

Pericarp structure in *Banisteriopsis* C.B.Rob. and *Diplopterys* A.Juss. (Malpighiaceae): new data supporting generic segregation

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RESUMO

(Estrutura do pericarpo de *Banisteriopsis* C.B.Rob. e *Diplopterys* A.Juss. (Malpighiaceae): novos dados suportando a segregação genérica). Recentes estudos moleculares indicaram que espécies de *Banisteriopsis* são mais apropriadamente enquadradas em *Diplopterys*. Ambos os gêneros são membros neotropicais de Malpighiaceae, família com grande diversidade na América do Sul, especialmente em savanas (cerrado). Estudos com anatomia de órgãos reprodutivos de Malpighiaceae de cerrado são muito raros. Assim, o presente trabalho descreve a morfologia, anatomia e ontogênese do pericarpo de *B. campestris*, *B. oxyclada*, *B. stellaris*, e *D. pubipetala*, comparando-os e identificando características que possam dar suporte à segregação genérica. Os frutos de *Banisteriopsis* estudados apresentam estrutura muito similar entre si, enquanto que *D. pubipetala* mostra várias peculiaridades. O fruto das quatro espécies é esquizocárpico e formado por três samarídeos com ala dorsal; *D. pubipetala* apresenta também alélulas sobre o núcleo seminífero. O mesocarpo das *Banisteriopsis* apresenta grupos de células alongadas e espessadas, enquanto essas células formam uma faixa contínua em *D. pubipetala*; o endocarpo de *D. pubipetala* prolifera muito, formando um aerênquima não observado em *Banisteriopsis*. Além da homogeneidade estrutural do pericarpo das *Banisteriopsis*, todas as características distintivas referidas sustentam a manutenção de *D. pubipetala* em um gênero diferente.

Palavras-chave: *Banisteriopsis campestris*, *Banisteriopsis oxyclada*, *Banisteriopsis stellaris*, *Diplopterys pubipetala*, Ontogênese

ABSTRACT

(Pericarp structure in *Banisteriopsis* C.B.Rob. and *Diplopterys* A.Juss. (Malpighiaceae): new data supporting generic segregation). Molecular studies have indicated that some species of *Banisteriopsis* would be more appropriately placed in *Diplopterys*. Both of these genera are neotropical members of the Malpighiaceae, a family that is widely diverse in South America, especially in the Brazilian savanna (*cerrado*). Studies concerning the anatomy of the reproductive organs of Malpighiaceae from the *cerrado* are very rare. Thus, the present work describes the morphology, anatomy, and ontogeny of the pericarp of *B. campestris*, *B. oxyclada*, *B. stellaris*, and *D. pubipetala*, comparing them and identifying characteristics that could support the segregation of genera. The fruits of *Banisteriopsis* studied here demonstrate structures very similar among themselves, while *D. pubipetala* shows numerous differences. The fruits of all species are schizocarpic and formed by three samara with dorsal wings, while the fruits of *D. pubipetala* also have lateral winglets on the seed chamber. The mesocarp of the *Banisteriopsis* species has groups of thick and elongated cells, while these cells form a continuous band in *D. pubipetala*; the endocarp of *D. pubipetala* proliferates markedly, forming an aerenchyma layer not seen in *Banisteriopsis*. Besides the homogeneous structure of the pericarp of the *Banisteriopsis* species, all referred distinctive characteristics support the maintenance of *D. pubipetala* in a different genus.

Key words: *Banisteriopsis campestris*, *Banisteriopsis oxyclada*, *Banisteriopsis stellaris*, *Diplopterys pubipetala*, Ontogeny

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Introduction

Banisteriopsis and *Diplopterys* are stigmaphylloid closely related genera within Malpighiaceae (Gates 1982; Davis *et al.* 2001; Anderson & Davis 2006), a family comprising approximately 66 genera and 1,200 species (Anderson 1990). Both genera are principally found in South America, but have representatives in North and Central America too. Many species occur in the Brazilian central plains, especially in the savanna (Gates 1982).

According to Gates (1982), *Diplopterys* comprised only four species. The genus was described as a small genus, differing from *Banisteriopsis* principally because of fruit characteristics. The fruits of *Banisteriopsis* are schizocarps, composed of three samaras with well developed dorsal wings, while in *Diplopterys* there are three nuts in which the dorsal wings are extremely reduced, and many winglets or lateral crests occur on the samaras.

In a molecular and phylogenetic study, Anderson & Davis (2006) concluded that *Banisteriopsis* was not a monophyletic group and that the subgenus *Pleiopterys* contained species that could be more appropriately included in *Diplopterys*. Accordingly, these authors amplified *Diplopterys* by adding species heretofore composing *Pleiopterys*, thus making both genera monophyletic. *Diplopterys sensu* Anderson & Davis (2006) included a new combination *D. pubipetala* (A.Juss.) W.R.Anderson & C.Cav.Davis; this species was previously named *Banisteria pubipetala* A.Juss., which was in *Banisteria* sect. *Anisopterys* Griseb., a synonym of *Banisteriopsis* sect. *Anisopterys* (Griseb.) B.Gates according to Gates (1982).

Anderson (1979) stated that the floral morphology of Malpighiaceae is quite homogeneous, but their fruits demonstrate extreme diversity. Dry or fleshy, and dehiscent or indehiscent fruits can be encountered in the family (Barroso *et al.* 1999). As such, fruit characteristics have traditionally been used in the delimitation of the *taxa* within the family, and to define the tribes and genera. However, Davis *et al.* (2001) highlighted that the circumscription of *taxa* using fruit characteristics are artificial, because fruit traits can be homoplastic. Thus detailed studies of the morphology and anatomy of these fruits, especially ontogenetic analyses that have been very rare for this group, are interesting to recognize these homoplasies.

