

## Scientific Electronic Archives

Issue ID: Sci. Elec. Arch. Vol. 13 (10)

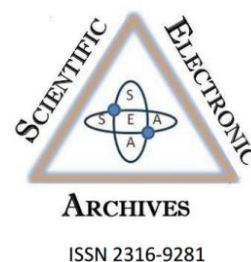
October 2020

DOI: <http://dx.doi.org/10.36560/131020201267>

Article link

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## Taxonomic Considerations About the Anatomical Description of Gynecium *Oxypetalum appendiculatum* Mart. (Apocynaceae)

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**Abstract.** The flowers of Apocynaceae are considered as the most complex among the angiosperms, due to the occurrence of synorganization culminated in the origin of a new floral structure, the gynostege, formed by the union of androceu and gynecium, which guarantees an optimization in the reproduction process. The gynostegium is widespread in the genera of the subfamily Asclepiadoideae, being cited as an artifact of taxonomic significance in the group. *Oxypetalum* R.Br. It is the largest neotropical genus of Asclepiadoideae and is included in Clado MOOG, which presenting difficulties regarding the taxonomic positioning of its species. Few studies have investigated the floral anatomy of *Oxypetalum* and these did not address the taxonomic significance of the anatomy of the gynostegium in resolving the positioning of individuals in the group. This work seeks to investigate the anatomy of the gynecium and the gynecium of *Oxypetalum appendiculatum* in order to contribute to the group's taxonomy. With this, we seek to show key anatomical parameters for the easy identification of gaps in that group. Buds and flowers at pre-anthesis, anthesis, and post-anthesis were collected to analyze histologically. Material was fixed in arnovsky's solution and gradually dehydrated in an ethanol series. Samples were infiltrated, and embedded in hydroxyethyl methacrylate. Circa 5 µm cross and longitudinal sections were cut using a rotary microtome, stained with Toluidine Blue O. All the slides were analyzed with optical and stereo microscopes and images were captured with a photomicroscope or a trinocular photo stereomicroscope. Gynoecium comprises two carpels fused at style and style head, with free ovaries. The five receptive parts of stigma are located on the gynostegium, more precisely behind the translators. At a higher level, the stigma begins to divide, appearing bifid, towards the apex. The gynoecium of *Oxypetalum appendiculatum* is an extremely complex structure due to the synorganization that occurred between the corona, the anthers and the gynecium itself. The result of this union is the gynostegium, which provides morphoanatomical characters that assist in delimiting this taxon and other species of this genus.

**Keywords:** Asclepiadoideae, Bifid attachment, Anatomy, Taxonomy, Gynostegium.

### Introduction

The flowers of Apocynaceae are considered by Wyatt and Broyles (1994) as the most complex among the angiosperms, due to the occurrence of unions between the floral elements of the same whorl and between elements of different whorls. This synorganization culminated in the origin of a new floral structure, the gynostege, formed by the union of androceu and gynecium, which guarantees an optimization in the reproduction process (Endress et al., 2014; Vieira et al., 2012). The gynostegium is widespread in the genera of the subfamily Asclepiadoideae, being cited as an artifact of taxonomic significance in the group (Endress & Bruyns, 2000; Kunze, 1994; Liede & Kunze, 1993; Valente & Costa, 2005). The most significant

difference is the longitudinal conformation of the anthers around the stylet and the stigma, guaranteeing characteristics peculiar to each species (Kunze & Wanntorp, 2008; Rapini et al., 2011).

*Oxypetalum* R.Br. It is the largest neotropical genus of Asclepiadoideae, with approximately 120 species, and is included in Clado MOOG (composed by Metastelmatinae Endl. Ex Meisn., Orthosiinae Liede and Rapini, Oxypetalinae E. Fourn., And Gonolobinae G. Don ex Liede), which presenting difficulties regarding the taxonomic positioning of its species (Farinaccio & Goyder, 2016). Recent phylogenetic analyzes (Ezcurra et al., 2008; Farinaccio & Keller, 2014; Rapini et al., 2010) transferred species from other genera Oxypetalinae

to *Oxypetalum*, causing the number of species to increase considerably, with the morphology of the gynoecium together with the gynostegium they provided data to assist in these taxonomic findings (Farinaccio, 2008; Rapini et al., 2011).

