apically dilating the style branches (Figure 4, lower row). Truncate or more or less rounded style branches with style trichomes only at the very tips of the style branches (mostly an apical, seldom a subapical tuft of style trichomes) characterize eight further style types (Figure 4, upper and middle rows). Pump mechanisms are found across the whole family (Figure 14).

A large number of style types (22 of 49) is involved in a combination of pump and brushing mechanisms (Figure 5). In general, we can predict the relative involvement of pumping and brushing, respectively, from the relative length of the hairy zone of the style. The special pump mechanism – confined to members of Carduoideae (Dicomeae and Cardueae) and realized by five style types that are characterized by a ring of trichomes beneath a hairy stylar part (Figure 5, lower row) – is essentially a pump mechanism (and treated as such in Figure 14). However, to some extent, the short-pilose distal stylar part acts as pollen presenter and pollen grains are brushed out by a collar of long trichomes, so that we arranged the corresponding style types in this comparative consideration in Figure 5.

It has to be mentioned that in staminate flowers with sterile styles, pure brushing and pure pump mechanisms as well as combinations of pump and brushing mechanisms occur (Figure 14).

FINAL REMARKS

Modern sequence analysis has become more and more established in recent years and the detailed and refined analyses provide a basis for the present classifications of Asteraceae. Nevertheless, style morphology and anatomy seems to be meaningful as well as in systematic and taxonomic considerations on a higher level. Furthermore, the style of the Asteraceae, seems in terms of its biological relevance, i.e., its functionality, to be an efficient concept for success, due to which the family owes its immense diversity of species. Along with other special flower structures (stamen-corolla tube, anther tube) and their growth behaviour, the style serves as a successful pollen presenter in the mechanisms of pollen portioning. As a rule, the mechanisms of secondary pollen presentation ensure a gradual delivery of small pollen portions to pollinators over time. Generally speaking, the size of the pollen portion is correlated with many other parameters of flower functions (see the multifactorial net of correlations concerning flower functions; Fig. 164 in Leins & Erbar, 2010). The delivery of numerous small pollen portions, the dense inflorescences (capitula), the reduction to a single ovule per flower, the long time of pollen presentation during anthesis at the level of each flower, capitulum, and individual plant (often bearing multiple capitula), as well as the open structure of the pseudanthium, which allows pollinators to move freely, collectively contribute to the ecological generalization of most Asteraceae. This generalist strategy, in terms of flower and pollination ecology, is further supported by the high number of pollen application sites available to pollinators. They have a large spectrum of different pollinators such as beetles, bees, wasps, flies and butterflies. This can lead to a considerable loss of pollen, since some of these flower-visiting insects may be unreliable concerning the repeated visit of the same plant species. Nevertheless, loss of pollen through "vagabonds" can be reduced by limiting the amount of pollen offered by a flower at a time by the mechanisms of secondary pollen presentation (pollen portioning; see, e.g., Leins & Erbar, 2006, 2010).

Secondary pollen presentation is also found among Asteraceae closest relatives in the Asterales. The sister family Calyceraceae exhibits a pure, simple deposition mechanism (Erbar, 1993): The pollen grains