The phylogeny of some groups of Malpighiaceae is still not clear, even with the aid of molecular analyses, and detailed evaluations of fruit morphology and development would be very useful in clarifying the evolution of the *taxa* of this large family (Davis *et al.* 2001; Davis & Anderson 2010).

In view of provide additional ontogenetic data about the fruits of Malpighiaceae, the present work describes the morphology, anatomy, and ontogeny of the pericarp of *Banisteriopsis campestris* (A.Juss.) Little, *B. oxyclada* (A.Juss.) B.Gates, *B. stellaris* (Griseb.) B.Gates, and *Diplopterys pubipetala* (A.Juss.) W.R.Anderson & C.Cav.Davis, and

evaluates structural characteristics of the fruits that can be highlighted as peculiarities between species of *Banisteriopsis* and *Diplopterys*.

Materials and methods

The species *Banisteriopsis campestris* (A.Juss.) Little, *B. oxyclada* (A.Juss.) B.Gates, *B. stellaris* (Griseb.) B.Gates, and *Diplopterys pubipetala* (A.Juss.) W.R.Anderson & C.Cav.Davis were selected for detailed examination. These species were collected in the Brazilian *cerrado* at km 3 of the Hipólito Martins Highway, municipality of Botucatu, SP, Brazil (23°02'51"S, 48°31'18"W); Mina Road, municipality of Botucatu, SP, Brazil (22°42'38"S, 48°18'35"W); and in residual savanna areas in the municipality of Botucatu, SP, Brazil (22°51'22"S, 48°29'29"W and 23°03'28"S, 48°15'51"W). Fertile branches were registered in the BOTU Herbarium under the numbers 25304, 24292, 24289, and 24291 respectively.

Morphological characteristics of the fruits were described and illustrated based on 50 samples collected at random from a minimum of five different individual plants. The nomenclature used to describe the fruits was based on Roth (1977) and Barroso *et al.* (1999). In order to measure the insertion angle of the wings on the seed chamber, fruits with samaras still attached to the torus were examined. The fruits were photographed with an Olympus digital camera and the angles measured directly on printed photographs.

For anatomical studies, samples of ovaries and fruits in various stages of development were fixed in FAA 50 (Johansen 1940) and subsequently stored in 70% ethyl alcohol.

To prepare permanent slides, the specimens were dehydrated in an ethanol series, embedded in methacrylate Leica™ (Paiva *et al.* 2011), sectioned using a rotary microtome, stained with 0.05% toluidine blue O at pH 4.7 (O'Brien *et al.* 1964, modified), and mounted in synthetic resin. The seed chambers of the mature fruits were boiled for an hour in water and glycerin (1:1) before dehydration.

Semi-permanent slides were prepared using samples sectioned on a Ranvier microtome, clarified with a 20% solution of sodium hypochlorite, and stained with safranin and astrablue in aqueous solution (Bukatsch 1972). The material was subsequently mounted in glycerin jelly (Dop & Gautié 1928).

Histochemical tests were performed by hand-sectioning fresh or fixed materials that were submitted to the following stains or reagents with their respective controls: ruthenium red to identify polysaccharides and pectins (Jensen 1962); phloroglucinol with hydrochloric acid to identify lignified cell walls (Sass 1951); Sudan IV to identify lipidic substances; Lugol solution to stain starch; and ferric chloride with sodium carbonate to identify phenolic substances (Johansen 1940). The slides were analyzed under light microscopy.

The results were documented with photomicrographs that were captured using an Olympus digital camera coupled

to an Olympus light microscope; diagrams and drawings were prepared using a *camara lucida* coupled to a light microscope or stereomicroscope.

Results

Ovary of the floral bud

The ovaries of all of the four species studied are tri-locular, trilobular, with one ovule per locule. The dorsal face of each carpel is free and projected forward, giving the ovary a generally triangular outline in a cross section. The ovary has a regular outline in the three *Banisteriopsis* species (Fig. 1-3), but is irregular in *Diplopterys pubipetala* (Fig. 4). The carpels are adnate to the pyramidal torus at the base (Fig. 7-8, 10-11), becoming free at the apex.

The outer epidermis is uniseriate, and is formed by small cubical cells with thin walls, dense cytoplasm, and large and very visible nuclei (Fig. 9, 12). Many non-glandular trichomes can be seen, some still differentiating and others already mature. Two types of trichomes can be seen: one unicellular and "T" branched (Fig. 5) and another multi-seriate with a multicellular peduncle and a single ramified apical cell with thick walls (Fig. 6); this apical cell is very similar to the first trichome.

The ovarian mesophyll is formed of layers of isodiametric cells of varying sizes (Fig. 9), which are smaller and denser in the dorsal region (Fig. 12). Cells undergoing mitosis in various planes can be seen throughout the mesophyll, and so the number of cell layers is very variable, varying from six to ten. In the three species of *Banisteriopsis*, the subepidermal layer is impregnated with phenolic substances that mask the cell contents, except in the dorsal region and in the torus (Fig. 7-8); however, these subepidermal cells are not distinct in *D. pubipetala* (Fig. 12).