Few studies have investigated the floral anatomy of *Oxypetalum* (Valente, 1977; Silva et al., 2008; Vieira et al., 2012), and these did not address the taxonomic significance of the anatomy of the gynostegium in resolving the positioning of individuals in the group. According to Vieira and Shepherd (2002), the apex of the stylet and the conformation of the epidermis of the gynostegium may be useful in identifying subspecies of *Oxypetalum*, suggesting that such structures should be investigated.

Considering the conflicting nomenclatural history in Oxypetalineae and the emergence of new species of *Oxypetalum*, this work seeks to investigate the anatomy of the gynoecium and the gynostegium of *Oxypetalum appendiculatum* Mart. in order to contribute to the group's taxonomy. With this, we seek to show key anatomical parameters for the easy identification of gaps in that group.

## Methods

### *Obtaining Botanical Material*

Botanical materials of *Oxypetalum appendiculatum* were collected in a rocky outcrop (campo rupestre) within the boundaries of the town of Lavras, Minas Gerais. Herborized specimens were deposited at the ESAL Herbarium, Federal University of Lavras (voucher number ESAL 27.511).

### *Anatomical Analysis*

Buds and flowers at pre-anthesis, anthesis, and post-anthesis were collected to analyze histologically. Material was fixed in arnovsky's solution for 24 hours (Karnovsky 1965), gradually ehydrated in an ethanol series (10%, 20%, 30%, 40%, 50%, and 60%) and conserved in ethanol 70%.

Samples were dehydrated in a growing ethanol series, infiltrated, and embedded in hydroxyethyl methacrylate (Leica®) to make up permanent slides. Circa 5 µm cross and longitudinal sections were cut using a rotary microtome, stained with Toluidine Blue O (pH 4.7) (O'BRIEN et al., 1964), and mounted in Acrilex 500® colorless glass varnish (PAIVA et al., 2006). All the slides were analyzed with optical and stereo microscopes and images were captured with a photomicroscope or a trinocular photo stereomicroscope.

## Results and discussion

### *Bud and Flower Morphology*

Flowers are pentamerous. Five sepals and five petals alternate with each other. The latter alternate with the five corona elements and five stamens; calyx is dialysepalous with green sepals; corolla is gamopetalous and lobed at apex, petals are discolor, their abaxial surface is yellowish green

and the adaxial one, yellowish green with prevailing vinaceous tinges (Figure 1A); corona is greenish cream (Figure 1A) with rounded lobes located around the gynostegium, fused at base but free at apex (Figure 1A – C, AB).

The pentagonal gynostegium is composed by the fusion of anthers to the style head (Figure 1B-E); gynostegium apex is prolonged into a vinaceous, bifid appendage ramified above the median portion (Figure 1B-C). Each of the five anthers is covered with an appendage named denticle (Figure 1B - DE); filament is sessile (Figure 1B); anthers present a winged, laterally suboblong expansion called anther wing. The junction of two adjacent wings forms a guide rail.

Gynoecium comprises two carpels fused at style and style head, with free ovaries. The five receptive parts of stigma are located on the gynostegium, more precisely behind the translators (see retinaculum - brownish) (Figure 1B, E - GI).

### *Anatomical Description of the Gynostegium*

The gynoecium is composed of two carpels, free in the ovary and stylus. These are fused at the apex of the stylus and stigma forming the gynostegium (Figure 2A). Around the gynostegium, the five anthers are enshrined.

