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Style head in Apocynaceae: a very complex secretory activity performed by one tissue

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ABSTRACT

Nuptial glands are very diverse and associated with different pollination mechanisms. The greater the specificity in the pollen transfer mechanism from anther to stigma, the greater the morphological elaboration of flowers and functional complexity of the nuptial glands. In Apocynaceae, pollination mechanisms reached an extreme specificity, a fact that was only possible due to an extreme morphological synorganization and a profusion of floral glands. Although these glands are of different types, the vast majority have secretory cells only in the epidermis. In general, these epidermal cells produce many different compounds at the same time, and previous studies have demonstrated that in the style head, the functional complexity of epidermis has become even greater. Four types of style head are found in the family, which have different degrees of functional complexity in relation to the secretion produced and pollen dispersal mechanism. The secretion is fluid in types I, II and III, and the pollen is dispersed and adhered to the pollinator by the secretion produced by the style head. In type IV, the secretion hardens and acquires a specific shape, moulded by the spatial constraints of the adjacent floral organs. This evolutionary alteration is accompanied by changes in the structure and arrangement of the secretory cells, as well as in pollen aggregation and position of stigma. Histochemical analysis has shown that the secretion is mixed and highly complex, especially in the style head type IV, where the secretion, called translator, is formed by a rigid central portion, which adheres to the pollinator, and two caudicles that attach to two pollinia. The translator has a distinct composition in its different parts. Further studies are needed to answer the new questions that have arisen from the discovery of this highly functional complexity of the secretory tissue.

Key words: glands; histochemistry; pollinarium; secretion; synorganization; translator.

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Introduction

Most environments around the world these days are dominated by flowering plants.[1] The success of angiosperms and their contemporary distribution are directly related to their rapid growth and reproduction,[2-4] the latter being highly diverse, mediated by several types of pollinators.

The interaction with pollinators, in most types of pollination, is associated with floral rewards.[5-7] The rewards sought by pollinators are mostly pollen and/or nectar;[5,7] however, many flowers have other types of floral rewards, such as fragrance, oil, or resin,[6,8-10] and others do not offer any type of reward, pollinated instead by deceit.[11] However, the pollination process (and consequent fertilization of the ovules) does not involve only floral rewards. Many other types of substances are necessary to enable the pollen deposited on the stigma to germinate and its pollen tube to reach the ovule inside the ovary.[12] The tissue system responsible for nourishing and guiding pollen tubes through the gynoecium is called transmitting system.[12,13]

The secretions involved from the removal of pollen from the anther by a pollinator to the fertilization of the ovule are called nuptial secretions. These secretions are produced by different glands, and the greater the degree of specialization for sexual reproduction of the species to occur, the greater the quantity and/or degree of functional complexity of these nuptial glands.[12]

Apocynaceae

Apocynaceae, with an emphasis on asclepiads, stand out for having one of the most complex pollination mechanisms among angiosperms, which is related to a highly elaborate floral morphology[14] and numerous defensive and nuptial glands.[12] Some species of Apocynaceae have up to 11 types of glands in a single flower, making the flowers of this family the richest in secretions among angiosperms.[12]

Despite the high number of floral glands in the family, almost all external glands have the secretory activity restricted to epidermal cells, regardless of their function, as observed in glandular trichome, colleter, staminal wing gland, extragynoecial compitum, stylar canal, obturator and style head, in addition to some glands that are often exclusively epidermal in the family, such as nectaries and osmophores.[12,15-19]

This particular case reflects an evolution directly related to the plasticity of the protodermis which, together with the emergence of the pollinia, seems to have been mainly responsible for the high
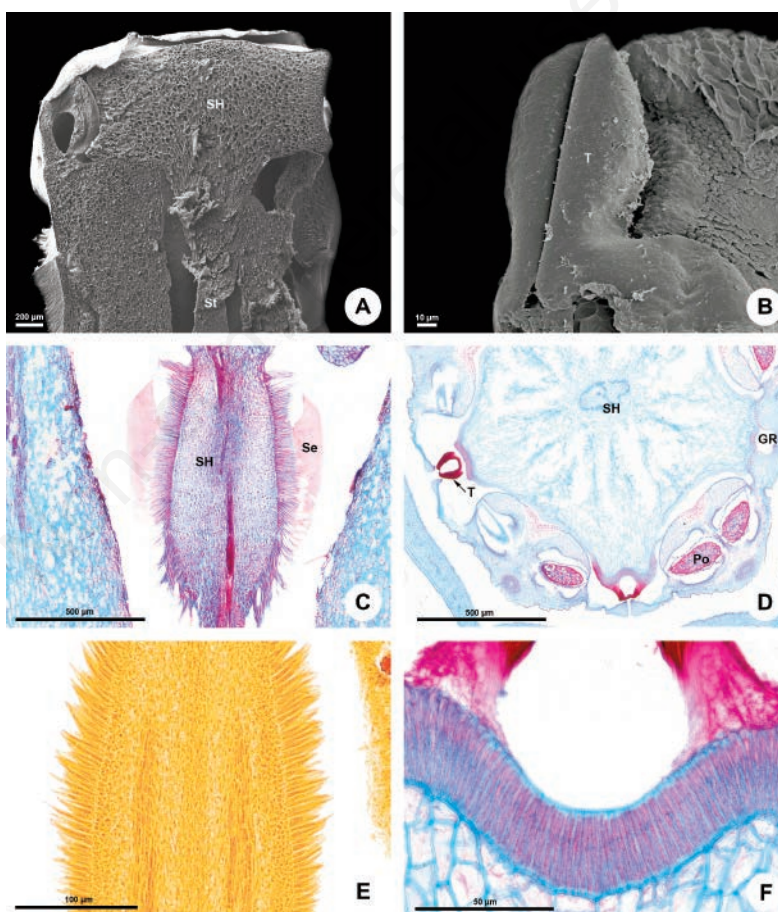

Figure 1. Style head in Apocynaceae. **A,D,F**) *Blepharodon bicuspidatum* E. Fourn. **B**) *Peplonia axillaris* (Vell.) Fontella & Rapini. **C**) *Plumeria rubra* L. **E**) *Aspidosperma australe* Müll. Arg. **A,B**) Scanning electron microscopy. **C-F**) Light microscopy. **A**) Longitudinal section of the flower; note the style head at the top of the style. **B**) Detail of the translator. **C**) Style head with sticky secretion. **D**) Style head with a rigid secretion (translator); note the guide rail that is positioned below the translator. **E**) Secretory epidermis composed of trichomes. **F**) Secretory epidermis constituted of elongated palisade cells.: GR, guide rail; Po, pollinium; SH, style head; Se, secretion; St, style; T, translator.

radiation of the family,[14,20] giving rise to thousands of species distributed almost worldwide.[21,22]

Among the different parts of the flower having secretory tissues, the style head stands out for its fundamental importance for pollination in Apocynaceae.[15,22-24]

Style head

Structure and function

Usually, the stigma is located in the terminal portion of the gynoecium, which, in the vast majority of angiosperms, corresponds to the top of the style.[25] However, throughout the evolutionary history of Apocynaceae, there was a displacement of the receptive portion of the gynoecium from the terminal portion to a subapical region.[23,26] This shift is related, at least partially, to the appearance of an expansion in the terminal portion of the style, called style head (Figure 1A).