The vasculature in the three species of *Banisteriopsis* examined is limited to three ventral bundles serving the ovary in the basal region, which separate into six bundles as they approached the apex of the ovary (Fig. 1-3, 7-8). In *D. pubipetala*, there are six ventral bundles at the base of the ovary (Fig. 10), but these join, forming just three bundles nearer the apex (Fig. 11). In addition to these ventral bundles, all four species show two lateral bundles for each carpel, placed near to the ventral bundles (Fig. 1-4). The bundles are amphicribal, with the ventral bundles being larger and more differentiated than the lateral ones.

The inner epidermis is initially uniseriate, but periclinal cell divisions can be seen in this region, giving rise to two cell layers (Fig. 13).

The torus constitutes the septum and it is parenchymatous and composed of large cells with ample vacuoles (Fig. 14-15). Immersed in this region, there are numerous phenol-containing idioblasts (Fig. 7-8) and druse crystals (Fig. 14-15).

Ovary of anthesis flowers

The outer epidermis appears very similar at anthesis to that observed in the floral bud. An intense formation of non-glandular trichomes can be observed as well as a thickening of the distal cell wall (Fig. 16-18), which takes on a verrucose aspect. The epidermal cells accumulate phenolic substances in the region where the carpels are fused, except in *D. pubipetala*.

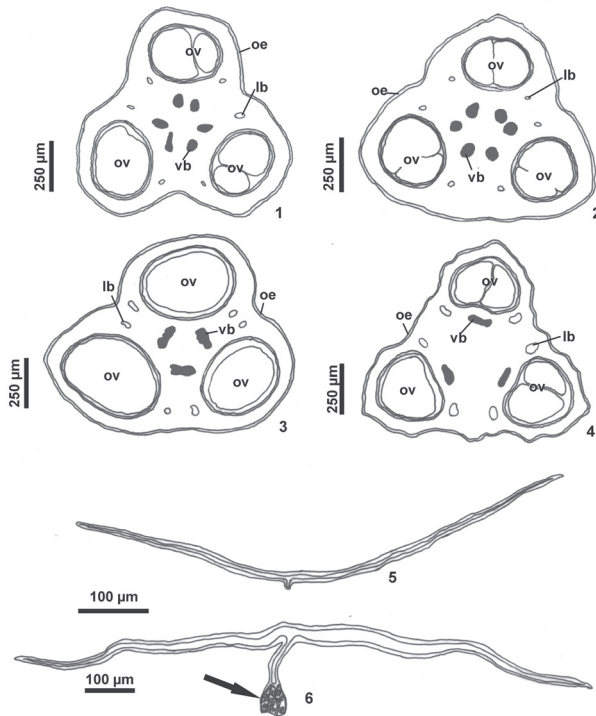
Significant modification occurs during development of the ovarian mesophyll, which was divided into three regions for description purposes. The outer mesophyll is composed by one layer of subepidermal cells containing phenolic substances in all of the *Banisteriopsis* species, being interrupted in the dorsal region of each carpel and in the torus; internally, numerous layers of vacuolated cells can be seen with thin walls and evident nuclei, accompanied by numerous idioblasts containing druses (Fig. 16). The median mesophyll has groups of cells that retain the meristematic nature, with very dense cytoplasm, and only a small elongation (Fig. 16); numerous phenolic idioblasts can be seen among these cells. In the inner mesophyll, layers composed of cells that have begun to elongate horizontally can be seen adjacent to the inner epidermis (Fig. 16, 19); three cell layers occur in *B. campestris* and *B. oxyclada* (Fig. 16, 20), with up to two cell layers in *B. stellaris* (Fig. 19, 21); and up to five layers can be observed in the ventral region of all three species. In *D. pubipetala*, the mesophyll is parenchymatic, showing cells with thin walls, vacuoles, and evident nuclei; in the median region of the mesophyll there is a continuous band of more compact and denser cells, with voluminous nuclei occupying a large portion of the lumen; this is quite different from the situation seen in the species of *Banisteriopsis*, which have blocks of meristematic cells in the median mesophyll (Fig. 17).

As a result of periclinal divisions in the inner epidermis, approximately two cell layers are formed in all of the lateral and dorsal region of this epidermis (Fig. 20) in *Banisteriopsis* species; the largest numbers of divisions can be seen in the ventral region, forming up to three layers in *B. campestris*, and six layers in *B. stellaris* (Fig. 21) and *B. oxyclada* (Fig. 22). These layers are horizontally elongated and accumulate phenolic substances in the apical two thirds of the ovary. Many periclinal divisions can be observed in *D. pubipetala*, forming many layers that appear to be stacked, with irregularly distributed cells containing phenolic substances (Fig. 23).

The dorsal region of each carpel demonstrates an accumulation of meristematic cells that form a salience on the ovarian wall (Fig. 23). Groups of meristematic cells forming numerous projections along the lateral sides of the carpels are also observed in *D. pubipetala*.

Young fruit

During fruit development, the growth of the free portions of the carpels is very notable; the mericarps are developed still adnate to the torus (Fig. 24).



Figures 1-6. Diagrams of transverse sections of the median region of ovaries from floral buds, with details of the non-glandular trichomes. 1. *Banisteriopsis campestris*. 2. *Banisteriopsis oxyclada*. 3. *Banisteriopsis stellaris*. 4. *Diplopterys pubipetala*. 5-6. *Banisteriopsis oxyclada*. 5. Unicellular non-glandular trichome. 6. Non-glandular trichome with multicellular peduncle (arrow). lb, lateral bundle; oe, outer epidermis; ov, ovule; vb, ventral bundle.

The exocarp does not undergo many alterations in the young fruit and remains uniseriate (Fig. 24-27). It has stomata (Fig. 26) and still produces non-glandular trichomes that are impregnated with lignin or phenolic substances. The exocarp cells develop vacuoles, and often contain phenolic substances.

