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Corona development and floral nectaries of Asclepiadeae (Asclepiadoideae, Apocynaceae)

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ABSTRACT

Flowers of Asclepiadoideae are notable for possessing numerous nectaries and elaborate coronas, where nectar can accumulate but is not necessarily produced. Given the complexity and importance of these structures for reproduction, this study aimed to analyze the ontogeny of the corona, the structure and position of nectaries and the histochemistry of the nectar of species of Asclepiadeae. Two types of coronas were observed: androecial [C(is)] and corolline (Ca). The development of the C(is)-type of corona initiates opposite the stamens in all species examined with the exception of *Matelea* in which it begins to develop as a ring around the filament tube. Despite their morphological variation, coronas typically originate from the androecium. A notable difference among the studied species was the location of the nectaries. Primarily, they are located in the stigmatic chamber, where nectar composed of carbohydrates and lipids is produced. A secondary location of nectaries found in species of *Peplonia* and *Matelea* is within the corona, where nectar is produced and stored, composed of carbohydrates and lipids in *Peplonia* and only carbohydrates in *Matelea*. The functional role of nectar is related to the location of its production since it is a resource for pollinators and inducers of pollen germination.

Keywords: asclepiads, histochemistry, ontogeny, nectar, structural diversity

Introduction

The floral complexity of Asclepiadoideae is mainly due to the different shape and structures of the corona. Despite its considerable systematic value in Apocynaceae (Endress & Bruyns 2000; Fishbein 2001), there have been few studies related to this structure (Hofmann & Specht 1986; Kunze 1990; 1997; Liede & Kunze 1993; Kunze & Wanntorp 2008).

The corona is formed in the region between the bases of the gamopetalous corolla and the filament tube (Endress & Bruyns 2000) after the initial development stages of these two whorls (Hofmann & Specht 1986; Kunze 1990). In the mature flower, the parts with staminal and/or corolline origin cannot be distinguished (Endress & Bruyns 2000). The terminology of the coronal structures differs from group to group and even from author to author, this being the most commonly used structure for taxa classification in Asclepiadoideae (Woodson 1941; Rao & Ganguli 1963; Liede & Kunze 1993).

The subfamily Asclepiadoideae is the most diverse in secretory structures within a flower among the angiosperms (Demarco 2017a), and the presence of nectar in different locations is related to a specialized pollination mechanism considered one of the most intricate in the angiosperms (Kunze 1991). Many structures are involved in guiding the insect during pollination, such as the guide rail formed

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by staminal wings, corona, corolla and the nectar holders (Kunze 1991; 1995; 1999; Demarco 2008).

In Asclepiadoideae, the primary location of the nectariferous tissues is in the interstaminal areas of the filament tube (stigmatic chambers), and nectar can accumulate (nectar holders) in the staminal corona or at the area where the corolla tube is connected to the gynostegium (Galil & Zeroni 1965; Christ & Schnepf 1985; Kunze 1991; 1997; Demarco 2017a). The position of the nectaries, however, has been controversial.

Nectar can be found in the stigmatic chamber, known as primary nectary, and in cup-shaped structures formed by the corona. Some authors assume that only the nectariferous stigmatic chamber is secretory and that nectar runs through an intricate capillary system to the nectar holder (Galil & Zeroni 1965; Bookman 1981; Kunze 1997); however, in some species secretory tissue has been described on the staminal corona, also known assecondary nectary (Rao & Ganguli 1963; Valente & Silva 1984; Bruyns 1993; Kunze 1995; 1999; Demarco 2005).

Considering the different locations of nectar presentation, it is expected that they have a variable composition and may play different roles depending on the species since nectar with double function, as a resource for the pollinator and inducer of pollen germination, has already been reported in many asclepiads (Galil & Zeroni 1965; Eisikowitch 1986; Kunze 1991). Furthermore, the nature of the exudate of these nectaries was previously considered heterogeneous (Christ & Schnepf 1985; Vieira & Shepherd 2002). Nevertheless, a comparative analysis among the different nectaries in the same flower was never carried out in order to evaluate the composition and function of the nectar.

The aim of this work was to evaluate the origin of the corona and examine the position of the floral nectaries in species of Asclepiadeae. This investigation involved an ontogenetic study, a description of the structure and a comparative histochemical evaluation of the nectaries in flowers of Asclepiadinae and from the New World endemic clade MOG (Metastelmatinae, Oxypetalinae and Gonolobinae) (Rapini *et al*. 2006).

Materials and methods

The species of Asclepiadeae (*sensu* Endress *et al*. 2014) selected to this work were *Asclepias curassavica* L. (subtribe Asclepiadinae; D. Demarco 52, 66, 68), *Matelea denticulata* (Vahl) Fontella & E.A.Schwarz (subtribe Gonolobinae; D. Demarco 37, 38), *Oxypetalum banksii* subsp. *banksii* Roem. & Schult. (subtribe Oxypetalinae; D. Demarco 57, 70), and *Peplonia axillaris* (Vell.) Fontella & Rapini (subtribe Metastelmatinae; D. Demarco 35, 48, 49). All species were collected at the Parque Estadual da Serra do Mar – Núcleo Picinguaba, Ubatuba, São Paulo, Brazil. Vouchers were deposited at the Herbarium of the Universidade Estadual de Campinas (UEC), Campinas, São Paulo, Brazil.

Floral buds and mature flowers of all species were fixed in formalin–acetic acid–alcohol (FAA) solution for 24 h (Johansen 1940), buffered neutral formalin (BNF) in 0.1 M sodium phosphate buffer (pH 7.0; Lillie 1965) and ferrous sulfate–formalin (FSF; Johansen 1940) solution for 48 h, and then stored in 70 % ethyl alcohol.

For micromorphological analysis, mature flowers fixed in FAA were isolated, dehydrated in ethanol series, CO_2-critical point dried, mounted and coated with gold. The observations and recordings of images were performed using a Jeol JSM 5800 LV 10 kV SEM (Jeol, Tokyo).

Based on morphological changes during floral development, seven stages were stablished, avoiding conflicts caused by the different sizes of flowers among species: 1) floral meristem; 2) primordia of petals, stamens, and carpels completely wrapped by the calyx; 3) beginning of corolla elongation; 4) buds with half the final length; 5) pre-anthesis; 6) anthesis; 7) post-anthesis. Thirty floral buds and mature flowers of each species were isolated, dehydrated in a butyl series (Johansen 1940), embedded in Paraplast, and transversely and longitudinally sectioned at 10-14 μm on a Microm HM340E rotation microtome (Microm International, Walldorf, Germany). The sections were stained with astra blue and safranin (color index [C.I.] 50240; Gerlach 1984), and mounted in synthetic resin.