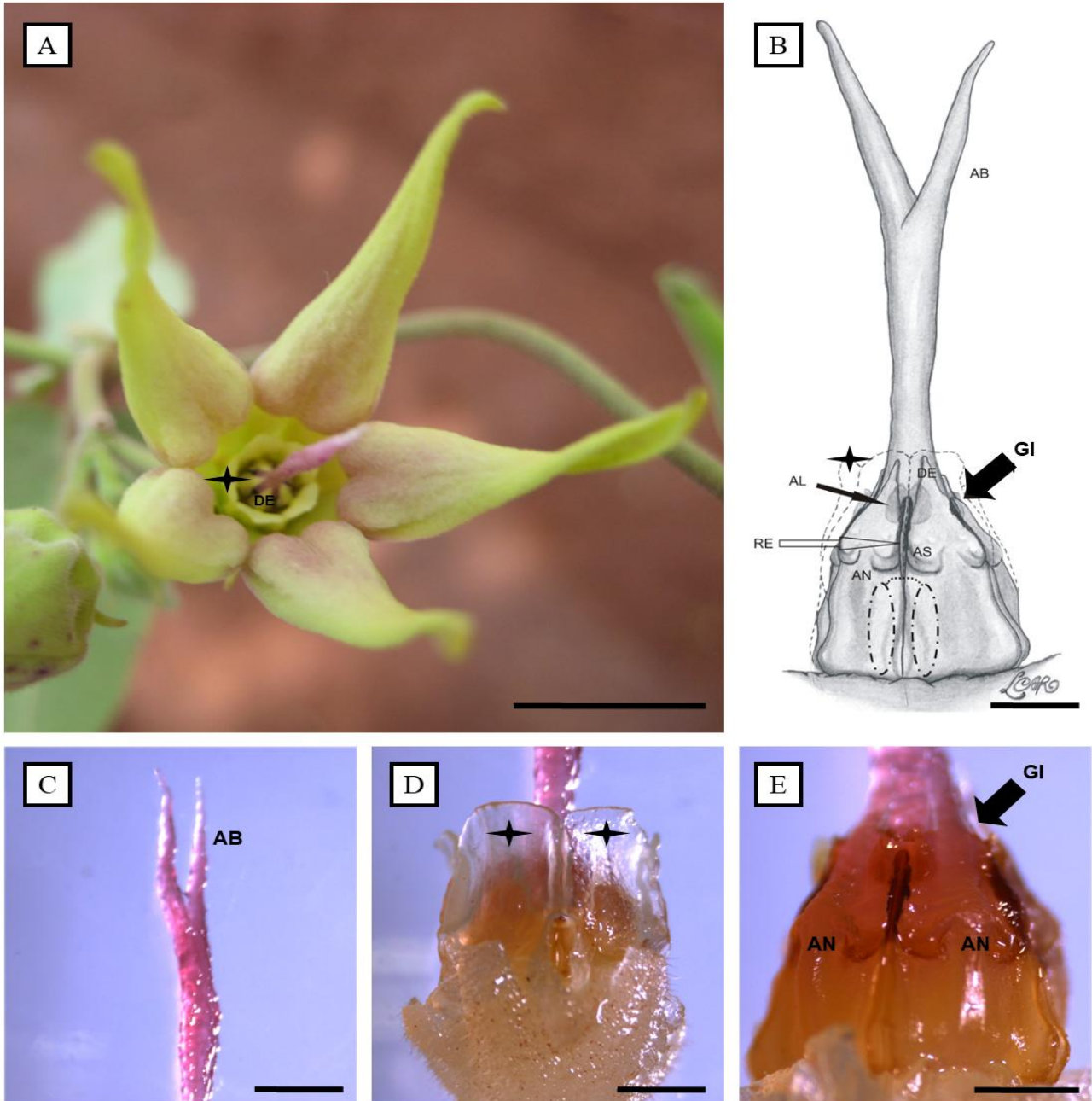
In the region corresponding to the base of the ovary, we noticed that it has uni-stratified epidermis, cells of variable shape and size, presenting a larger diameter in the periclinal direction, covered by a thin, smooth cuticular layer, in some striated stretches. Occurrence of numerous multi-celled multi-cell hairs, with a frequency of 1-2 smaller cells at the base (Figure 2A).

In the central part, we observe two carpels and separated from each other by a central opening. The dorsal walls of the ovary, close to the vascular bundles, are not delimited because their cells are still closely related to the cells of the peripheral parenchymal tissue. The ventral walls of the carpels, juxtaposed to the central opening, as well as the placental regions are made up of a compact parenchyma of smaller cells, with thin walls, without meatus (Figure 2C, D, E).