The style head is ubiquitous in Apocynaceae,[12,22,24] and is one of the main morphological synapomorphies of the family. It forms from a postgenital fusion of the apices of the two carpel primordia during flower development.[15,23,27] Nevertheless, the developmental moment of the fusion is distinct in the species. The precocious formation of the style head is an indication of its morphological and functional complexity.[12,15,23,25] In the most basal clades, style fusion is late and occurs in intermediate phases of flower development.[28,29] On the other hand, fusion is precocious in the most derived clades and occurs in the beginning of gynoecium development.[15,25] After the fusion of styles, the apical portion expands and differentiates a secretory epidermis that will release a sticky secretion (Figure 1 B-F), responsible for helping the pollen transfer from anther to stigma.[12,15,23,25,29,30]

The structure and function of the epidermis of the style head (Figure 1 E,F), as well as its modifications throughout the evolutionary history of the family, are directly linked to a complex pollination mechanism that is unique among eudicots. Fallen[23] described four morphological types of style head (Figures 2 and 3). These types are mostly related to the complex secretory system of the epidermis that covers it.

In type I (Figure 2A), the entire secretory surface is also stigmatic, formed by trichomes that produce a viscous fluid for pollen transfer/capture and also act as a receptive surface (Figure 2C). In this type of style head, pollen deposited on any portion of the secretory surface can germinate and the pollen tubes penetrate the gynoecium.

In type II (Figure 2B), there is a spatial and functional separation of the specialized cells of the epidermis. The stigma is displaced to a basal portion of the side of the style head, while the remaining body is retained, the only function of which is to produce a secretion for pollen transfer (Figure 2D). This separation of functions alters the flower pollination mechanism and the location of pollen deposition on the style head. In type II, the style head has
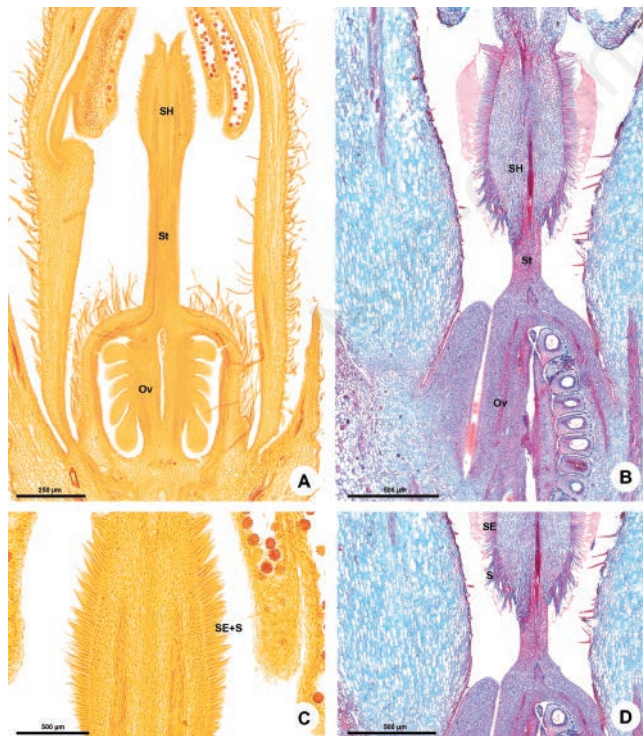

Figure 2. Style head in Apocynaceae. **A,C)** *Aspidosperma australe* Müll. Arg; style head type 1. **B,D)** *Plumeria rubra* L.; style head type 2. Ov, ovary; SE, secretory epidermis; S, stigma; SH, style head; St, style.
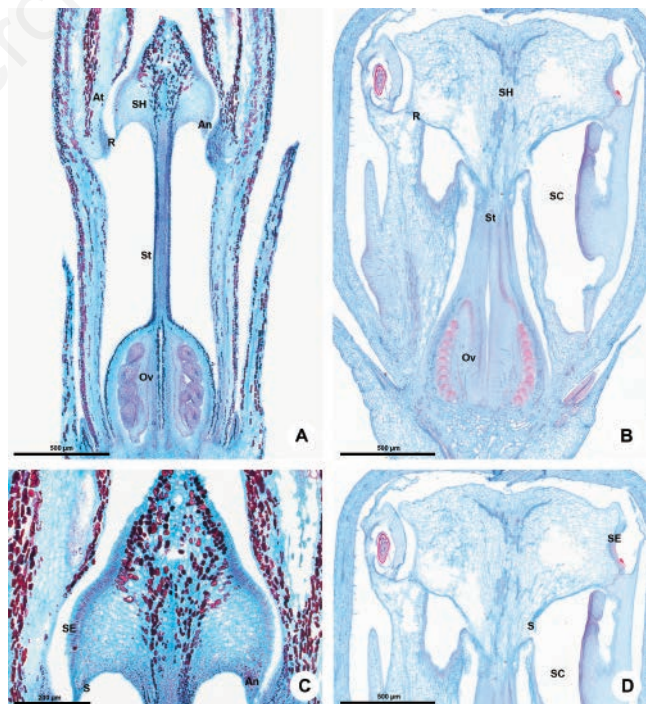

Figure 3. Style head in Apocynaceae. **A,C)** *Mandevilla tenuifolia* (J.C. Mikan) Woodson; style head type 3; note the cell projection (retinaculum) that fuses the anther base to style head. **B,D)** *Blepharodon bicuspidatum* E. Fourn; style head type 4; note the adnation between androecium to gynoecium (retinaculum) and the stigmatic chamber, where the pollinium will be inserted. An, annulus; At, anther; Ov, ovary; R, retinaculum; S, stigma; SC, stigmatic chamber; SE, secretory epidermis; SH, style head; St, style.

often an upper ring of long trichomes, which helps to isolate the pollen storage region from the region where the adhesive secretion is produced. The secretory region is composed of trichomes producing lipids and mucilage and occupies the main portion of the style head. The stigmatic region is located at the base of the style head and has non-secretory trichomes (Figure 2D).