For histochemical analysis, different treatments were performed to highlight the major chemical classes in the composition of the secretion: ruthenium red for acidic mucilage (Gregory & Baas 1989), tannic acid and ferric chloride for mucilage (Pizzolato 1977), periodic acid–Schiff's (PAS) reaction (pararosaniline C.I. 42500) for carbohydrates (McManus 1948), Sudan Black (C.I. 26150) and Sudan IV (C.I. 26105) for lipids (Pearse 1985), Nile blue (C.I. 51180) for acidic and neutral lipids (Cain 1947), copper acetate and rubeanic acid for fatty acids (Ganter & Jollés 1969; 1970), ferric chloride for phenolic compounds (Johansen 1940), and Dragendorff's (Svendsen & Verpoorte 1983) and Wagner's (Furr & Mahlberg 1981) reagents for alkaloids. Fixation with ferrous sulfate–formalin was also used in order to detect the presence of phenolic compounds in nectar composition. The slides were mounted with glycerin gelatin. The presence of glucose in the nectar was detected by the use of glucose enzymatic test strips in contact with the nectar holders and stigmatic chambers.

The tests control of hydrophilic substances and lipophilic substances were carried out according to Demarco (2017b). All photomicrographs were taken using an Olympus BX51 microscope (Melville, USA).

The terminology adopted to describe the corona in the present paper follows the terms proposed by Liede & Kunze (1993). "C" for corona, which can be differentiated in: "a" for annular; "i" for interstaminal; "s" for staminal; and "is" for interstaminal + staminal. Some flowers of *Blepharodon bicuspidatum* E.Fourn. were hand-pollinated to verify the action of the primary nectar as inductor of pollen germination.

Results

The flowers of Asclepiadeae have five monadelphous stamens with five stigmatic chambers in the filament tube in an interstaminal position behind the guide rails and delimited by the staminal wings (Fig. 1A-D). The corona is also present with different morphologies between androecium and corolla (Fig. 2A-F). The corona of *A. curassavica*, *O. banksii* subsp. *banksii* and *P. axillaris* is formed by staminal and interstaminal projections [C(is)] (Fig. 2A-C, F), while in *M. denticulata* the different parts of the corona form a ring at the base of the gynostegium (Fig. 2D-E). Additionally, *M. denticulata* presents an annular corona (Ca) at the base of the corolla tube (Fig. 2D).

Nectaries

Stigmatic chamber

All flowers present nectariferous stigmatic chambers (Fig. 3A-E), elongated in most species (Fig. 1C) and very short in *M. denticulata* (Fig. 3E). The style head covers these chambers in its upper portion (Fig. 1C). In all species the secretory tissue corresponds only to the epidermal cells (Fig. 3A-E).

The secretory epidermis is composed of one layer of squared to slightly elongated cells with thin cell walls, dense cytoplasm and large nucleus. The cuticle is thin, and a periplasmic space is observed below the outer periclinal cell wall (Fig. 3A, D). This epidermal layer is continuous until the base of the style head (Fig. 3B), where the stigma is found. In the androecium region, which corresponds to the distal portion of the filaments (Fig. 3C), the anthers are free in *A. curassavica, O. banksii* subsp. *banksii* and *P. axillaris* and the space between the anthers forms an opening in the chamber (Fig. 3B). In *M. denticulata*, an opening is absent due to the union of the bases of the anthers.

The cells start their secretory activity in pre-anthetic flowers and continue to secrete until post-anthesis. The secretion appears to be dense and accumulates within the chamber (Fig. 3B-C, E). The primary nectaries of *A. curassavica*, *M. denticulate*, *O. banksii* subsp. *banksii* and *P. axillaris* correspond to the stigmatic chamber and produce a heterogeneous exudate composed of carbohydrates, including glucose and mucilage, and lipids (Tab. 1). Nectar stimulates the pollinium germination (Fig. 1B), and the pollen tubes grow to the stigma below the style head (Fig. 1D).

Corona

The corona in *A. curassavica*, *M. denticulate*, *O. banksii* subsp. *banksii* and *P. axillaris* (Fig. 2A-F) originates from the androecium. This structure is formed in the third and fourth stages of the floral development at the base of the filament tube (Fig. 4A-E). Initially, the meristem activity is observed opposite the filament vascular bundle (Fig. 4B) in *A. curassavica*, *O. banksii* subsp. *banksii* and *P. axillaris* and forms the staminal portion of the corona (Figs. 4C-G, 5A-B). The formation of the interstaminal corona (Fig. 4F) begins later. In *M. denticulate*, the meristem activity initiates simultaneously along the entire base of the filament tube, originating a ring [C(is)] around the gynostegium (Figs. 2E, 5C-D). Although the adnation of the corona with the corolla tube is observed in *P. axillaris* (Figs. 2C, 5B), it is secondary and the corona originates exclusively from the androecium. In *M. denticulate*, another type of corona is also formed by an annular thickening (Ca) at the base of the corolla tube (Figs. 2D-E, 4D, 5E).

The corona is strictly related to the regions where nectar accumulates and is available to the pollinator, forming nectar holders of differing shapes and positions. In *A. curassavica*, the nectar holder is a cup-shaped structure formed by the staminal corona, and in *O. banksii* subsp. *banksii* and *P. axillaris*, the nectar is stored in interstaminal cavities formed by the adnation of the corona to the corolla tube (Figs. 2C, 5B). In *M. denticulate*, the C(is) corona is well developed in the interstaminal region, projecting itself between the base of the anthers (Fig. 2E). Part of the nectar in this species is stored in this region between the ring and the filament tube and is also observed between the rings of the C(is) and Ca corona (Figs. 2D-E, 5E).

The nectar available in the nectar holders has different origins in the studied species. In the corona of *A. curassavica* and *O. banksii* subsp. *banksii* (Fig. 2F) a secretory tissue is absent, and the nectar found in the nectar holders comes from the nectariferous stigmatic chambers (primary nectaries). On the other hand, the corona in *P. axillaris* (Figs. 2B, 4F-G, 5A) and *M. denticulate* (Fig. 5C-E) present secretory zones, and the nectar available is exclusively produced by these secondary nectaries.

Only the staminal corona (Cs) has a secretory activity in *P. axillaris* (Fig. 4F). The secretory portion is continuous along the entire staminal coronal surface under the level of the base of the anthers (Fig. 4G) and is composed of one layer of rectangular epidermal cells, continuous with the filaments tube, and square-shaped epidermal cells at the free portion of the corona (Figs. 4F-G, 5A). The epidermal cells are covered by a thin cuticle with thin cell walls, dense cytoplasm and a periplasmic space at the distal portion (Fig. 5A), where the secretion accumulates before it is released outwards. A hypodermis without secretory activity with cytoplasm slightly stained is observed under the rectangular epidermal cells (Fig. 5A).