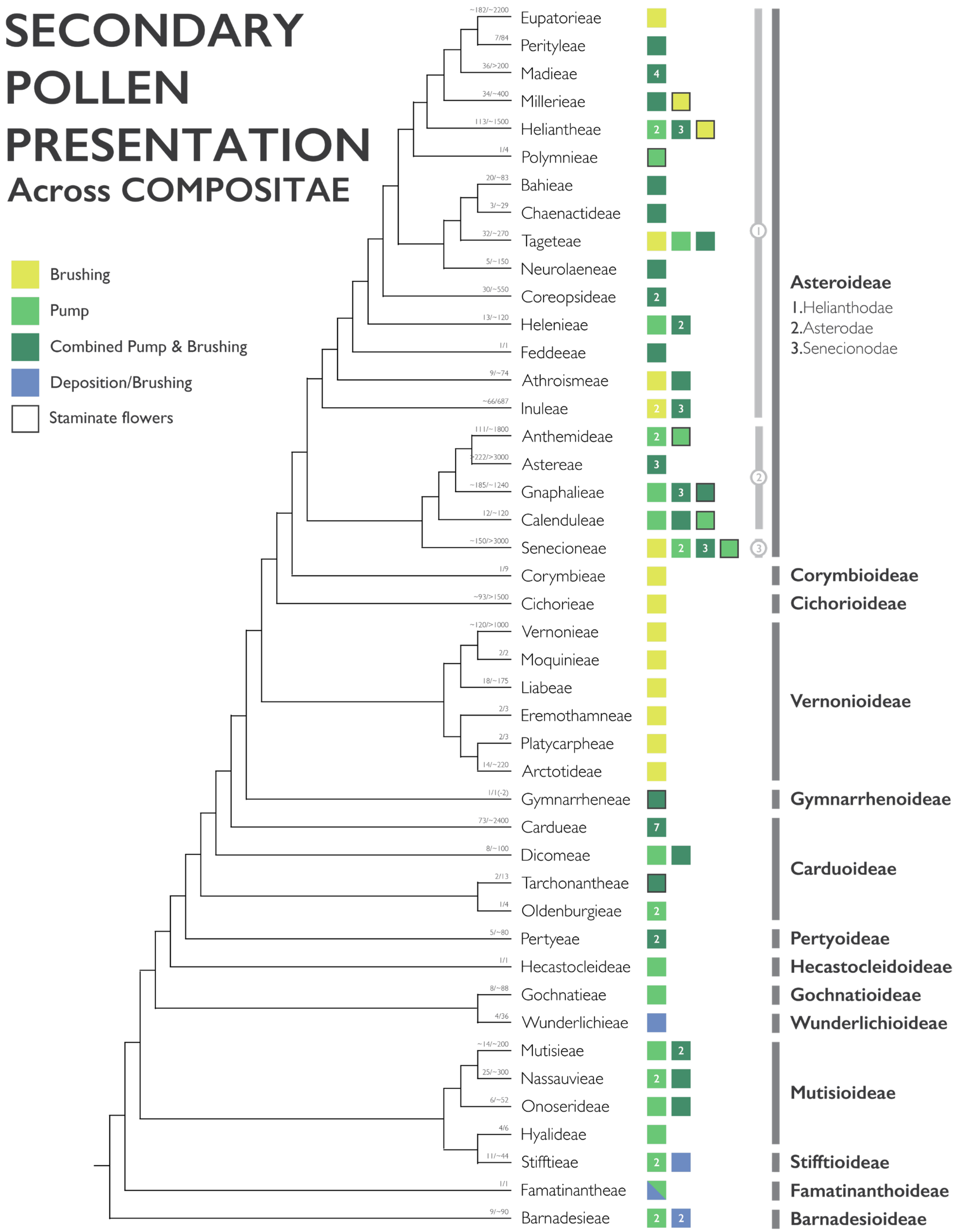


Figure 14. Generalized phylogenetic tree of Asteraceae (based on Funk et al., 2009b; Panero et al., 2014; Mandel et al., 2019) onto which the different mechanisms of secondary pollen presentation are plotted. The numerals inside boxes indicate number of style types exhibiting the corresponding mechanism. As regards closely related families, Calyceraceae exhibit a pure deposition mechanism, and Goodeniaceae a cup mechanism (confined to this family).