In type III (Figure 3A), the structure of the secretory epidermis and stigma is very similar to that of type II. However, it is possible to observe a compartmentalization of the sites where secretion production occurs and the site of pollen deposition. This compartmentalization is due to a spatial restriction generated by adnation between the androecium and gynoecium, forming the gynostegium (Figure 3A). In this type of style head, as in the previous ones, the secretory epidermis is constituted of trichomes (Figures 3C and 4A), which all produce lipids and mucilage. The evolutionary novelty of this type of style head is that its base projects downwards, forming an annulus (Figure 3 A,C) which is related to the mechanism of pollen removal from the pollinator. When the pollinator retracts the proboscis after collecting the nectar at the bottom of the floral tube, the annulus scrapes it, retaining the pollen under the style head. In this type of style head, the stigma has moved a little more and is located from the side of the annulus to the inferior surface of the style head, the exact position where the pollen is accumulated (Figure 3C). The receptive surface is also formed by non-secretory cells.

The main histological alteration occurs in the type IV (Figure 3B), where the secretory epidermis is now formed by elongated palisade cells (Figures 1F and 3D) and the secretion, which is fluid in the other types of style head, now takes on a rigid consistency and form, called a translator (Figures 1B and 4B). For this significant evolutionary alteration to occur, a series of functional changes were necessary. In type IV, the epidermis has different cells producing different amounts of secretion with different composition in a synchronized manner (Figure 4 C-F). The secretion, after being released to the outside, hardens and takes a specific shape (Figure 1B). Different cells are activated at different times, and their metabolism is distinct from each other, even though they are all contiguous. The translator takes on a certain shape, moulded by the spatial constraints of the floral parts that are adjacent to the secretory portion (Figure 4 E,F). In this type of style head, the stigma moves further downwards and is observed almost entirely on the style, below the style head (Figure 3D). The receptive surface is formed by non-secretory cells.

Style head evolution

The style head type I is morphologically the simplest type and was the first to appear in the evolution of Apocynaceae, found only in the most basal species in the tribe Aspidospermateae (Figure 5).[31] In these species, the epidermis of the style head has a dual function: production of secretion for pollen transference and recep-
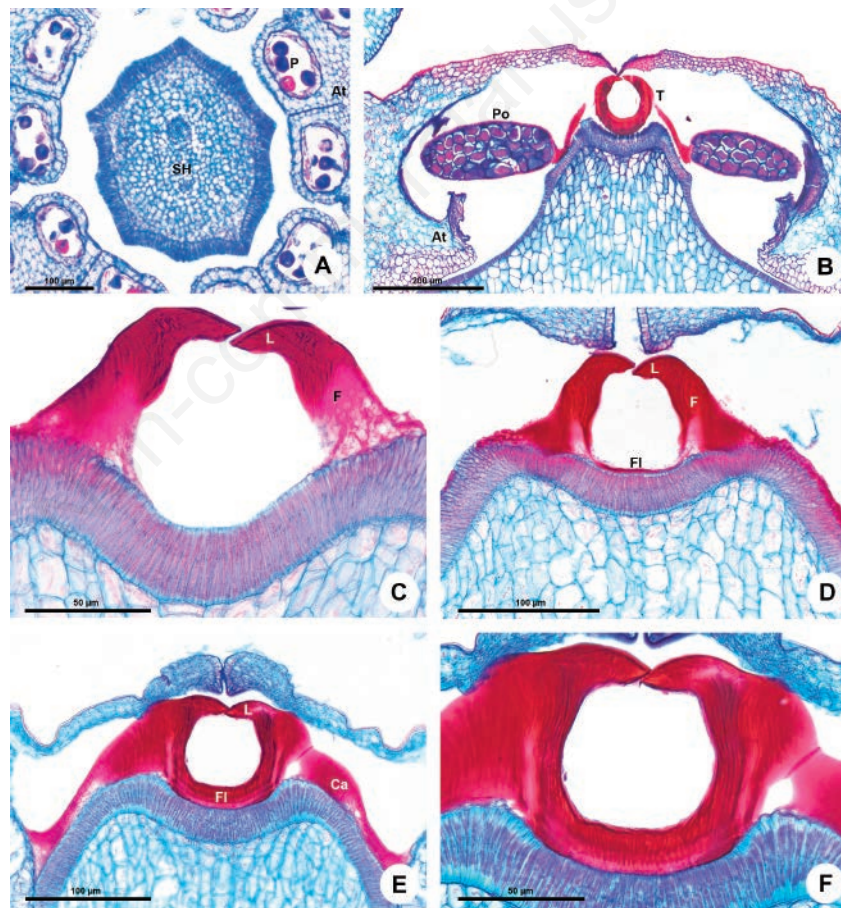

Figure 4. Style head in Apocynaceae. **A)** *Tabernaemontana catharinensis* A.DC; note pollen, inside the anther, as monads. **B-F)** *Blepharodon bicuspidatum* E. Fourn. **B)** Pollinarium composed of translator and two pollinia. **C-F)** Translator morphogenesis, beginning with the secretion of the ledges and flanks of the corpusculum (**C**), followed by the secretion of corpusculum floor and caudicles (**D,E**). **F)** Striations of the corpusculum correspond to the secretion of each epidermal cell. At, anther; Ca, caudicle; F, flank; Fl, floor; L, ledge; P, pollen; Po, pollinium; SH, style head; T, translator.

tive surface. Type II appeared in the sequence and is found in the other lineages of the rauvolfioid grade. The epidermis is also composed of trichomes, but the secretory trichomes are only responsible for producing the secretion involved in pollen transfer, with the stigma restricted to the basal portion of the style head (Figure 5). Type III evolved in the apocynoid grade and shows a small specialization in relation to the position of the stigma at the base of the style head under an annulus (Figure 5), although the structure and function of the secretory epidermis is similar to that of type II. On the other hand, the epidermis of the style head type IV is structural and functionally distinct from that of the others. The rigid secretion (translator) has a specific shape. The translator has a very constant morphology in each taxon and has been used to classify the genera at tribal and/or infratribal levels.[15,24,32] Its different parts are associated with its function. In general, there is a portion that will adhere to the pollinator, and another portion that will adhere to pollen. This type of style head is found in the most derived subfamilies: Periplocoideae, Secamonoideae and Asclepiadoideae (Figure 5),[12,15,33-35] the last being the most diverse clade with more species than the rest of the family combined.[36] The early formation of the style head in these derived clades is directly related to the high complexity of their secretory activity, which begins early in floral development.[12,15]