The staminal corona (Cs) in *P. axillaris* has a flat base (Fig. 2C) and does not present a cup-shaped structure as in *A. curassavica*. Therefore, the secretion released flows to the interstaminal cavities (Fig. 2C, 5B), located under the

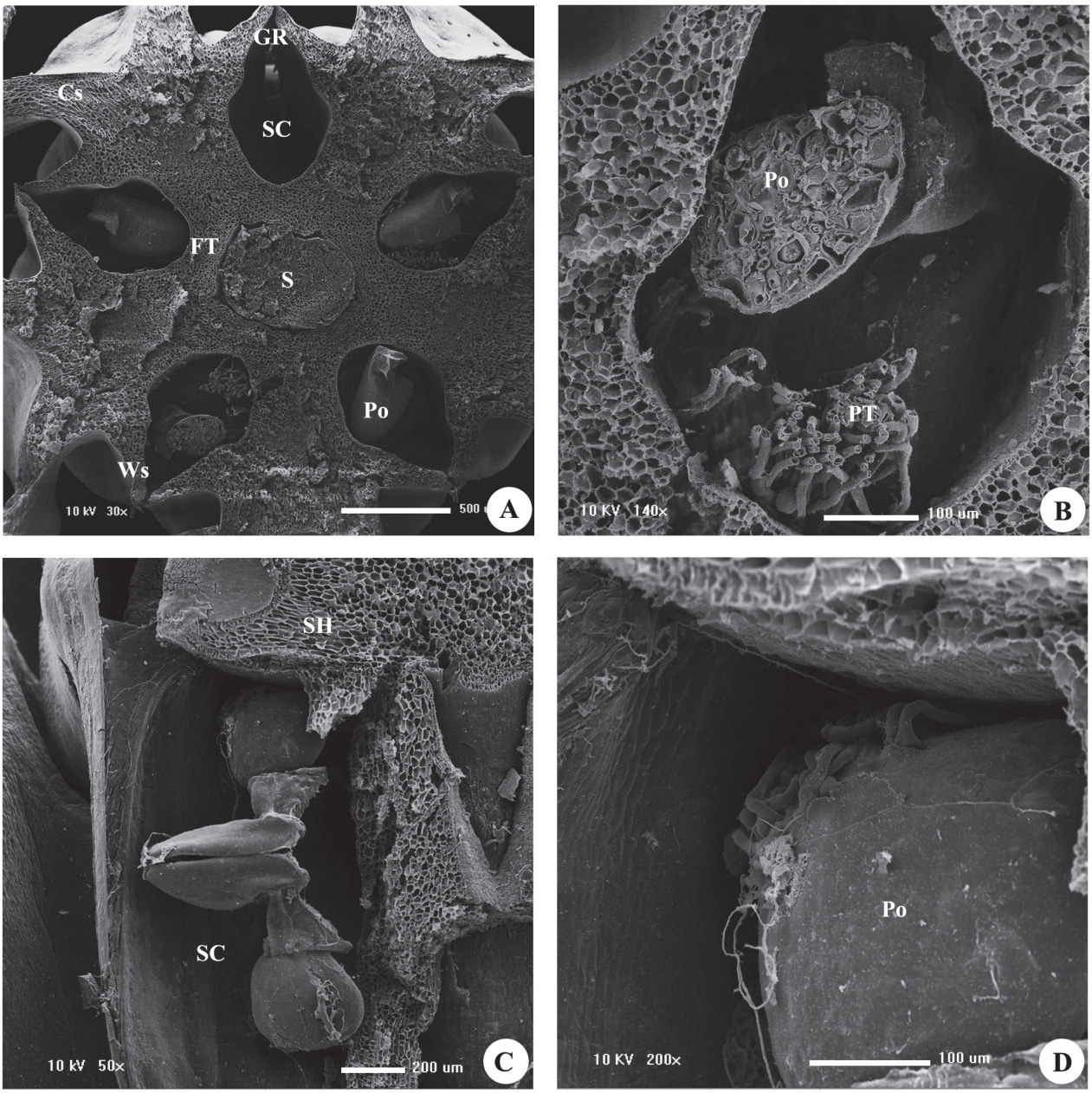

Figure 1. SEM micrographs showing hand-pollinated flowers of *Blepharodon bicuspidatum*. **A.** Transverse section of the flower with pollinia in the stigmatic chambers located in the filament tube, alternate the anthers, behind the guide rails formed by two adjacent staminal wings. **B.** Detail of A, showing the stigmatic chamber and the pollinium with pollen tubes. **C.** Longitudinal section of the stigmatic chamber with a pollinarium. **D.** Detail of C, showing the germinated pollinium with the pollen tubes growing towards the stigma below the style head. Cs = staminal corona; FT = filament tube; GR = guide rail; Po = pollinium; PT = pollen tubes; S = style; SC = stigmatic chamber; SH = style head; Ws = staminal wing.

guide rail and stigmatic chamber. The secretion from the corona is composed of carbohydrates, including glucose and mucilage, and lipids (Tab. 1).

The C(is) corona in *M. denticulate* has secretory activity in the lower half of the ring (Fig. 5E). The secretory portion is lobed, and both the epidermal and parenchyma tissues are secretory (Fig. 5C-E). A layer of elongated epidermal cells is organized in palisade, and several layers of secretory parenchyma, up to fifteen, can be found at the lobed region and around five layers in the regions between the lobes. All secretory cells have thin cell walls with dense cytoplasm (Fig. 5D). The epidermal cells are covered by thin cuticle and present periplasmic spaces where the secretion accumulates before being released through the cell wall and cuticle. The