The outer mesocarp of the three species of *Banisteriopsis* examined is formed by up to four cell layers in some regions, with large juxtaposed cells that accumulate phenolics (Fig. 26). The median mesocarp is more ample and composed of parenchyma cells (Fig. 26, 28), with numerous druses and phenolic idioblasts. The groups of meristematic cells immersed in this parenchyma begin to differentiate at this stage, becoming elongated in different directions, having thickened cell walls and forming many phenolic idioblasts (Fig. 26, 28). The inner mesocarp is composed of a variable number of cell layers that become tangentially elongated (Fig. 28) and show thickening of the cell walls.

The mesocarp of *D. pubipetala* is different from that of *Banisteriopsis*, although it can also be divided into three regions (Fig. 25). The outer mesocarp is formed by large juxtaposed cells (Fig. 27), among which phenolic idioblasts and occasional druses can be seen. The median mesocarp is composed of a continuous layer of cells that are elongated in numerous planes, although the transverse plane predomina-

tes; their cell walls become thicker (Fig. 27, 29). The inner mesocarp is composed of two or three layers of irregularly shaped cells with vacuoles and thin walls, containing phenolic substances (Fig. 29).

Small and inconspicuous lateral vascular bundles develop between the outer and median mesocarp in all four species (Fig. 26).

The endocarp in the three species of *Banisteriopsis* has longitudinally elongated cells with thick cell walls, vacuoles, and inconspicuous nuclei (Fig. 28). The endocarp forms approximately three cell layers in *B. campestris*; in *B. oxyclada* it has from two to six layers; and in *B. stellaris* from two to four cell layers.

The endocarp of *D. pubipetala* proliferates intensely forming many cell layers; initially, these cells are juxtaposed, but during development they become branched, forming large intercellular spaces and sometimes accumulating phenolic substances (Fig. 29-30), constituting the seed cushion.

The projection of the dorsal region is greatly increased in size and produces the dorsal wing that is seen in all of the species (Fig. 31). In *D. pubipetala* accessory lateral projections are also formed, creating winglets whose structures are similar to the dorsal wings, although smaller (Fig. 25, 32).

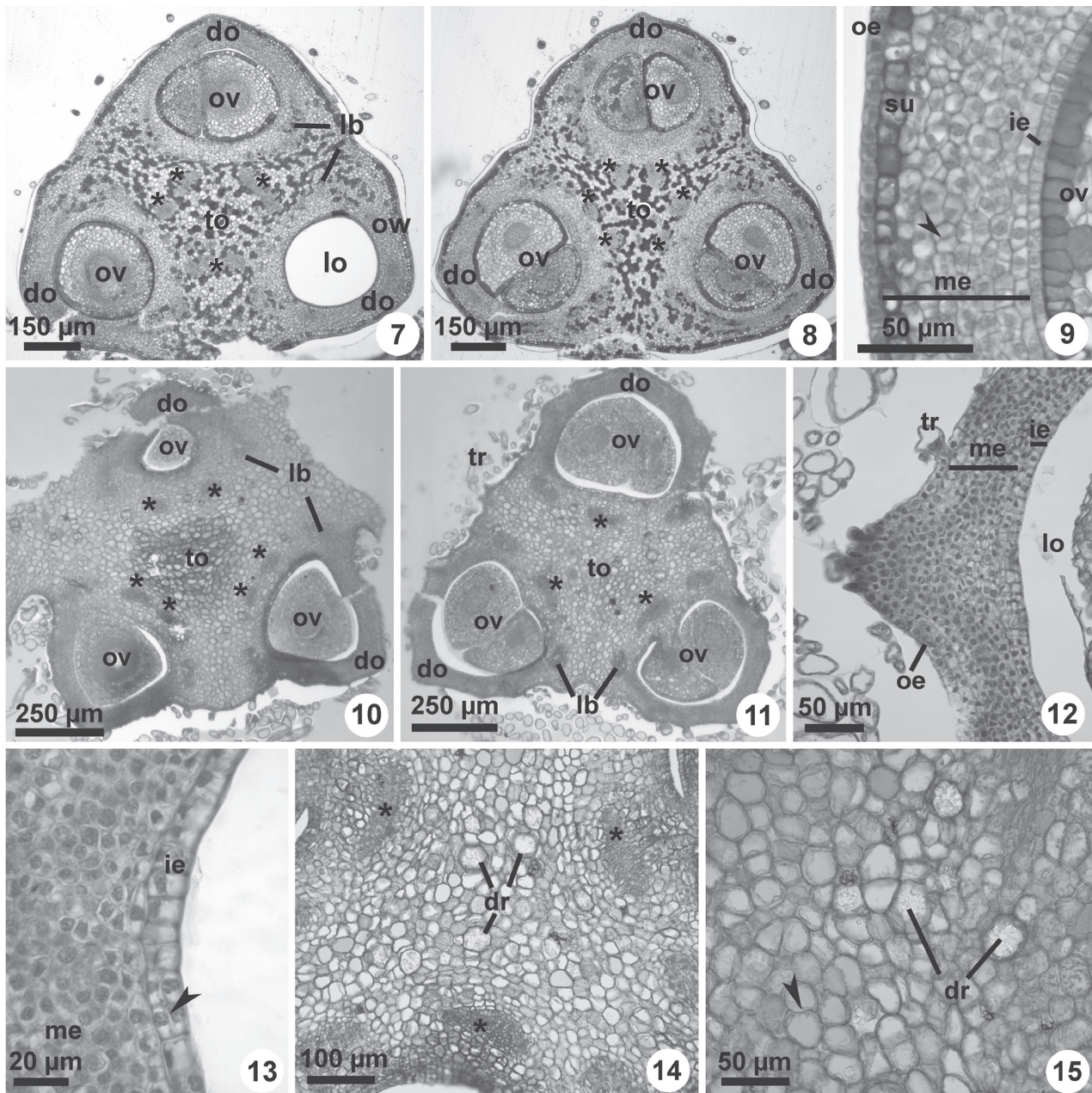
The wing initially has a meristematic aspect (Fig. 31, 33), with a uniseriate exocarp composed of well juxtaposed cells that have thin walls and dense cytoplasm. The mesocarp is composed of layers of cells with thin walls and dense cytoplasm in this phase. Intercellular spaces begin to form in the innermost region of the mesocarp, and many procambial strands can be seen immersed in this region (Fig. 33).