Pollen aggregation and pollinating apparatus

All these evolutionary alterations in the style head occurred concomitantly with profound changes in floral synorganization and distinct degrees of pollen aggregation, forming several types of pollen dispersal units. In species with style head types I, II and III, in which the secretion is fluid (Figure 1C), pollen is dispersed individually in units called monads (Figure 4A), eventually in tetrads.[24,37,38] Type IV evolved in the clades Periplocoideae and Secamonoideae + Asclepiadoideae. In Periplocoideae, the translator carries the pollen aggregated in tetrads or massulae ("loose pollinia") on a large spoon-like part.[24,37,39] The occurrence of secondary pollen presentation in this subfamily (*i.e.*, the relocation of pollen onto organs other than anthers) is directly related to the shape of its translator, in which the pollen is shed before floral anthesis.[24,33,37] In the subfamilies Secamonoideae and Asclepiadoideae, pollen reaches the maximum degree of aggregation, forming pollinia (Figure 4B).[12,15,24,25,30,37] In the pollinium, all grains formed in a pollen sac remain together and cohesive in a single unit. To be able to transfer this entire set of pollen, the epidermis of the style head reached the maximum of specialization and functional complexity of a secretory tissue.

The translator in Asclepiadoideae is formed by a rigid, clip-shaped central region, called corpusculum, and two lateral extensions that attach the corpusculum to pollinia, called caudicles (Figure 4B).[12,15,24,25,30,33,34,37]

The translator is initially secreted by some cells that form the ledges of the corpusculum. Then, other cells are activated and begin to secrete the flanks of the corpusculum (Figure 4C). In some species, these two halves, initially free from each other, are held in position by a secretion that stabilizes them, called ligament.[15,34] After the formation of both parts, cells in another position secrete the floor, uniting the halves and completing the
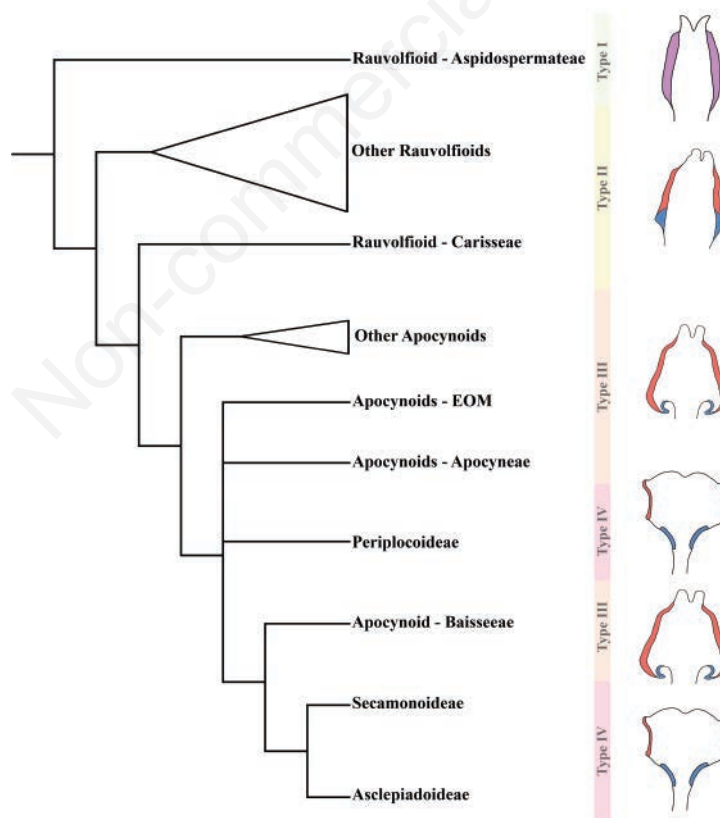

Figure 5. Simplified phylogenetic tree of Apocynaceae. Schematic drawings illustrate the type of style head found in the different clades, highlighting the stigma in blue, secretory epidermis in red and secretory epidermis + stigma in violet. Phylogenetic tree based on Fishbein *et al.*[31]

formation of the corpusculum (Figure 4D). At the same time, cells further away from this region begin to produce the caudicles (Figure 4 D,E). Unlike the corpusculum, the entire caudicles are secreted simultaneously by the cells. It is possible to verify each portion of secretion produced by each cell, aligning the corpusculum striation with the cells of the secretory tissue (Figure 4F). After the end of translator secretion, the anthers open in an apical region close to the end of the caudicle, and upon contact of each

pollinium with the respective caudicle, they adhere to each other (Figure 4B), forming the pollinarium (translator + pollinia). When the pollinator visits the flower to collect nectar, it attaches its proboscis or legs to the slit of the corpusculum, like a clasp, and removes the entire pollinarium from the flower. When collecting nectar from another flower (or, in some cases, the same flower), it inserts the pollinarium or part of it into the stigmatic chamber, whose opening is located immediately below the style head, where
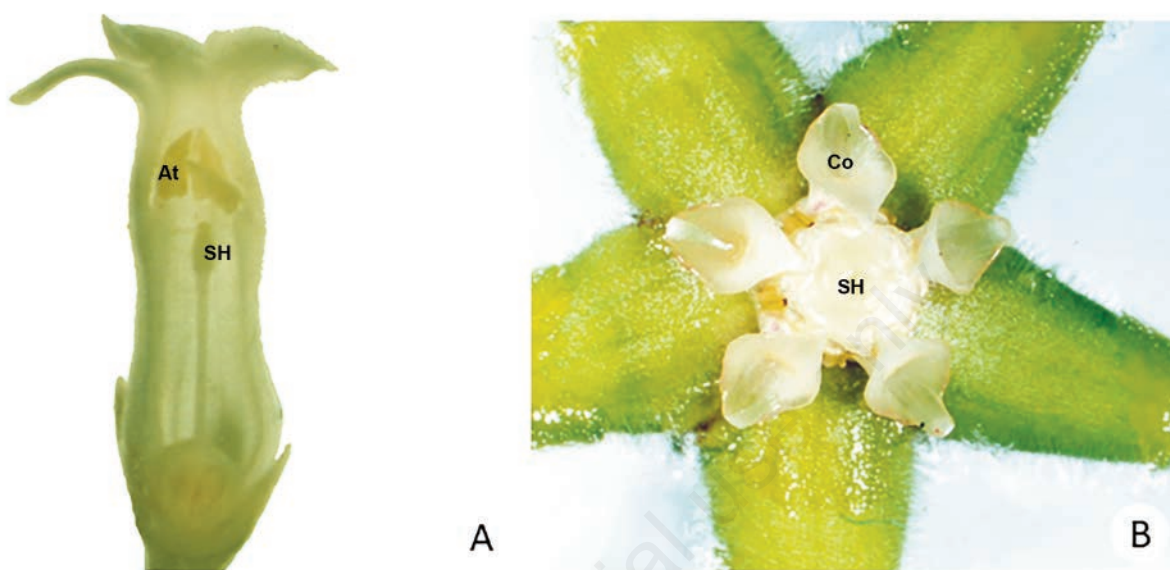