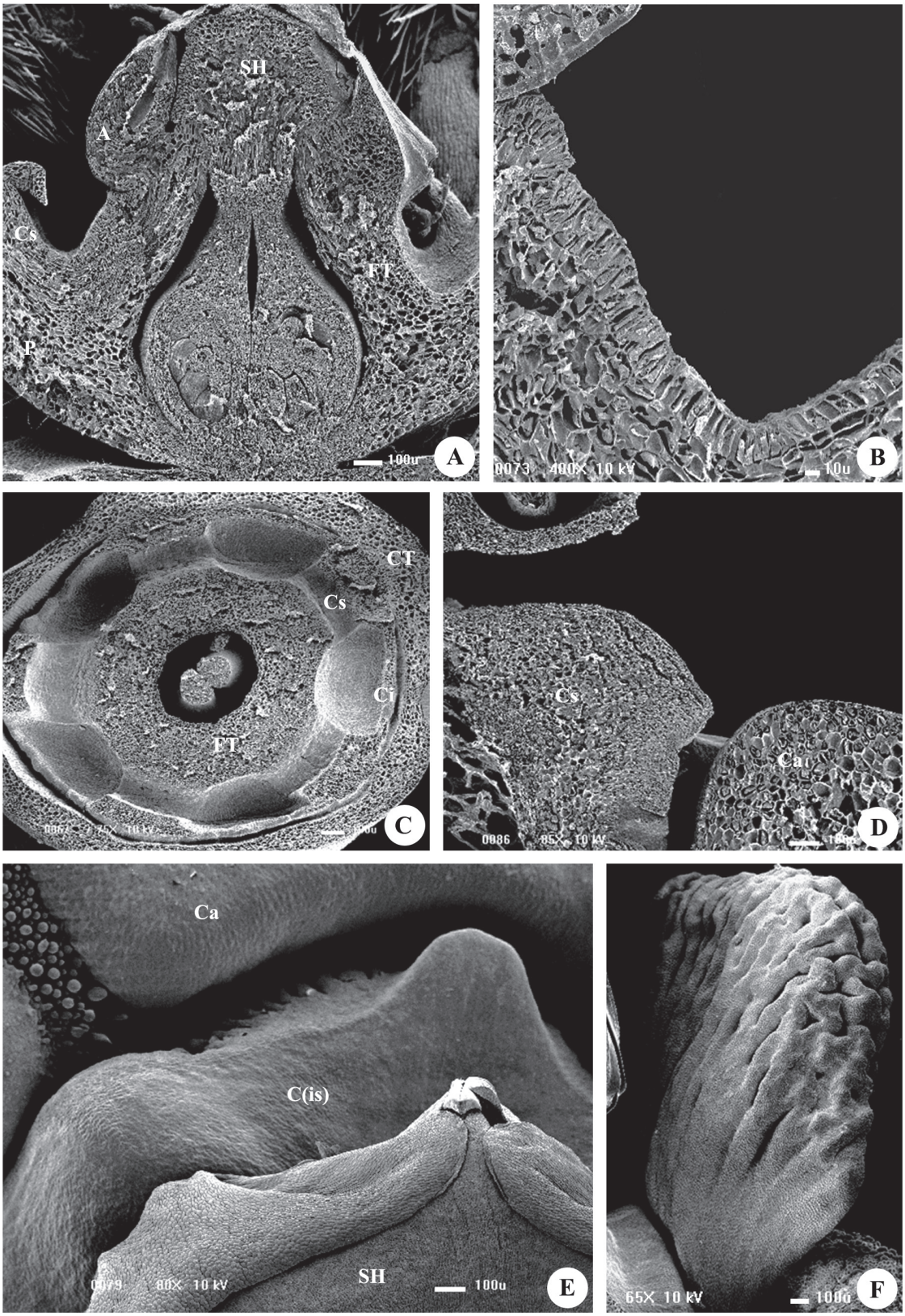

Figure 2. SEM micrographs showing mature flowers of Asclepiadeae. **A-B, D.** Longitudinal sections of the flowers. **C.** Transverse section of the flower. **A-C.** *Peplonia axillaris*. **D-E.** *Matelea denticulata*. **F.** *Oxypetalum banksii*. **A**. View of a sectioned flower. **B.** Secondary nectary in the staminal corona. **C.** Flower base showing the staminal corona postgenitally adnate to the corolla tube. **D-E.** General view of the ring-shaped corona (androecial corona) and annular corona (corolline corona). **F.** Staminal corona. A = anther; Ca = annular corona; Ci = interstaminal corona; $C_{(is)}$ = staminal + interstaminal corona; Cs = staminal corona; CT = corolla tube; P = petal; SH = style head.

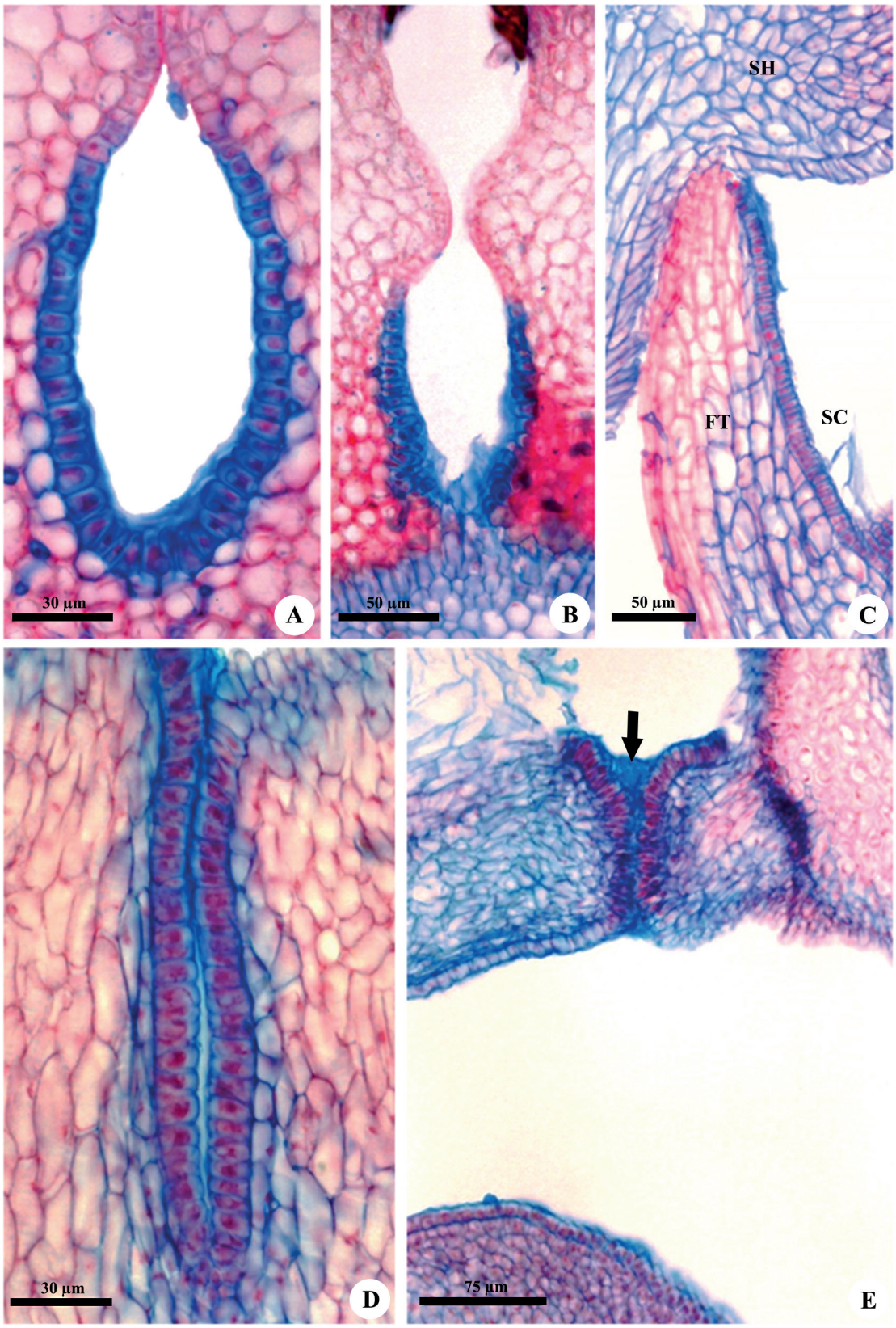

Figure 3. Structure of stigmatic chamber in flowers of *Peplonia axillaris* (**A-C**) and *Matelea denticulata* (**D-E**). **A-B, D.** Transverse sections. **C, E.** Longitudinal sections. **A, D.** Nectariferous epidermis in the stigmatic chamber. **B.** Opening of the stigmatic chamber in the stigma region. **C.** Filament tube and stigmatic chamber ending at the style head. Note secretion on the nectariferous epidermis. **E.** Short chamber completely filled with nectar (arrow). FT = filament tube; SC = stigmatic chamber; SH = style head.