The torus retains the same appearance as in the previous phases, although it undergoes a significant increase in volume, initially due to cell divisions in various planes, and later due to the increase of the size of cell vacuoles (Fig. 34). At the border between the torus and the pericarp of each mericarp there are approximately two layers of small flattened cells with thin walls that compose the abscission layer of the samaras (Fig. 35).

Mature fruits

The mature fruit is a schizocarp composed of three samaras attached to the torus. They are reddish when near maturity, becoming brown as dehydration proceeds. Each samara has a dorsal wing, small irregular saliences lateral to the seed chamber, and a persistent style (Fig. 36-38); *Diplopterys pubipetala* also has winglets on the seed chamber (Fig. 39). The angle between the dorsal wing and the torus of each samara is approximately 55° in *B. campestris*, 30° in *B. oxyclada*, 15° in *B. stellaris*, and 65° in *D. pubipetala*.

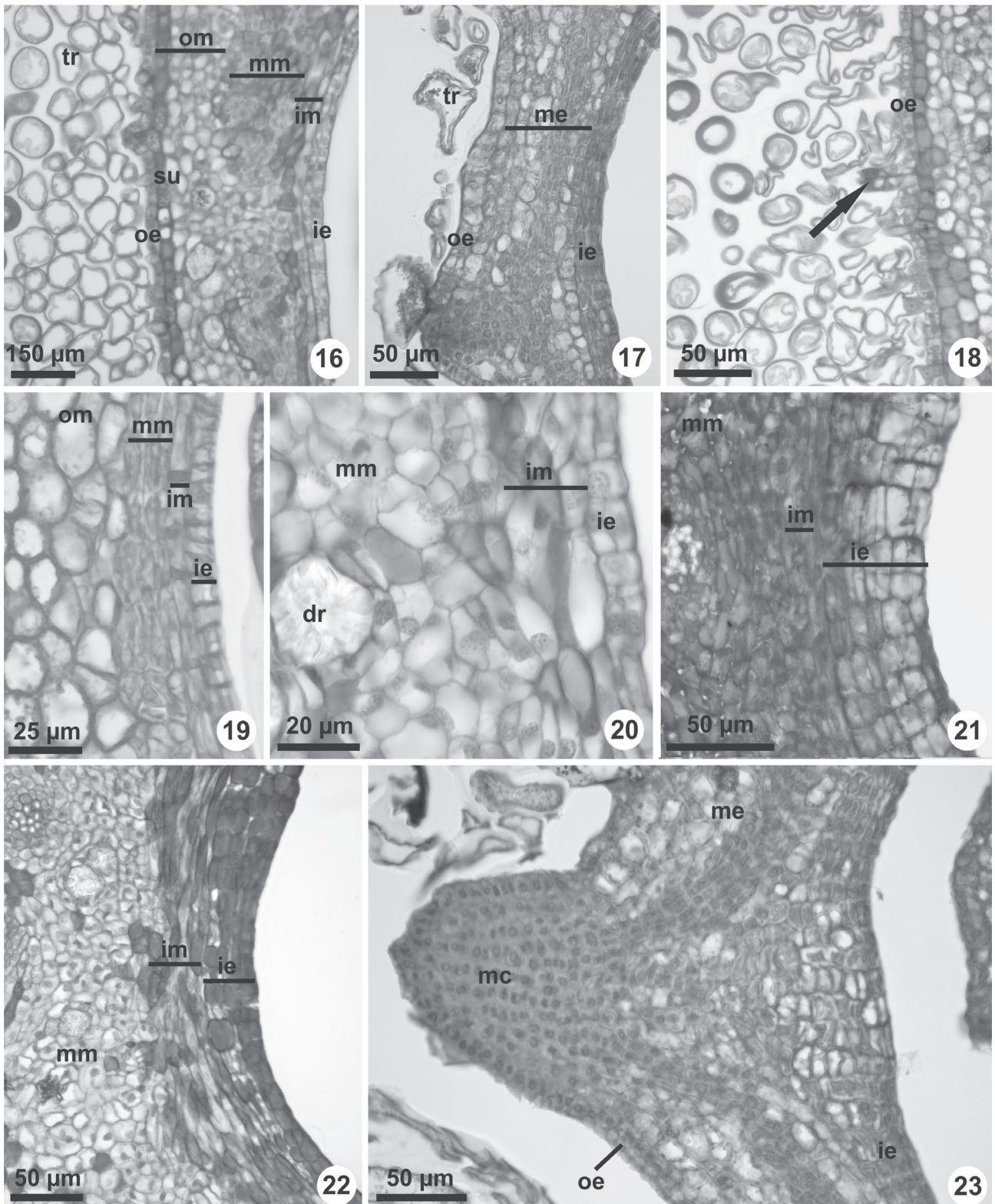
The exocarp remains uniseriate in the seed chamber, and it is formed by small cells of various shapes (Fig. 42) with their external periclinal walls covered by a thin cuticle; stomata are observed, and the cells often have phenolic contents. The unicellular and multicellular non-glandular trichomes still are seen with "T" shape and verrucose cell