Figure 6. Flowers of Apocynaceae. **A**) *Aspidosperma australe* Müll. Arg. Tubular flower. **B**) *Blepharodon bicuspidatum* E. Fourn. Flower showing a cup-shaped corona (nectar holder). At, anther; Co, corona; SH, style head.
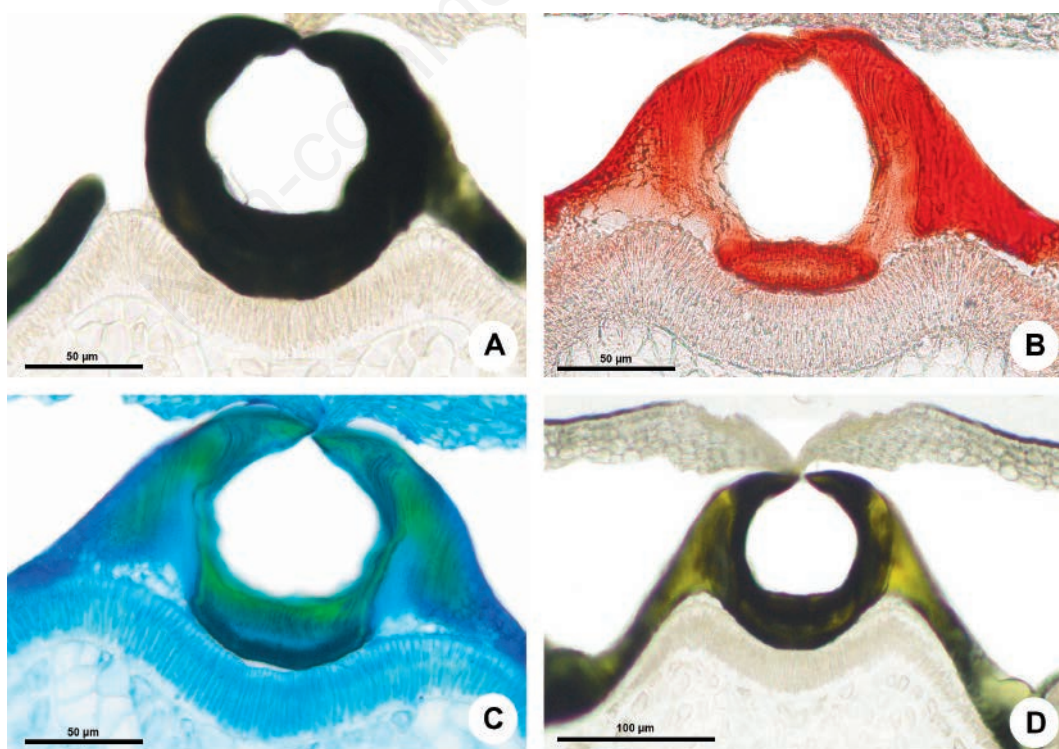

Figure 7. Histochemistry of the translator of *Blepharodon bicuspidatum* E. Fourn. **A-C**) Lipids identification with Sudan black B (**A**), Sudan IV (**B**) and Nile blue (**C**). **D**) Fatty acids detected using copper acetate and rubeanic acid.

the stigma is located (Figure 3D).[25,26,30,40]

This complex pollination mechanism is only possible due to a series of morphological modifications that occurred during the evolutionary history of Apocynaceae, such as changes in the secretory epidermis of the style head, position of stigma, formation of the gynostegium, in addition to several floral parts associated with directing the pollinator to the correct region for pollen removal and deposition, such as trichomes, nectar holders and corona.[17,25,30]

Style head secretion and pollination

The secretion produced by the style head was fundamental in the evolution of Apocynaceae since it is related to elaborate pollination mechanisms. Perhaps the key innovation for the family's reproductive success and increase of the morphological elaboration was the emergence of the style head with its peculiar secretory activity. Although the style head that first appeared is located at the apex of the style and corresponds to the stigma, as observed in most families, its function is far from a usual stigma.

Stigmas can be secretory, called wet stigma.[41] Thus, the stigma in Aspidospermateae occupies the expected position in any flower

and produces a secretion, like all wet stigmas. However, this secretion differs from the secretions of those stigmas. It is much more viscous, mainly formed by lipids. Due to this distinct composition, the secretion of the style head/stigma in this tribe not only has the function of capturing the pollen brought by the pollinator and stimulating its germination[41] but also helping the pollen dispersal of its own flower. The flowers with style heads of types I, II and III are tubular flowers (Figure 6A) that usually produce nectar at the bottom of the floral tube.[23,24,37,42,43] These three types of style head produce secretions that are fluid, lipid-rich, and highly viscous (Figures 1C and 2B). After the pollinator collects nectar at the bottom of the floral tube, it retracts the proboscis, which initially passes through the style head, where the secretion smears it. When the proboscis passes through the anthers, the pollen grains adhere to the secretion present on the proboscis. When the pollinator collects nectar from another flower, the proboscis is again smeared with the secretion; when the proboscis is retracted, the pollen is left on the lateral portion of the style head, removed by the stigmatic cells themselves (or annulus) in types II and III of style heads.