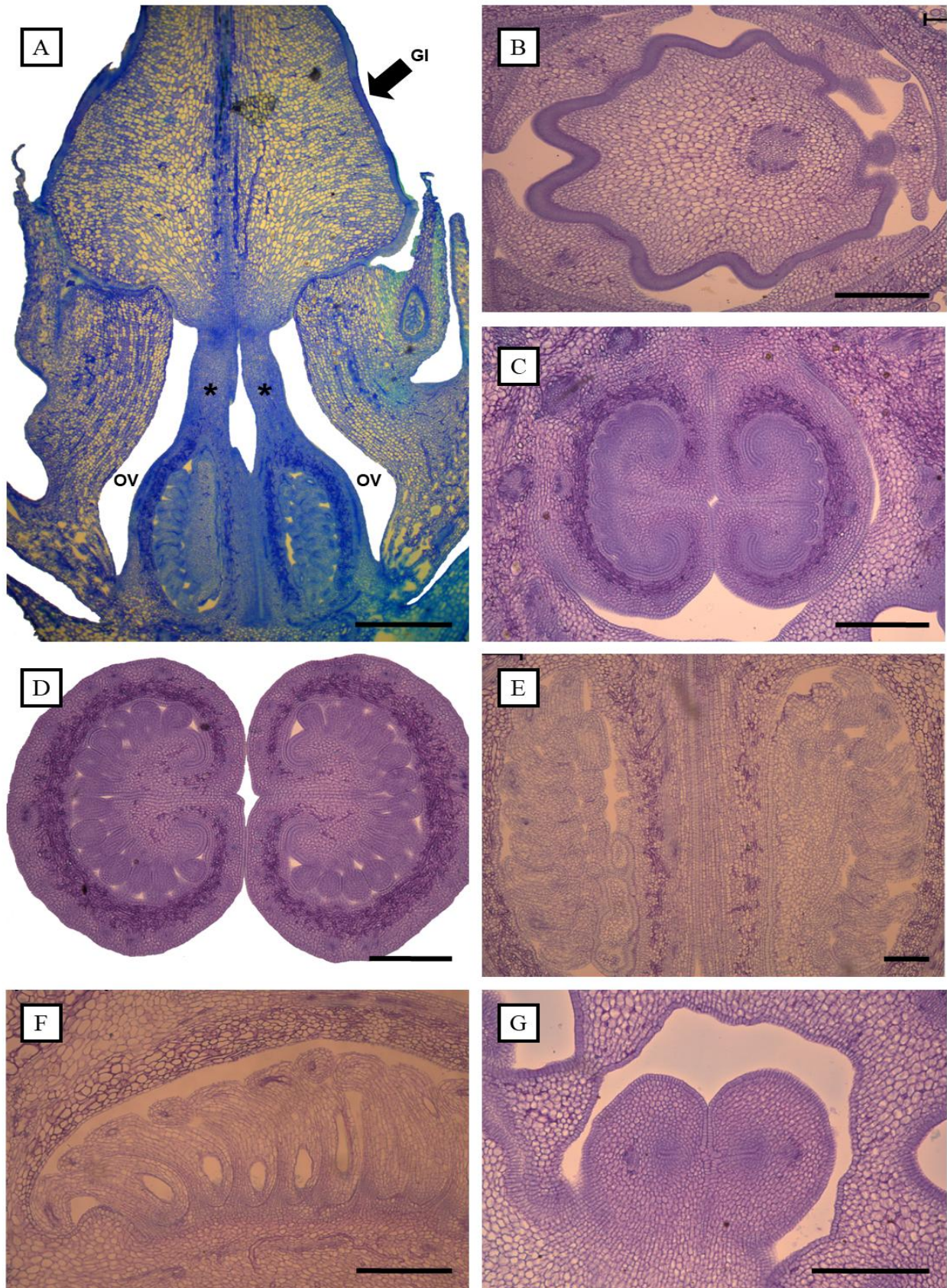
Placental regions have two pairs of vascular bundles, and laticifers also occur. Some ovules are also seen. The two carpels are free of each other in the basal region (Figure 2C, D). The carpel walls are covered by two epidermis: an adaxial or external epidermis, uni-stratified, contiguous to the inner wall of the gynostegium and with cells of rectangular section, larger diameter in the anticline direction, covered by a thin and smooth cuticle, and the abaxial epidermis or internal lining the internal wall of the locule, and is also uni-stratified, with rectangular cells, with a larger diameter in the periclinal direction, covered by a thin and smooth cuticular layer. The median part of the carpel wall presents several layers of small parenchymatic cells, heterodimensional, with thin walls and with rare meatus (Figure 2E, F).

The placenta is constituted in its internal part by a parenchymatic tissue, a nourishing tissue, with small cells, approximately cubic, of thin wall, with meatus and dairy products between them (Figure 2C, D). At the ovule insertion sites, the placental wall consists of an epidermis of cells rich in cell content, of variable shape and size, with a

relatively thick cuticle. This epidermis can be continuous or it can constitute projections for the fixation of the ovule, thus forming the funicles. Carpels show marginal placentation, with the eggs developing on the inner or abaxial surface (Figure 2E, F).



**Figure 1.** Flower and Gynostegium Morphology. A: Flowers at anthesis. B: General aspect of gynostegium. C: Bifid attachment of gynostegium. D: Coronas covering the gynostegium. E: anthers around the gynostegium. Detail for removed coronas. AB = Bifid attachment of gynostegium; AN = Anther; AL = Anther ala; AS = Anther wing; DE = Denticulo; GI = Gynostegium; RE = Retináculo; Black Star = Corona. SCALE BARS: 4 mm (A); 2 mm (B, C, D, E).