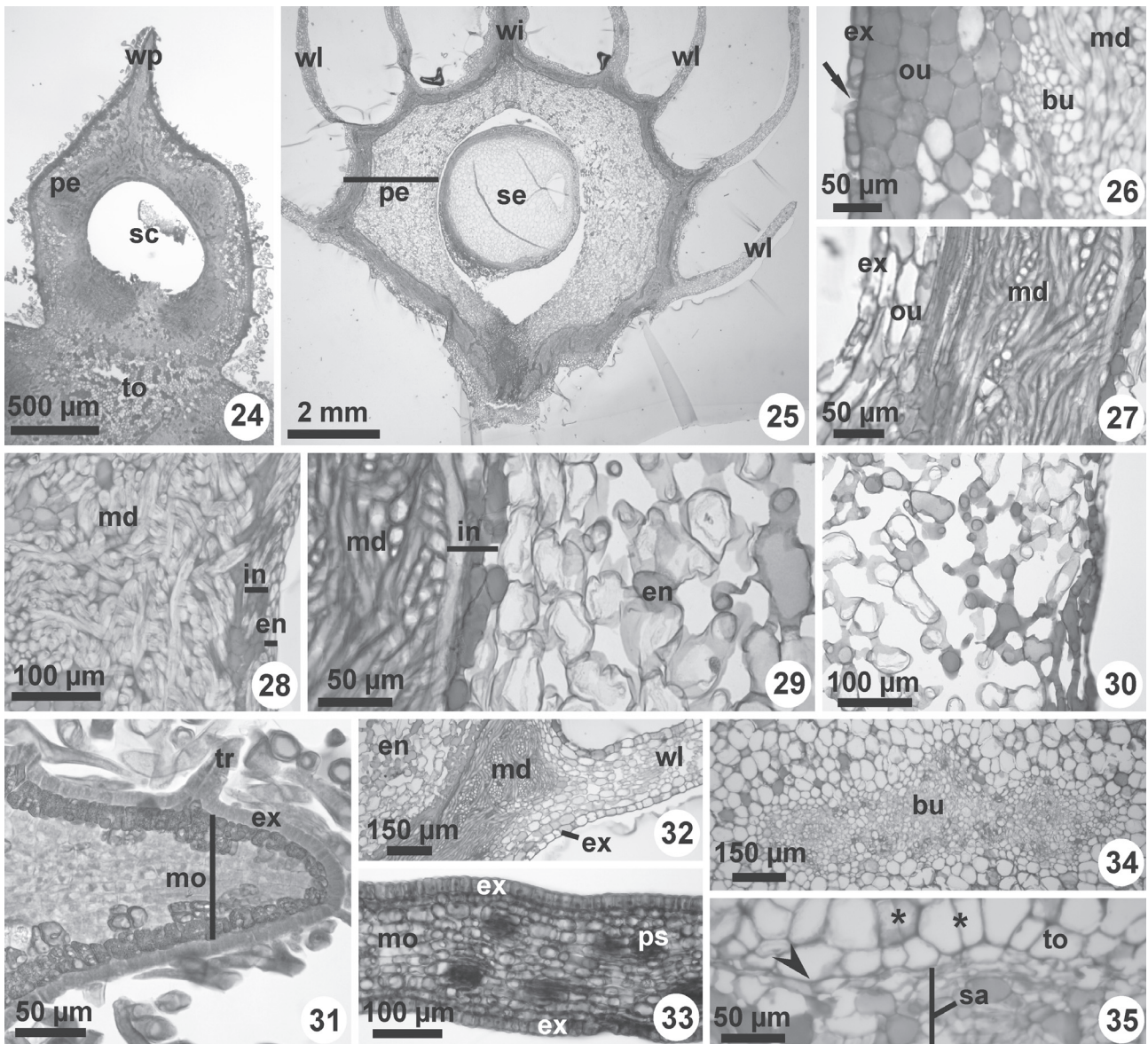
Figures 7-15. Ovaries in floral buds of *Banisteriopsis oxyclada* (7-9) and *Diplopterys pubipetala* (10-15). Transverse sections (7-14), longitudinal section (15). 7. General view of the basal portion of the ovary with two ventral bundles still united at the septa between the two carpels, and two ventral bundles already separated at the other septa. 8. General view of the apical portion of the ovary showing the six separate ventral bundles. 9. Detail of the ovarian wall; note meristematic aspect of the all cells, and the occurrence of cell divisions in the mesophyll (arrowhead). 10. General aspect of the base of the ovary with six separate ventral bundles. 11. General aspect of the median portion of the ovary, showing the ventral bundles fused into pairs between the adjacent carpels, forming three bundles. 12. Detail of the ovarian wall in the dorsal region of the carpel, clearly showing a projection in the ovarian wall. 13. Detail of the ovarian mesophyll highlighting the first periclinal divisions in the inner epidermis (arrowhead). 14-15. Aspect of the torus; cell divisions can be seen in 15 (arrowhead); note idioblasts containing druses. asterisk, ventral bundle; do, dorsal region; dr, druse; ie, inner epidermis; lb, lateral bundle; lo, locule; me, ovarian mesophyll; oe, outer epidermis; ov, ovule; ow, ovarian wall; su, subepidermal layer; to, torus; tr, non-glandular trichome.

walls (Fig. 44); in *B. oxyclada* trichomes without wall ornamentation are also observed. Trichomes are abundant on the fruits of all species, except in *D. pubipetala*, which has few trichomes, giving it a glabrous appearance. When the fruits are ready for dispersal the exocarp collapses on the seed chamber.