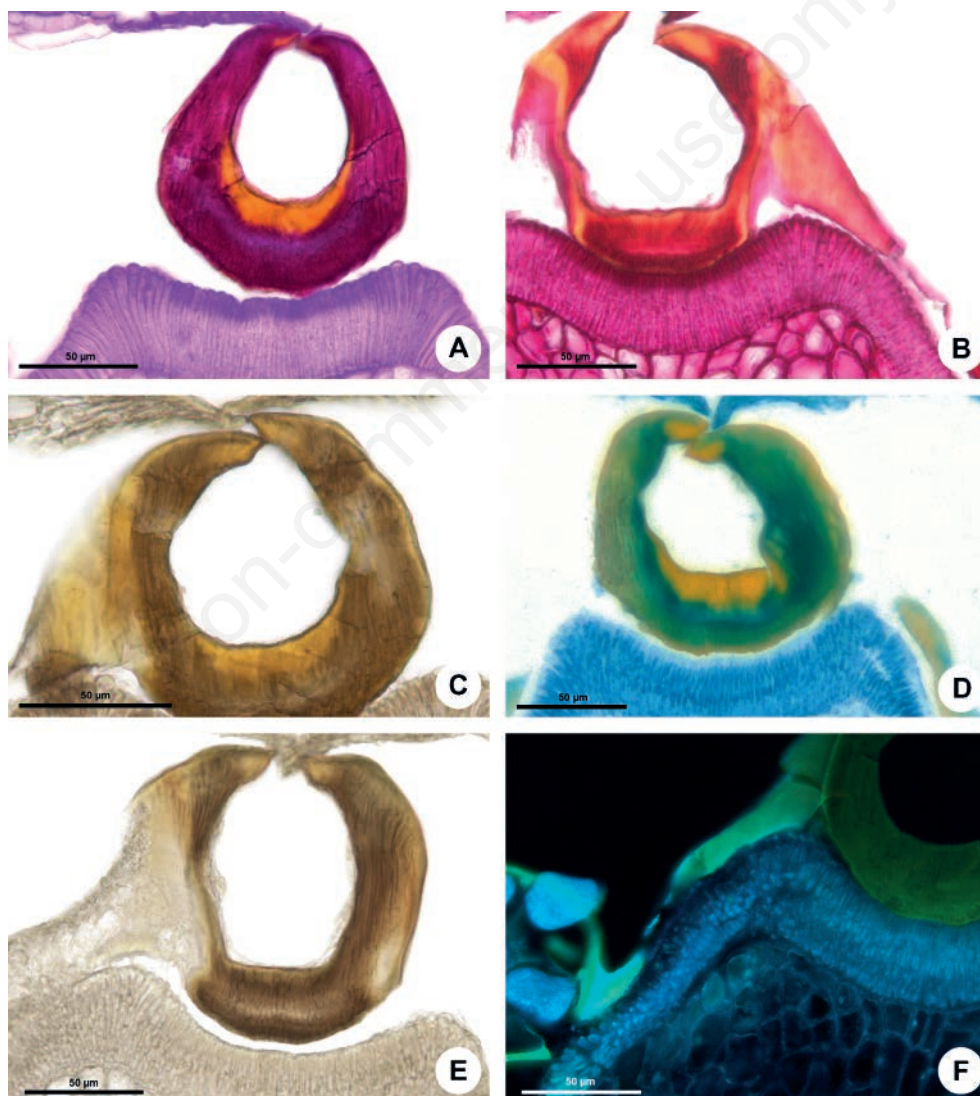In flowers containing style head type IV, nectar may be pro-

Figure 8. Histochemistry of the translator of *Blepharodon bicuspidatum* E. Fourn. **A)** Polysaccharides detected by PAS reaction. **B,C)** Mucilage stained with ruthenium red (**B**), and tannic acid and ferric chloride (**C**). **D)** Proteins identified with aniline blue black. **E,F)** Phenolic compounds detected using ferric chloride (**E**) and autofluorescence under UV (**F**).

duced in different regions. The place where nectar is presented to the pollinator (nectar holder; Figure 6B) compels the insect to insert the proboscis or leg into a slit formed by lateral projections of the stamen, called a guide rail (Figure 1D).[17] In this location, the pollinator becomes trapped and can only be released through an inward and upward movement. When the pollinator does this, its proboscis (or leg) attaches to the slit of the corpusculum and the entire pollinarium is removed from the flower. When collecting nectar from another flower, the same pollinator movement will insert the pollinarium carried on the proboscis (or leg) into the stigmatic chamber (Figure 3B) behind the guide rail.[17,24,25,30,44] This chamber produces nectar (Figure 3D) that stimulates pollen germination, and pollen tubes grow in the secretion towards the stigma.[12,17,45]

Secretion composition

The highly functional complexity of the secretory epidermis, perfectly synchronized with floral development during translator morphogenesis, is also noteworthy in its chemical composition.

The fluid secretion produced by style heads type I, II and III is basically composed of lipids and mucilage. Translators are also composed mainly of these two types of compounds (Figures 7 and 8),[12,15,46-48] but phenolic compounds and proteins have also been reported (Figure 8 D-F).[12,15,49] However, these compounds are not homogeneously distributed throughout the translator (Figure 8F). The corpusculum often has a composition and rigidity significantly different from those of the caudicles.[15,48] While the corpusculum has a large amount of proteins and phenolic compounds, they are present in low quantities, heterogeneously distributed along the caudicles (Figure 8 D-F).

In relation to lipids, the composition of the corpusculum and caudicles may be similar, as observed in *Blepharodon* (Figure 7), or may differ, as observed in *Asclepias*, *Matelea*, *Peplonia* and *Oxypetalum*, in which the corpusculum has a large amount of fatty acids, while the caudicles have neutral lipids.[15] This difference may be related to the movement of the caudicles after pollinarium removal from the flower, as observed in some species. During the pollinator's flight while carrying the pollinarium, the caudicles lose water and change their conformation. In this way, the pollinia change their position, facilitating their insertion into the stigmatic chamber of the other flower visited by the insect.[25,30,35,44,50] This movement of the caudicles does not always happen,[30] and, possibly, the composition difference between corpusculum and caudicles needs to be investigated case-by-case.

Future perspectives

Apocynaceae reached the highest degree of functional complexity of a secretory tissue. The occurrence of secretory epidermis as the only secretory tissue of the most external glands of the family may be related to the increased metabolic complexity of the stigmatic cells in Aspidospermateae and its consequent derivation in the other types of style head. The use of histochemistry allowed us to discover temporally and functionally distinct cellular activities in the same tissue and has opened up a new universe of questions about secretory activity in plants. Further studies are needed to answer how the compounds are distributed along the translators and which subcellular changes occurred in the secretory cells that triggered such changes in the secreted product. However, it is only the first step towards understanding the evolution of this tissue that allows the dispersal of large quantities of pollen at once (pollinium), an unparalleled case in eudicots that can only be compared to the pollination mechanisms of orchids.