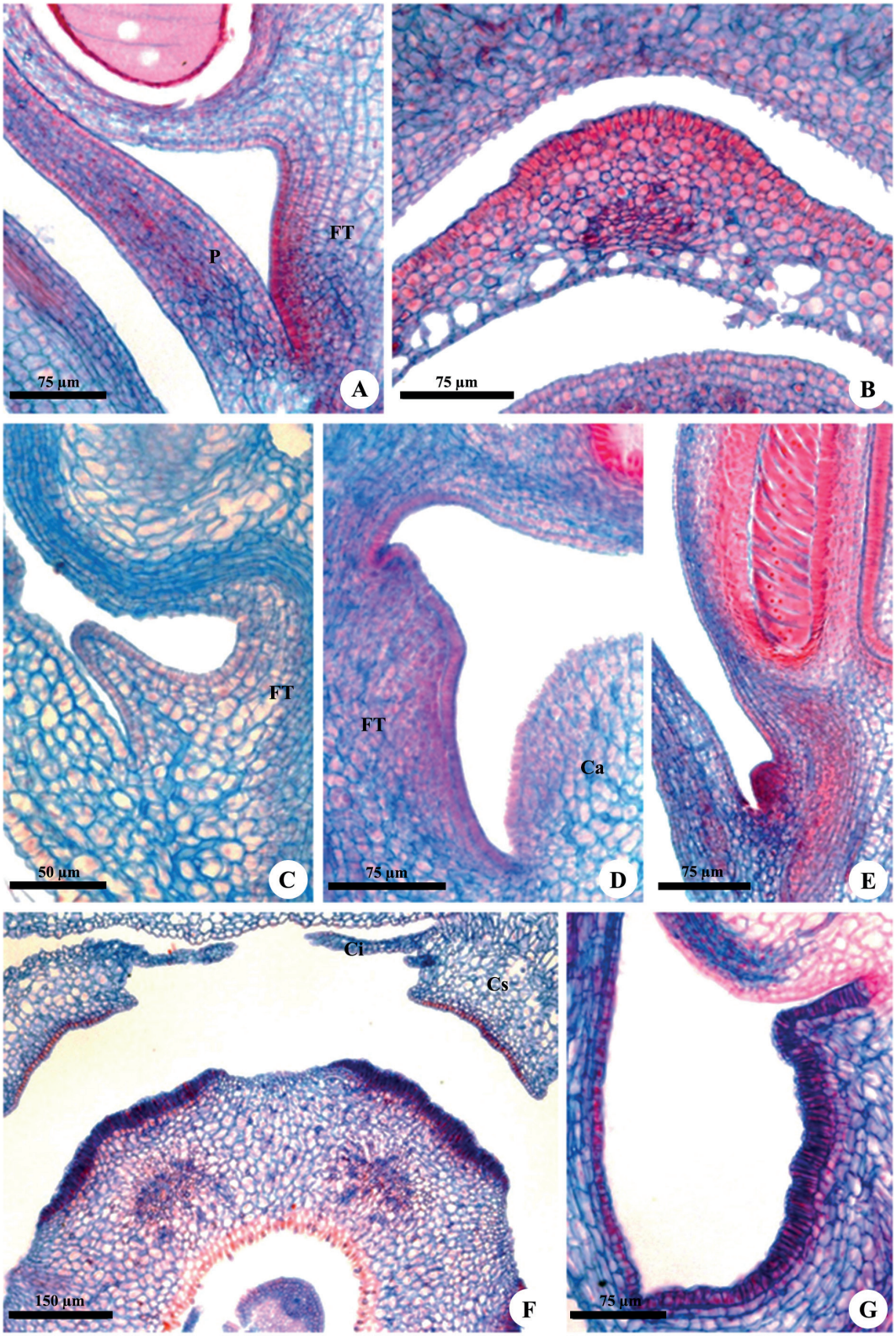

Figure 4. Ontogeny and structure of corona in flowers of Asclepiadeae. **A, C-E, G.** Longitudinal sections. **B, F.** Transverse sections. **A-B**. *Asclepias curassavica*. **C, F-G.** *Peplonia axillaris*. **D.** *Matelea denticulata*. **E.** *Oxypetalum banksii*. **A-E.** Corona origin at the base of the filament tube. **D.** Formation of the androecial corona [C(is)] continuous with almost the entire filament and annular corona developed. **F-G.** Secretory epidermis in the mature staminal corona (Cs) under the level of the anther. Ca = annular corona; Ci = interstaminal corona; Cs = staminal corona; FT = filament tube; P = petal.

Table 1. Histochemical tests for identification of the main metabolite classes present in the nectar of the primary and secondary nectaries of Asclepiadeae.

Histochemical test	Substance to be detected	Primary nectary		Secondary nectary	
		Ac, Pa	Md, Ob	Pa	Md
Ruthenium red	acidic mucilage	+ (Fig. 6A)	+	+	+ (Fig. 6B)
Tannic acid and ferric chloride	mucilage	+	+ (Fig. 6C)	+	+ (Fig. 6D)
PAS reaction	carbohydrates	+	+ (Fig. 6E-F)	+	+ (Fig. 6G)
Ferric chloride	phenolic compounds	-	-	-	-
Ferrous sulfate–formalin	phenolic compounds	-	-	-	-
Sudan black	lipids	+	+ (Fig. 6H)	+ (Fig. 6I)	-
Sudan IV	lipids	+ (Fig. 6K)	+	+ (Fig. 6J)	-
Nile blue	acidic and neutral lipids	+	+	+	-
Copper acetate and rubeanic acid	fatty acids	-	-	-	-
Dragendorff's reagent	alkaloids	-	-	-	-
Wagner's reagent	alkaloids	-	-	-	-

Note. Ac = *Asclepias curassavica;* **Md** = *Matelea denticulata;* **Ob** = *Oxypetalum banksii*; **Pa** = *Peplonia axillaris*; +:= presence; -:= absence).