**Figure 2.** Gynoecium Anatomy. A: Longitudinal section of the gynostegium, showing the ovaries and the stylets, both free. B: Carpel base evidencing the vascular bundles of each ovary. C-D: Cross section of the free ovaries. E-F: Longitudinal section of the ovary locus with details for the ovule. G: Apex of the stylus and base of the gynostegium. GI = gynostegium; OV = Ovule; \* = Stylets. SCALE BARS: 200 µm (A, B, C, D, F, G); 100 µm (E).

In the region that corresponds, more or less, to the base of the stylets, they are separated, and in cross section, they show an oval to circular contour, showing two collateral libero-woody bundles, one for each stylus, and are surrounded by some dairy products (Figure 2G). They consist of a unified stratified epidermis with small cells of uniform shape and size, thin membranes, smooth cuticle. Below the epidermis, a parenchyma is located with several layers of relatively small, heterodimensional cells and few intercellular spaces. In this parenchyma, and occupying opposite positions in the two stylets, we find, for each stylus, remains of the placental epidermal tissue, forming the stylet's canal, surrounded by smaller cells, constituting the nourishing tissue.

At the level of the membranous appendages, we observe the stigma, of subcircular section, with uni-stratified epidermis, of papillary cells, covered by a striated cuticle. The parenchyma with several layers of heterodimensional cells, with small intercellular spaces just below the epidermis, becomes more uniform up to the center, where it involves the two vascular bundles of the stylets, gathered there in one, and surrounded by elements of the liber; in the parenchyma band that joins the mentioned bundles, small groups of elements of the internal liber are observed. At a higher level, the stigma begins to divide, appearing bifid, towards the apex.

The pentagonal gynostegium found in *Oxypetalum appendiculatum* is widespread in the "Clado MOOG" of Asclepiadoideae. The gynostegium of Asclepiadoideae represents a highly synorganized complex structure, which is this fusion between the anthers and the stylus head preserved in numerous evolutionary changes (Rapini et al., 2011; Kunze & Wanntorp, 2008). The adnation of the anthers to the gynostegium gives a unique conformation capable of individualizing species of Oxypetalineae with precision (Kunze & Wanntorp, 2008).

Another important aspect in the delimitation of taxa in this group is the bifid attachment of the gynostegium. In a study carried out by Silva et al., (2008), 25 species of *Oxypetalum* had their segregation reinforced by the bifid attachment, where characteristics such as the length, the turning and the color of this region provide taxonomic subsidies. *Oxypetalum insigne* var. *insigne* (Decne.) Malme and *Oxypetalum globosum* C. Ezcurra & C. M. Martin are one of the few species in the group that do not have a bifid conformation of the annex, but are globose (Silva et al., 2008; Martin et al., 2017). The gynostegial crown, together with the bifid attachment, strongly differentiated from the stem parts is pointed out as a potential synapomorphism of the subtribe Asclepiadinae, into which *Oxypetalum* is inserted (Liede, 1994).

The unilocular and marginal placental ovaries found are quite common in *Oxypetalum*. Placental epidermal cells have characteristics of secretory cells as seen in *O. banksii* (Valente, 1977)

and *O. pachyglossum* Decne. (Vieira et al., 2012). The style is of the hollow type covered by glandular epidermis, as evidenced by Vieira et al., (2012), but after joining the dorsal portion, the caperle's stylets become solid, filled with isodiametric parenchymal cells.

## Conclusion

The gynoecium of *Oxypetalum appendiculatum* is an extremely complex structure due to the synorganization that occurred between the corona, the anthers and the gynostegium itself. The result of this union is the gynostegium, which provides morphoanatomical characters that assist in delimiting this taxon and other species of this genus.