Description of procedures

Plant material

Floral buds and mature flowers of *Aspidosperma australe* Müll. Arg., *Blepharodon bicuspidatum* E.Fourn., *Mandevilla tenuifolia* (J.C. Mikan) Woodson, *Peplonia axillaris* (Vell.) Fontella & Rapini, *Plumeria rubra* L. and *Tabernaemontana catharinensis* A.DC. were fixed in formalin, acetic acid and alcohol (FAA) for 24 h[51] or buffered neutral formalin in 0.1M sodium phosphate buffer (pH 7.0)[52] for 48 h, being subsequently stored in 70% ethanol.

Scanning electron microscopy

Flowers fixed in FAA were dehydrated in a graded ethanol series, critical point dried with CO_2, mounted on aluminum stubs, and coated with gold using a Balzers SCD-050 sputter coater (Balzers, Liechtenstein). Observations were carried out in a Zeiss Sigma VP scanning electron microscope (Carl Zeiss, Oberkochen, Germany).

Histological analysis

Fixed flowers were dehydrated through a tertiary butyl alcohol series,[51] embedded in Paraplast (Leica Microsystems Inc., Heidelberg, Germany), and serial sectioned at 12 μm thickness on a Leica RM2145 rotary microtome. Longitudinal and transverse sections were stained with either Flemming's triple stain[51] or Astra blue and Safranin.[53] The slides were mounted with Permount resin (Fisher Scientific, Pittsburgh, PA, USA).

Histochemistry

The main chemical classes of compounds in the secretion were detected using the following histochemical tests: Sudan black B and Sudan IV for lipids,[54] Nile blue for acidic and neutral lipids,[55] copper acetate and rubeanic acid for fatty acids,[56,57] ferric chloride for phenolic compounds,[51] Aniline blue black for proteins,[58] periodic acid-Schiff's (PAS) reaction for carbohydrates,[59] Ruthenium red for acidic mucilage[60] and tannic acid and ferric chloride for mucilage.[61] Autofluorescence under UV was also used for detection of phenolic compounds.[62] All observations and photographs were performed using a Leica DMLB light microscope equipped with an HBO 100 W mercury vapor lamp and a UV filter block (excitation filter BP340-380, dichromatic mirror RKP400, suppression filter LP425). The procedures of all tests were carried out according to Demarco.[62]

References

1. Coiffard C, Gomez B, Daviero-Gomez V, Dilcher DL. Rise to dominance of angiosperm pioneers in European Cretaceous environments. Proc Natl Acad Sci USA 2012;109:20955-9.
2. Stebbins GL. Why are there so many species of flowering plants? Bioscience 1981;31:573-7.
3. Midgley JJ, Bond WJ. How important is biotic pollination and

dispersal to the success of the angiosperms? Philos T Roy Soc B 1991;333:209-15.

4. Berendse F, Scheffer M. The angiosperm radiation revisited, an ecological explanation for Darwin's "abominable mystery". Ecol Lett 2009;12:865-72.

5. Nicolson SW, Nepi M, Pacini E. Nectaries and nectar. Springer, Dordrecht; 2007.

6. Tölke ED, Capelli NV, Pastori T, Alencar AC, Cole TCH, Demarco D. Diversity of floral glands and their secretions in pollinator attraction. In: Mérillon JM, Ramawat KG, Editors. Coevolution of secondary metabolites. Reference Series in Phytochemistry. Springer, Cham 2019; p. 1-46.

7. Sponsler D, Iverson A, Steffan-Dewenter I. Pollinator competition and the structure of floral resources. Ecography 2023;2023:e06651.

8. Vogel S. The role of scent glands in pollination. On the structure and function of osmophores. Translated by J.S. Bhatti, Amerind Publishing, New Delhi; 1990.

9. Armbruster WS. The role of resin in angiosperm pollination: ecological and chemical considerations. Am J Bot 1984;71:1149-60.

10. Boff S, Demarco D, Marchi P, Alves-dos-Santos I. Perfume production in flowers of Angelonia salicariifolia attracts males of Euglossa annectans which do not promote pollination. Apidologie 2015;46:84-91.

11. Lunau K, Wester P. Mimicry and deception in pollination. Adv Bot Res 2017;82:259-79.

12. Demarco D. Floral glands in asclepiads: structure, diversity and evolution. Acta Bot Bras 2017;31:477-502.

13. Tilton VR, Horner HT Jr. Stigma, style, and obturator of Ornithogalum caudatum (Liliaceae) and their function in the reproductive process. Am J Bot 1980;67:1113-31.

14. Endress PK. Development and evolution of extreme synorganization in angiosperm flowers and diversity: a comparison of Apocynaceae and Orchidaceae. Ann Bot 2016;117:749-67.

15. Demarco D. Secretory tissues and the morphogenesis and histochemistry of pollinarium in flowers of Asclepiadeae (Apocynaceae). Int J Plant Sci 2014;175:1042-53.

16. Demarco D. Staminal wing gland: a novel secretory structure of asclepiads. Botany 2017;95:763-72.

17. Monteiro MM, Demarco D. Corona development and the floral nectaries in Asclepiadeae (Asclepiadoideae, Apocynaceae). Acta Bot Bras 2017;31:420-32.

18. Ribeiro JC, Ferreira MJP, Demarco D. Colleters in Asclepiadoideae (Apocynaceae): protection of meristems against desiccation and new functions assigned. Int J Plant Sci 2017;178:465-77.

19. Medina MC, Sousa-Baena MS, Capelli NV, Koch R, Demarco D. Stinging trichomes in Apocynaceae and their evolution in angiosperms. Plants 2021;10:2324.

20. Wyatt R, Lipow SR. A new explanation for the evolution of pollinia and loss of carpel fusion in Asclepias and the Apocynaceae s.l. Ann Mo Bot Gard 2007;94:474-84.

21. Ollerton J, Liede-Schumann S, Endress ME, Meve U, Rech AR, Shuttleworth A. et al. The diversity and evolution of pollination systems in large plant clades: Apocynaceae as a case study. Ann Bot 2019;123:311-25.

22. Bitencourt C, Nürk NM, Rapini A, Fishbein M, Simões AO, Middleton DJ. et al. Evolution of dispersal, habit, and pollination in Africa pushed Apocynaceae diversification after the Eocene-Oligocene climate transition. Front Ecol Evol 2021;9:719741.

23. Fallen ME. Floral structure in the Apocynaceae: morphological, functional and evolutionary aspects. Bot Jahrb Syst 1986;106:245-86.