C(is) corona secretes exclusively carbohydrates, including glucose and mucilage (Tab. 1). The Ca corona of *M. denticulate* does not have any secretory tissue (Fig. 5E). The presence of glucose was detected in all nectaries, primary and secondary.

Discussion

All studied species have secretory cells in the stigmatic chamber. The species differ only in the presence of secretory tissue in the corona. The corona in *A. curassavica* has no secretory activity, and the nectar found in the staminal corona is secreted by the nectariferous stigmatic chamber and runs through the canals originating from the corona until the nectar holder (Galil & Zeroni 1965). Similarly, the nectar available in the nectar holders of *Oxypetalum banksii* and other species of *Oxypetalum* is secreted by the nectaries in the stigmatic chambers (Valente 1977; Vieira & Shepherd 2002).

In Asclepiadoideae the nectariferous tissue can be found at several regions, but its primary location is in the stigmatic chamber (Kunze 1991; Endress & Bruyns 2000; Demarco 2017a), as in the studied species. The nectar can be found in the stigmatic chamber, corona and corolla base (Kunze 1991). Woodson (1954) reported nectariferous cells in the staminal corona in *Asclepias*; however, for *A. curassavica*, Galil & Zeroni (1965) reported that there are only five nectaries in the stigmatic chambers and that the nectar runs through the capillary canals until the nectar holders, originated by the staminal corona. This point of view has been reported by many authors for *Asclepias* (Kevan *et al.* 1989; Wyatt & Broyles 1994*), Calotropis* (Eisikowitch 1986), 20 other species of Asclepiadinae and four of Metastelmatinae (Kunze 1997; Liede 1997). Furthermore, Kunze (1999) observed three secretory regions in *Gonolobus* species: stigmatic chamber, base of the filament tube and the outer portion of the ring formed by the corona.

Stigmatic chamber

The primary nectaries correspond to the secretory epidermis of the stigmatic chamber in the studied species. The epidermis is continuous until the distal portion of the filament tube in *A. curassavica*, *O. banksii* and *P. axillaris*; in *M. denticulata*, as well as in other species of Gonolobinae (Kunze 1995), the anthers are fused at the base, and the secretory tissue of the chamber is continuous until the connate portion, where the stigmatic chamber is covered by the style head. Nectariferous stigmatic chambers have already been reported for many other Asclepiadeae species (Galil & Zeroni 1965; Valente 1977; Kevan *et al.* 1989; Kunze 1997; Demarco 2005) and are likely present in the filament tube of all Asclepiadoideae flowers (Endress & Bruyns 2000).

In general, the primary nectary has one single layer of epidermal cells (Valente & Silva 1984; Kunze 1991; 1995; 1999; Valente & Costa 2005; Demarco 2017a), as observed in the present study; however, in *Cynanchum vincetoxicum* a layer of secretory subepidermal cells can be observed (Christ & Schnepf 1985), and three to four layers of these cells can be found in *Leptadenia* cf. *abyssinica* (Kunze 1991).

The histochemical analysis of the nectariferous stigmatic chambers detected the presence of carbohydrates, including glucose and mucilage, and lipids. Mucilage secretion has already been reported in the cells of the inner surface of the filament tube around the style and is continuous with the epidermis of the stigmatic chamber in *Oxypetalum banksii*