At fruit maturity, the outer mesocarp collapses (Fig. 40-41, 43); the median mesocarp is composed of parenchyma cells with phenolics that also collapse, and blocks of elongated, thickened cells (Fig. 40) become lignified. These thickened cells are living gelatinous fibers; the more internal region of the cell wall is impregnated with pectic substances. In *B.*



Figures 16-23. Ovaries of flowers at anthesis of *Banisteriopsis oxyclada* (16, 18, 20, 22), *Banisteriopsis stellaris* (19, 21), and *Diplopterys pubipetala* (17, 23). Transverse sections. 16-17. General view of the ovarian wall. 18. Detail of the outer epidermis with trichomes in various stages of differentiation (arrow, base of a multicellular non-glandular trichome). 19-20. Details of the inner mesophyll and inner epidermis in the lateral region of the carpel. 21-22. Detail of the inner mesophyll and inner epidermis in the ventral region of the carpel. 23. Aspect of the ovarian wall in the dorsal region of the carpel, showing a projection formed by the dorsal meristematic cells. dr, druse; ie, inner epidermis; im, inner mesophyll; mc, dorsal meristematic cells; me, ovarian mesophyll; mm, median mesophyll; oe, outer epidermis; om, outer mesophyll; su, subepidermal layer; tr, non-glandular trichome.



Figures 24-35. Pericarp during development of *Banisteriopsis oxyclada* (24, 31), *Banisteriopsis stellaris* (26, 28, 34-35), and *Diplopterys pubipetala* (25, 27, 29-30, 32-33). Transverse sections. 24-25. General view of one samara showing the beginning of growth of the wing; in 25, note some winglets on the seed chamber. 26. Detail of the exocarp, and the outer and median mesocarp; note a vascular bundle and the presence of stoma (arrow). 27. Detail of the exocarp, and the outer and median mesocarp. 28. Detail of the median mesocarp, inner mesocarp, and the endocarp. 29. Detail of the median mesocarp, inner mesocarp, and part of the endocarp. 30. Detail of the aerenchyma in the endocarp, the seed cushion. 31. Detail of the wing primordium, showing poorly differentiated tissues. 32. Aspect of the insertion region of the wing on the seed chamber. 33. General aspect of the young wing, showing several procambial strands. 34-35. Detail of the torus; in 35, note the presence of cell divisions (asterisks) and the abscission zone (arrowhead). bu, vascular bundle; en, endocarp; ex, exocarp; in, inner mesocarp; md, median mesocarp; mo, mesocarp; ou, outer mesocarp; pe, pericarp; ps, procambial strand; sa, samara; sc, seed chamber; se, seed; to, torus; tr, non-glandular trichome; wl, winglet; wp, wing primordium.

campestris, the parenchyma cells of the median mesocarp also become lignified, transforming it into a continuous layer of lignified cells. The median mesocarp of *D. pubipetala* is formed by a mass of lignified cells that are elongated in numerous directions, but predominantly in the transverse plane (Fig. 43); druses are observed in some of those cells. The inner mesocarp of all of the species of *Banisteriopsis* is composed of lignified cells elongated in the tangential plane; in *D. pubipetala*, these cells are parenchymatic and are totally flattened at maturity (Fig. 43). Vasculature becomes incons-

picuous in the mature fruits, and only the ventral bundles in the torus and some small lateral bundles immersed in the fibers of the seed chamber are clearly defined.

The multiseriate endocarp of all of the species of *Banisteriopsis* is formed by longitudinally elongated cells with thick and lignified walls. In *D. pubipetala*, the endocarp is formed by aerenchyma that becomes compressed in the mature fruit (Fig. 43).

The wing is delimited by the uniseriate exocarp and is formed by small cells of various shapes (Fig. 45); it also

has stomata and many non-glandular trichomes, except in *D. pubipetala*, which has very rare trichomes. In the four analyzed species, the outer mesocarp forms a uniseriate to biseriate subepidermal layer composed of large cells. The median mesocarp forms an aerenchyma composed of branched cells and many intercellular spaces. Many vascular bundles can be seen immersed in this region, forming two rows peripherally to the median mesocarp; these are small collateral bundles with a sheath of fibers, which are wider next to the aerenchyma (Fig. 45-46). The winglets of *D. pubipetala* are structurally similar to the dorsal wings, although much smaller in size.