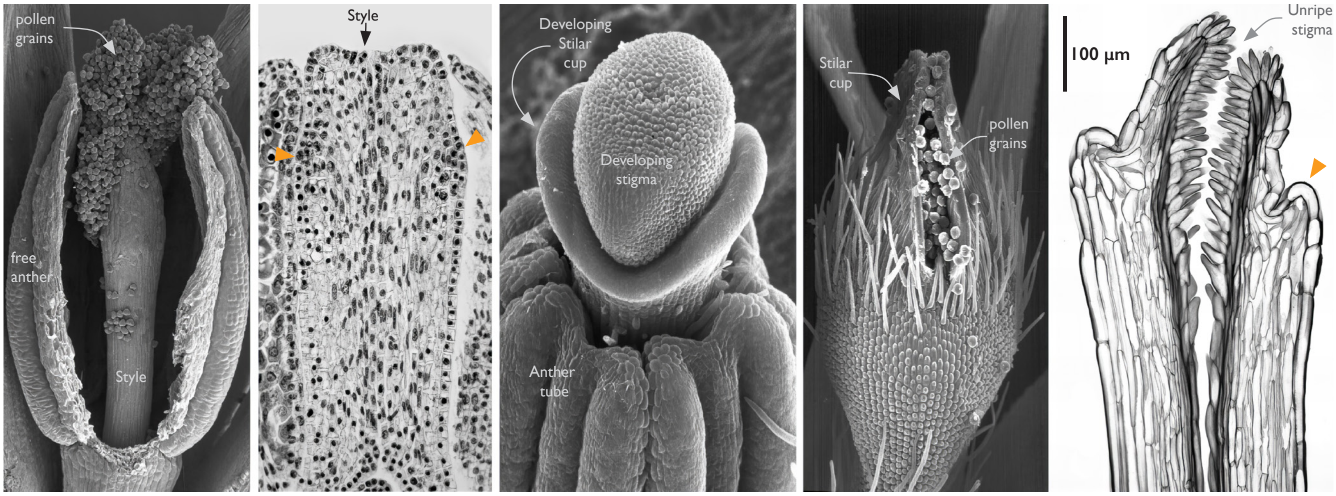


Figure 15. A. Simple deposition mechanism of secondary pollen presentation in *Acicarpha tribuloides* (Calyceraceae). B-D. *Brunonia australis* (Goodeniaceae). **B-C.** Early development of the stylar cup as outgrowth by cell divisions (orange arrows in B) beneath the stylar tip. **D.** Own pollen grains within the stylar cup before presented to pollinators by growth of the developing stigma. **E.** *Barnadesia polyacantha* (Asteraceae-Barnadesieae). The transverse bulges, typical of the *Barnadesia*-style type, correspond to the cup in Goodeniaceae in the position, but are formed by very late elongation of cells beneath the epidermis (orange arrow).

are deposited onto the style and the style emerging from the anther tube gradually presents the pollen on its outside. It merely requires a spreading of the emptied anthers for an unimpeded presentation of the pollen by lengthening of the style (Figure 15A; Erbar, 1993; Leins & Erbar, 2010). We assume that this mechanism is transferred as the first one to the basal Asteraceae. Nowadays Goodeniaceae are regarded as sister to Calyceraceae and Asteraceae. The cup mechanism of Goodeniaceae, confined to this family, is a specialized deposition mechanism (Erbar & Leins, 1988; Leins & Erbar, 2010). The cup-like structure beneath the tip of the style takes up the whole pollen mass (Figure 15D); later, the developing stigma gradually pushes out the pollen. The position of stylar cup and the bulges in *Barnadesia* are comparable, but there is a crucial difference in the ontogeny of both stylar structures. The stylar cup in Goodeniaceae is a very early outgrowth by cell divisions beneath the stylar tip Figure 15B-C; Erbar & Leins, 1988; Leins & Erbar, 1989), whereas in *Barnadesia*, the bulges are formed very late by elongation of cells beneath the epidermis (Figure 15E).

In conclusion, apart from the high diversity of style types within the Asteraceae, the specific and economical delivery of pollen grains onto pollinators through the mechanisms of secondary pollen presentation may be one of the key factors underlying the great evolutionary success of the family, in terms of species richness and distribution in diverse habitats almost anywhere in the world.

ACKNOWLEDGEMENTS

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