## References

- ENDRESS, P. K. Diversity and evolutionary biology of tropical flowers. Cambridge: Cambridge University Press. p. 387, 1994.
- ENDRESS, M. E.; BRUYNS, P. V. A revised classification of the Apocynaceae s.l. The Botanical Review, Bronx, v. 66, n. 1, p. 1-56, 2000.
- EZCURRA, C., J. FONTELLA PEREYRA, & L. KINOSHITA. 2008. Apocynaceae (incl. Asclepiadaceae). Pp. 1090–1143 in Catálogo de las plantas vasculares del Cono Sur vol. 2, eds. F. O. Zuloaga, O. Morrone, and M. J. Belgrano. Monographs in Systematic Botany from the Missouri Botanical Garden. St. Louis: Missouri Botanical Garden.
- FARINACCIO, M. A. 2008. Sistemática molecular de *Oxypetalum* R.Br. (Apocynaceae, Asclepiadoideae). Tese de Doutorado. São Paulo: Universidade de São Paulo. Available from: <http://www.teses.usp.br/teses/disponiveis/41/41132/tde-02062008-143035/>.
- FARINACCIO, M.A. & KELLER, H.A. Novelty in *Oxypetalum* (Apocynaceae-Asclepiadoideae) for the Argentine flora. Phytotaxa. v. 184, p. 109–114, 2014.
- FARINACCIO, M. A. & GOYDER, D. A synopsis of *Oxypetalum* (Apocynaceae) in Bolivia, with the description of one new species and a key to species in Bolivia. Phytotaxa. v. 267, p. 17–28, 2016.
- KARNOVSKY, M.J.A. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. The Journal of Cell Biology. v. 27, p. 137-138, 1965.
- KUNZE, H. Ontogeny of the translator in the Asclepiadaceae s. str. Plant Systematics and Evolution, New York, v. 193, n. 1-4, p. 223-242, 1994.
- KUNZE, H & WANNTORP, L. Corona and anther skirt in *Hoya* (Apocynaceae, Marsdenieae). Plant Systematic and Evolution. v. 271, p. 9-17, 2008.
- LIEDE, S.; ALBERS, F. Tribal disposition of Asclepiadaceae genera. Taxon. v. 43, n. 2, p. 201-231, 1994.
- LIEDE, S. & KUNZE, H. A descriptive system for corona analysis in the Asclepiadaceae. Plant Systematics and Evolution, New York, v. 185, n. 3-4, p. 99–284, 1993.

O'BRIEN, T.P.; FEDER, N. & MCCULLY, M.E. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*. v. 59, p. 368-373, 1964.

MARTÍN, C.M.; ZANOTTI, C.A. & EZCURRA, C. A New Species of *Oxypetalum* (Apocynaceae, Asclepiadoideae) from Northern Argentina. *Systematic Botany*. v. 42, v.3, p.578-583, 2017.

PAIVA, J.G.A.; FANK-DE-CARVALHO, S.M.; MAGALHÃES, M.P.; GRACIANO-RIBEIRO, D. Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. *Acta Botanica Brasilica*. v. 20, p. 257-264, 2006.

RAPINI, A.; KOCH, I.; KINOSHITA, L.S.; SIMÕES, A.O. & SPINA, A.P. Apocynaceae. Pp. 617–644 in *Catálogo de plantas e fungos do Brasil* vol. 1, eds. R. C. Forzza and P. Leitman. Rio de Janeiro: Jardim Botânico do Rio de Janeiro. 2010.

SILVA, N.M.F.; VALENTE, M.C.; PEREIRA, J.F.; FILHO, G.M.A. & ANDRADE, L.R. Morfoanatomia de espécies brasileiras de *Oxypetalum* (Asclepiadoideae-Apocynaceae). *Rodriguésia*. v. 59, n. 4, p. 915-948, 2008.

RAPINI, A.; FONTELLA-PEREIRA, J. & GOYDER, D. J. Towards a stable generic circumscription in *Oxypetalinae* (Apocynaceae). *Phytoaxa*. v. 26, p. 9-16, 2011.

VALENTE, M. da C. A flor de *Oxypetalum banksii* Roem. Et Schult. subsp. *Banksii*: estudo da anatomia e vascularização (Asclepiadoideae). *Rodriguésia*, Rio de Janeiro. v. 29, n. 43, p. 161-283, 1977.

VALENTE, M. da C. & COSTA, C.G. Estudo anatômico da flor de *Marsdenia loniceroides* E.Fournier (Asclepiadoideae-Apocynaceae). *Rodriguésia*, Rio de Janeiro, v. 56, p. 51-66, 2005.

VIEIRA, M.F.; FONSECA, R. & SHEPHERD, G. Morfologia floral e mecanismos de polinização em espécies de *Oxypetalum* R.Br. (Apocynaceae, Asclepiadoideae). *Revista Brasileira de Biociências*. v. 10, n. 3, p. 314-321, 2012.

WYATT, R.; BROYLES, S. B. Ecology and evolution of reproduction in milkweeds. *Annual Review of Ecology and Systematics*. v. 25, p. 423-441, 1994.