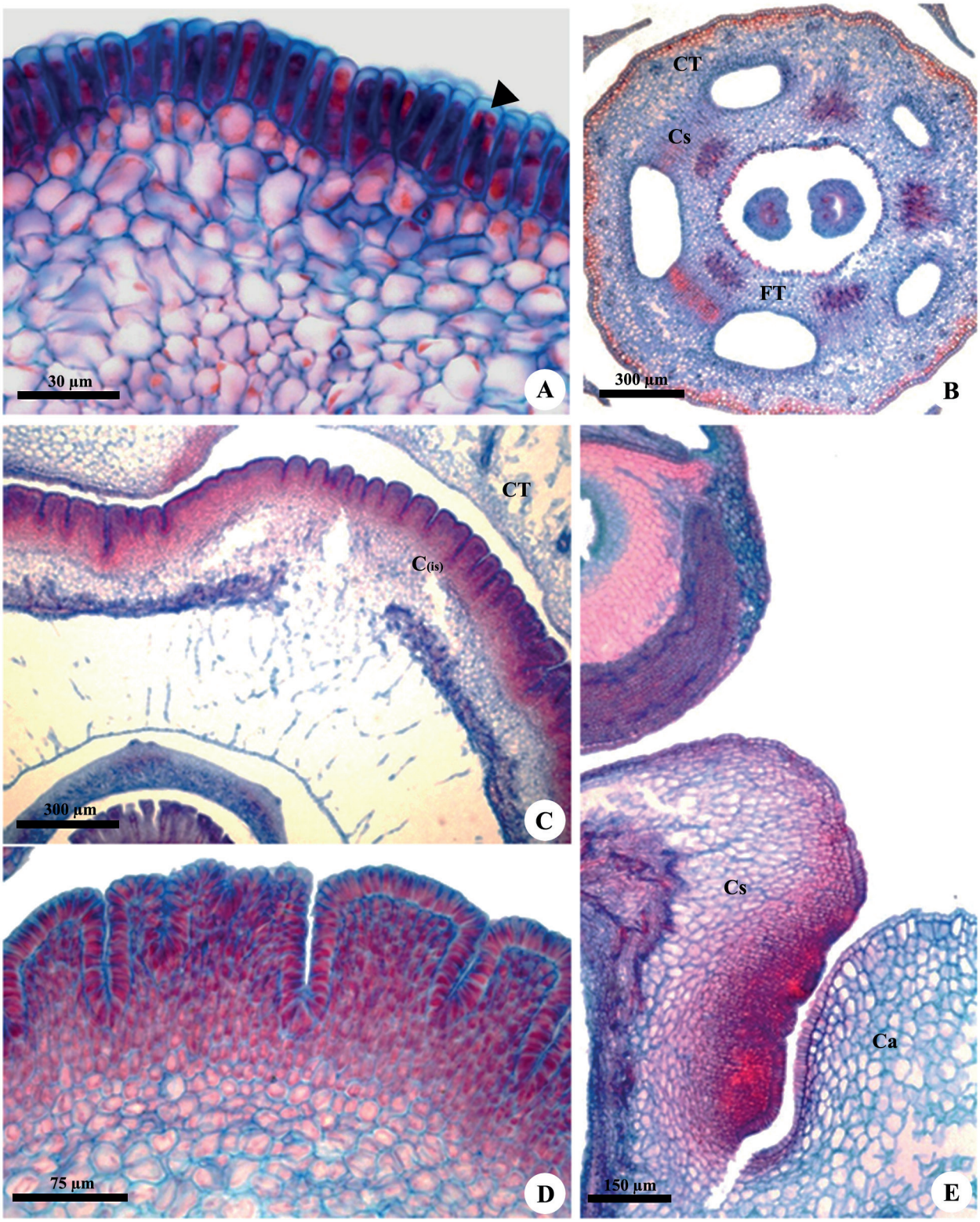

Figure 5. Structure of corona in flowers of *Peplonia axillaris* (**A-B**) and *Matelea denticulata* (**C-E**). **A-D.** Transverse sections. **A.** Secretory epidermis of the staminal corona along with the filament tube (arrowhead = periplasmic space). **B.** Interstaminal cavities formed by the adnation of corona to the corolla tube. **C.** General view of the androecial corona. **D.** Detail of nectariferous tissues. **E.** General view of C(is) and Ca coronae in longitudinal sections. Ca = annular corona; C(is) = androecial corona forming a ring around the filament tube. Cs = staminal corona; CT = corolla tube; FT = filament tube.

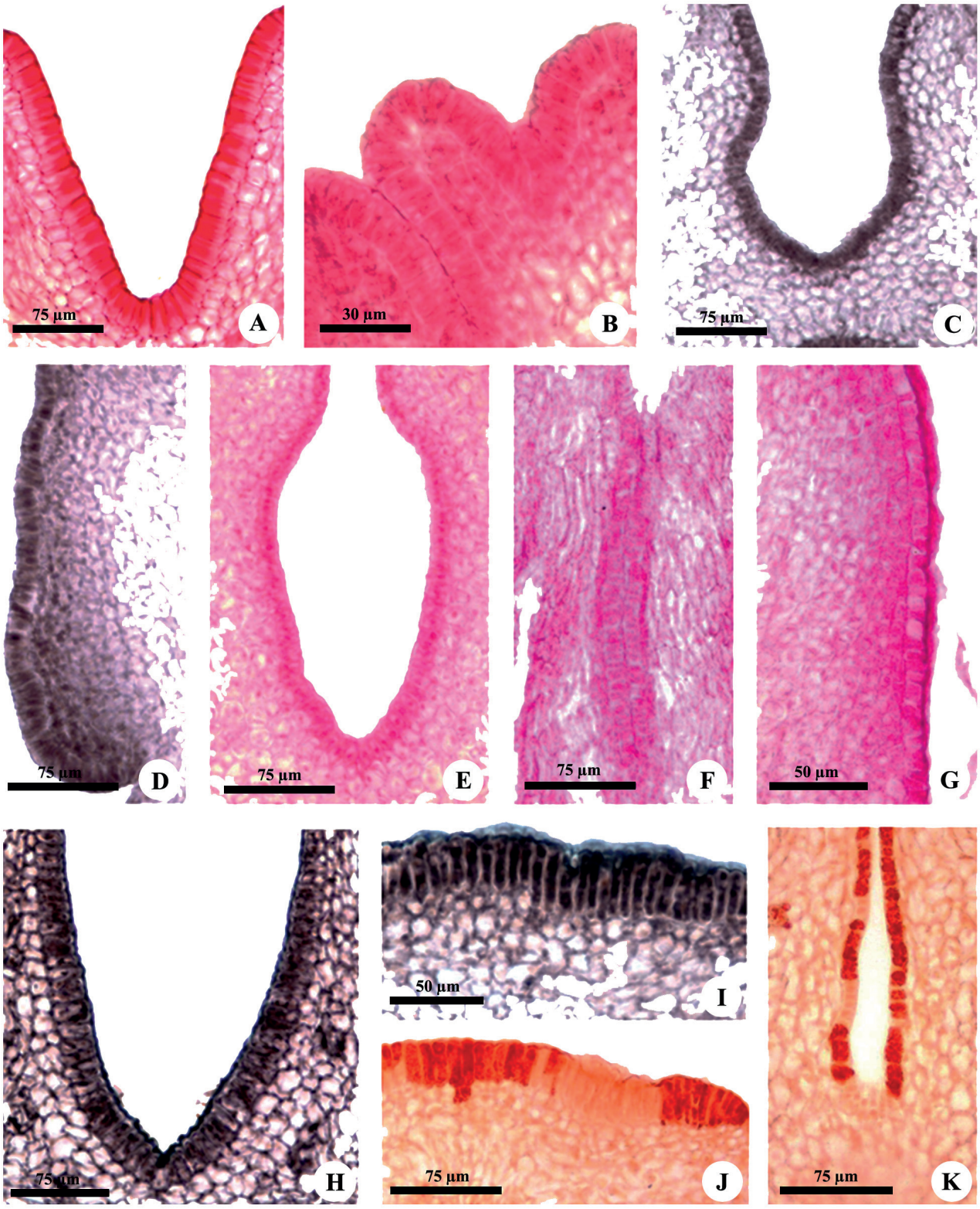

Figure 6. Histochemical tests carried out in flowers of Asclepiadeae. **A.** *Asclepias curassavica*. **B, D, F-G.** *Matelea denticulata*. **C, E, H.** *Oxypetalum banksii*. **I-K.** *Peplonia axillaris*. **A-C, E-F, H-K.** Transverse sections. **D, G.** Longitudinal sections. **A, C, E-F, H, K.** Stigmatic chamber (primary nectary). **B, D, G, I-J.** Corona (secondary nectary). **A-B.** Ruthenium red. **C-D.** Tannic acid and ferric chloride. **E-G.** Periodic acid–Schiff's (PAS) reaction. **H-I.** Sudan black. **J-K.** Sudan IV.

(Vieira & Shepherd 2002). Christ & Schnepf (1985) also detected sugars, lipophilic substances and polysaccharides in *C. vincetoxicum*; these authors also reported electron-dense material within the vacuoles of secretory cells of *Hoya carnosa* and *H. obovata* (Christ & Schnepf 1988). The presence of lipids in the nectar composition may be related to the viscosity of the secretion, which is responsible for keeping it at least partially attached to the walls of the stigmatic chamber.

Corona

The corona type in species of Asclepiadoideae is controversial, especially due to the terminological divergence among different authors and the lack of studies on floral ontogeny (Woodson 1941; Rao & Ganguli 1963; Hofmann & Specht 1986; Liede & Kunze 1993; Endress & Bruyns 2000; Kunze & Wanntorp 2008). The corona can originate from the corolla tube or from the androecium (Liede & Kunze 1993; Kunze 1995).