24. Endress ME, Bruyns PV. A revised classification of Apocynaceae s.l. Bot Rev 2000;66:1-56.

25. Endress PK. Diversity and evolutionary biology of tropical flowers. University Press, Cambridge; 1994.

26. Demarco D. [Glândulas de órgãos vegetativos aéreos e florais de espécies de Asclepiadeae (R.Br.) Duby (Asclepiadoideae, Apocynaceae) de Mata Atlântica do Estado de São Paulo].[PhD dissertation in Portuguese]. Universidade Estadual de Campinas; 2008.

27. Endress PK, Jenny M, Fallen ME. Convergent elaboration of apocarpous gynoecia in higher advanced dicotyledons (Sapindales, Malvales, Gentianales). Nord J Bot 1983;3:293-300.

28. Walker DB. Postgenital carpel fusion in Catharanthus roseus (Apocynaceae). I. Light and scanning electron microscopic study of gynoecial ontogeny. Am J Bot 1975;64:457-67.

29. Demarco D. [Estruturas secretoras florais e coléteres foliares em espécies de cerrado de Aspidosperma Mart. e Blepharodon Decne. (Apocynaceae s.l.)].[Master's thesis in Portuguese]. Universidade Estadual de Campinas,; 2005.

30. Kunze H. Structure and function in asclepiad pollination. Plant Syst Evol 1991;176:227-53.

31. Fishbein M, Livshultz T, Straub SCK, Simões AO, Boutte J, McDonnell A, Foote A. Evolution on the backbone: Apocynaceae phylogenomics and new perspectives on growth forms, flowers, and fruits. Am J Bot 2018;105:495-513.

32. Swarupanandan K, Mangaly JK, Sonny TK, Kishorekumar K, Chand Basha S. The subfamilial and tribal classification of the family Asclepiadaceae. Bot J Linn Soc 1996;120:327-69.

33. Kunze H. Evolution of the translator in Periplocaceae and Asclepiadaceae. Plant Syst Evol 1993;185:99-122.

34. Kunze H. Ontogeny of the translator in Asclepiadaceae s.str. Plant Syst Evol 1994;193:223-42.

35. Kunze H. Floral morphology of some Gonolobeae (Asclepiadaceae). Bot Jahrb Syst 1995;117:211-38.

36. Rapini A, van den Berg C, Liede-Schumann S. Diversification of Asclepiadoideae (Apocynaceae) in the New World. Ann Mo Bot Gard 2007;94:407-22.

37. Endress ME, Meve U, Middleton DJ, Liede-Schumann S. Apocynaceae. In: Kadereit J, Bittrich V, Editors. Flowering plants. Eudicots. The families and genera of vascular plants. Springer, Cham 2018; p. 207-411.

38. Livshultz T, Hochleitner S, Lakata E. Pollen transfer efficiency of Apocynum cannabinum (Apocynaceae): a comparative perspective. J Poll Ecol 2018;22:35-48.

39. Nilsson S, Endress ME, Grafström E. On the relationship of the Apocynaceae and Periplocaceae. Grana 1993;2:3-20.

40. Wiemer AP, Sérsic AN, Marino S, Simões AO, Cocucci AA. Functional morphology and wasp pollination of two South American asclepiads (Asclepiadoideae-Apocynaceae). Ann Bot 2012;109:77-93.

41. Heslop-Harrison Y. Stigma characteristics and angiosperm taxonomy. Nord J Bot 1981;1:401-20.

42. Woodson RE, Moore JA. The vascular anatomy and comparative morphology of Apocynaceae flowers. Bull Torrey Bot Club 1938;65:135-66.

43. Rao VS, Ganguli A. Studies in the floral anatomy of the Apocynaceae. J Indian Bot Soc 1963;42:419-35.

44. Kunze H, Liede S. Observations on pollination in Sarcostemma (Asclepiadaceae). Plant Syst Evol 1991;178:95-105.

45. Vieira MF, Shepherd GJ. Oxypetalum banksii subsp. banksii: a taxon of Asclepiadaceae with an extragynoecial compitum. Plant Syst Evol 2002;233:199-206.

46. Demeter K. [Vergleichende Asclepiadeenstudien].[Article in

OPEN ACCESS

German]. Flora 1922;15:130-76.

47. Safwat FM. The floral morphology of Secamone and the evolution of the pollinating apparatus in Asclepiadaceae. Ann Mo Bot Gard 1962;49:95-129.

48. Schnepf E, Witzig F, Schill R. [Über Bildung und Feinstruktur des Translators der Pollinarien von Asclepias curassavica und Gomphocarpus fruticosus (Asclepiadaceae)].[Article in German]. Trop Subtrop Pflanzenwelt 1979;25:1-33.

49. Vijayaraghavan MR, Cheema K. Ontogenetical and histochemical studies on the translator apparatus in Calotropis procera R.Br. I. The retinaculum. Acta Histochem 1977;59:15-20.

50. Kunze H. Pollination ecology in two species of Gonolobus (Asclepiadaceae). Flora 1999;194:309-16.

51. Johansen DA. Plant microtechnique. McGraw-Hill, New York;1940.

52. Lillie RD. Histopathologic technic and practical histochemistry. McGraw-Hill, New York;1965.

53. Gerlach D. [Botanish Mikrotechnik: eine Einführung].[Book in German]. Georg Thieme, Stuttgart;1984.

54. Pearse AGE. Histochemistry: theoretical and applied. C. Livingstone, Edinburgh 1985.

55. Cain AJ. The use of Nile Blue in the examination of lipids. Quart J Microsc Sci 1947;88:383-92.

56. Ganter P, Jollès G. [Histochimie normale et pathologique].[Book in French], vol. 1. Gauthier-Villars, Paris; 1969.

57. Ganter P, Jollès G. [Histochimie normale et pathologique]. [Book in French], vol. 2. Gauthier-Villars, Paris; 1970.

58. Fisher DB. Protein staining of ribboned epon sections for light microscopy. Histochemie 1968;16:92-6.

59. McManus JFA. Histological and histochemical uses of periodic acid. Stain Technol 1948;23:99-108.

60. Gregory M, Baas P. A survey of mucilage cells in vegetative organs of the dicotyledons. Isr J Bot 1989;38:125-74.

61. Pizzolato TD. Staining of Tilia mucilages with Mayer's tannic acid-ferric chloride. Bull Torrey Bot Club 1977;104:277-9.

62. Demarco D. Histochemical analysis of plant secretory structures. Methods Mol Biol 2023;2566:291-310.

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