The corona in *A. curassavica*, *O. banksii* and *P. axillaris* is C(is) and in *M. denticulata* is C(is) + Ca. The presence of annular corona (Ca), which is formed by the annular thickening of the corolla tube, is restricted to Ceropegieae and in Asclepiadeae is present only in Gonolobinae (Liede & Kunze 1993; Kunze 1995; Endress *et al.* 2014), where *Matelea* is inserted. This type of corona has been reported just once for Marsdenieae (Liede & Kunze 1993). The C(is) corona formed in the region between the bases of the corolla tube and the filament tube, which is considered by Endress & Bruyns (2000) the most complex type of corona, originates from the base of the filament tube. Although the corona in *Oxypetalum* and *Peplonia* is adnate to the corolla, this is a secondary adnation and its origin is exclusively from the androecium. Androecial corona has already been reported for several genera of Asclepiadoideae (Rao & Ganguli 1963; Hofmann & Specht 1986; Liede & Kunze 1993; Kunze 1995; 1997; Valente & Costa 2005).

In general, the staminal corona (Cs) is more developed than the interstaminal corona (Ci), as observed in *A. curassavica*, *O. banksii* and *P. axillaris*; however, in *M. denticulata* the corona forms a ring around the filament tube. This ring is also present in other species of *Matelea*, as well as in *Fischeria*, *Gonolobus*, *Holostemma*, *Sarcostemma* and *Trichosacme* (Rao & Ganguli 1963; Kunze 1995; 1999; Valente 1995).

Secondary nectaries were found in the corona of *P. axillaris* and *M. denticulata*. In *P. axillaris*, the nectary corresponds to the epidermis of the staminal corona, while in *M. denticulata* the entire lateral surface of the ring [C(is)] is secretory and composed of epidermis and many layers of subepidermal secretory cells. Only the epidermis is secretory in the corona of *Blepharodon bicuspidatum* (Demarco 2005); however, in *Gonolobus fraternus*, *G. chloranthus* and *Matelea argentinensis*, secretory subepidermal cells can also be found. The presence of secondary nectaries is a common feature of Asclepiadoideae (Rao & Ganguli 1963; Valente & Silva 1984; Bruyns 1993; Kunze 1995; 1999; Valente 1995; Demarco 2005); however, the first thorough documentation of its secretory activity was carried out in *Gonolobus fraternus* and *G. chloranthus* (Kunze 1999).

The constitution of the coronal secretion shows some differences among the species. The staminal corona in *P. axillaris* secretes a heterogeneous exsudate, composed of carbohydrates and lipids; however, the corona in *M. denticulata* secretes only carbohydrates. This difference in composition can be related to the type of pollinator of each of these species.

Function

The position of the nectar holders are of great importance for pollinaria removal by the proboscis or legs of the pollinators. In flowers where the nectar is available in the staminal corona, the pollinarium is removed by the pollinator's legs and when the nectar is under the guide rail, it is removed by the proboscis (Pant *et al.* 1982; Eisikovitch 1986; Lumer & Yost 1995; Vieira & Shepherd 1999); however, this mechanism may vary and the pollinarium can be removed by both the legs and proboscis in some species (Macior 1965; Frost 1965; Pant *et al.* 1982). The availability of nectar at the base, under the guide rail, is a common feature in Asclepiadoideae and considered by Kunze (1997) to be a plesiomorphic character.

Despite the presence of secretory tissue in the staminal corona of *P. axillaris*, the secretion accumulates in interstaminal cavities formed by the adnation of the corona to the corolla tube, promoting the pollinarium removal by the pollinator's proboscis. The same has been observed for *Oxypetalum*, in which the secretion of the nectariferous stigmatic chamber also accumulates in the interstaminal cavities (Vieira & Shepherd 1999). The secretion in *M. denticulata* accumulates in the interstaminal regions and between the C(is) and Ca corona rings. The nectar in *Gonolobus chloranthus* and *G. fraternus* produced at the base of the filament tube probably functions as a resource for the pollinator, allowing pollinarium removal by its proboscis, while the nectar secreted by the corona accumulates between the C(is) and Ca rings, enhancing the probability of pollinarium removal by the legs of Hymenoptera, which collect this nectar (Kunze 1999).

The nectar may have a double function: resource for the pollinator and inducer of pollen germination (Galil & Zeroni 1965; Eisikowitch 1986; Kunze 1991). These two functions can be carried out by the nectar produced in the nectariferous stigmatic chamber, as in *A. curassavica* and *O. banksii*, or by different nectaries. In *M. denticulata* and *P. axillaris*, the secretion in the stigmatic chamber probably stimulates the pollen germination, while the resource for the pollinator is produced by the corona (secondary nectary).

These distinctive functions have also been described for species of *Blepharodon*, *Gonolobus* and *Matelea* (Kunze 1995; 1999; Demarco 2005).

Many studies have reported the induction of pollen germination by the nectar produced in the stigmatic chamber. Pollen germination does not occur in dry chambers, and the stimulus is related to nectar concentration (Jaeger 1971; Pant *et al*. 1982; Eisikowitch 1986; Kevan *et al*. 1989; Wyatt & Broyles 1994). This concentration may vary during the day (Wyatt & Shannon 1986). If the pollinium is inserted when the nectar concentration is not suitable, germination will only take place when it is appropriate (Eisikowitch 1986). Nectar concentration above 30 % inhibits germination in *Asclepias* (Wyatt & Broyles 1994).

There is no need of contact between pollinium and stigma in order to induce pollen germination (Kevan *et al*. 1989; Kunze 1991), and retention of the nectar inside an open chamber is probably related to its viscosity due to the abundant presence of lipids in its composition. However, the presence of mucilage and lipids in the stigmatic chamber may also be related to germination stimulus and guidance of pollen tubes to the stigma. The pollen tubes grow towards the style head when a pollinium is left into the guide rail or in the stigmatic chamber (Kunze 1991). According to Vieira & Sheperd (2002), the mucilage in this chamber acts as a guide for the pollen tubes.

Conclusion

Although the studied species have different types of corona, they all have the same origin from the androecium, which highlights the importance of ontogenetic studies in a group in which the mature structures are too complex to establish a concise interpretation since postgenital fusion is very common among Apocynaceae. A precise interpretation of the corona is of great importance for the taxonomy of Apocynaceae since this is the most common structure used for taxa classification in Asclepiadoideae. Besides the importance for taxonomy, the position and structure of the corona, as well as the nectaries, regulates the access of pollinators of different sizes to the flower and influences the position the pollinaria to attach to the pollinator's body.

Beyond the morphology of the nectaries, the difference in the composition of nectars in the studied species is noteworthy and seems to differ according to the place where it is produced, which may be related to their pollinator type and has been previously described for other genera. In all species the primary nectar composition indicates its double function, as a resource for the pollinator and inducer for pollen germination. In this study, we observed that for species where primary and secondary nectaries are present, such as *Matelea denticulata* and *Peplonia axillaris*, each nectary produces a secretion with a different function, which seems to enhance the reproductive success of the group.

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