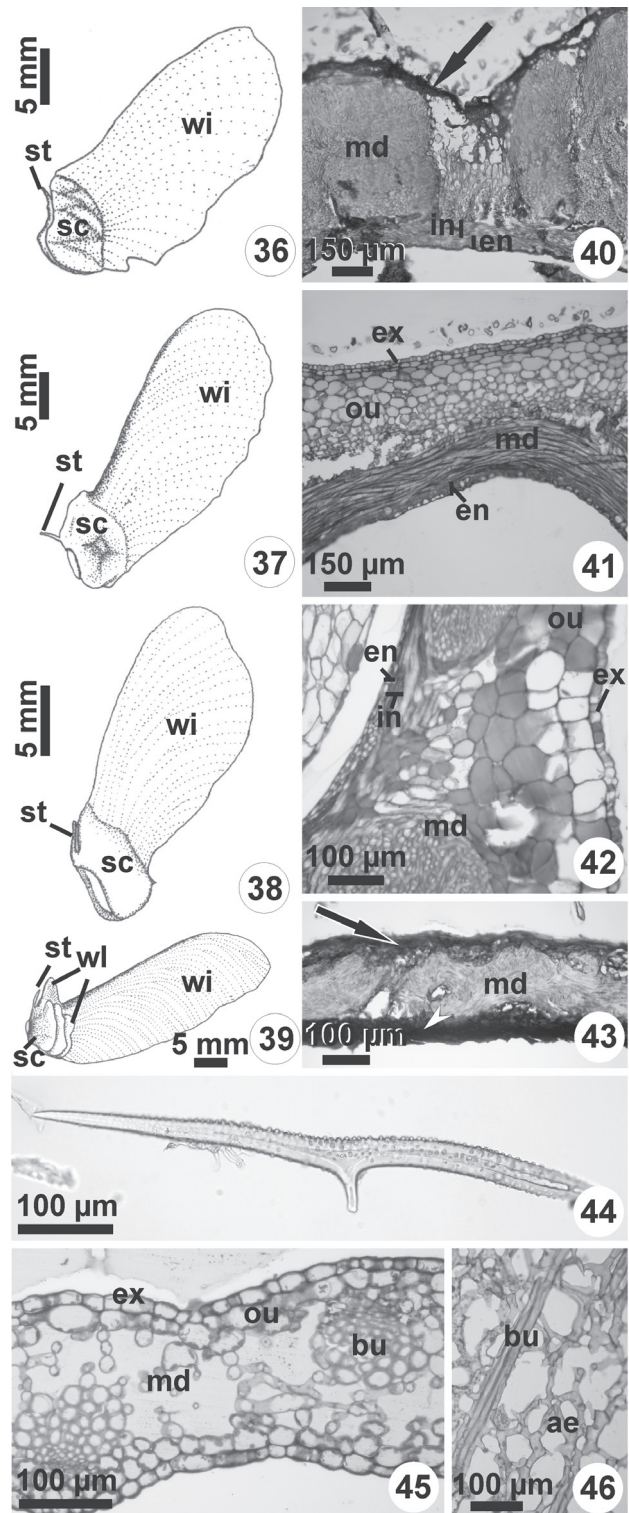
The torus does not become modified at maturity; it is parenchymatous and demonstrates many phenolic idioblasts and druses. At dispersion, the abscission layer ruptures liberating the samaras. In some parts in which the abscission tissue is not differentiated, the separation occurs between the parenchyma cells of the torus and the thick lignified cells of the mericarp.

Discussion

The structure and development of the pericarp in the species of *Banisteriopsis* examined here was very homogeneous. One of the few distinctive characteristics among the different species studied is the occurrence of lignified parenchymatic cells in the mature pericarp of *B. campestris*; in *B. oxyclada* and *B. stellaris*, these cells are not lignified and collapse at maturity. However, the pericarp of *D. pubipetala* demonstrated significant peculiarities during development, as well as in the mature structure.

The two genera studied are considered by various authors to be very closely related from a phylogenetic point of view (Gates 1982; Davis *et al.* 2001; Anderson & Davis 2006). Davis & Anderson (2010) placed both genera in the stigmaphylloids clade, but *Banisteriopsis* and *Diplopterys* are not sister taxa. *Diplopterys pubipetala* was previously included in *Banisteriopsis* sect. *Anisopterys* by Gates (1982), and has been included in *Diplopterys* after Anderson & Davis (2006). These authors removed *D. pubipetala* and another 24 species from *Banisteriopsis* in response to phylogenetic analyses based on molecular data that indicated that the subgenus *Pleiopterys* of *Banisteriopsis* was not monophyletic and was intermingled with *Diplopterys*. Significant disparities in pericarp structure and development are highlighted among the three species of *Banisteriopsis* examined and *D. pubipetala*, including the occurrence of winglets, an inner aerenchymatous endocarp, and thinner lignified layers in the endocarp of the last one. All of these characteristics corroborate the interpretation of Anderson & Davis (2006) and reinforce that *D. pubipetala* should not be included in the same genus as the other species described here.

One aspect that stands out in the present study is the presence of three ventral bundles at the base of the ovary of the *Banisteriopsis* species that later split into six bundles



Figures 36-46. Mature fruits of *Banisteriopsis campestris* (36, 40), *Banisteriopsis oxyclada* (37, 41, 46), *Banisteriopsis stellaris* (38, 42, 44-45), and *Diplopterys pubipetala* (39, 43). 36-39. General view of the samaras. 40-41, 43. Transverse sections of the mature pericarp (arrow, outer collapsed layers; arrowhead, inner collapsed layers). 42. Pericarp in pre-maturation phase. 44. Aspect of unicellular non-glandular trichome with verrucose wall. 45. Transverse section of the mature wing. 46. Median longitudinal section of the mature wing, showing the branched aspect of the aerenchyma cells. ae, aerenchyma; bu, vascular bundle; en, endocarp; ex, exocarp; in, inner mesocarp; md, median mesocarp; ou, outer mesocarp; sc, seed chamber; st, style; wi, dorsal wing; wl, winglet.

in the median region of the ovary. On the other hand, the ovary of *D. pubipetala* has six ventral bundles at the base of the ovary that fuse to form just three bundles apically. The phenomenon of three vascular bundles subdividing into six was also reported for *Camarea affinis* St.Hil. (Mamede 1993) and *Janusia guaranitica* (St.Hil.) A.Juss. (Lorenzo 1981). The peculiar vasculature in the carpels of *D. pubipetala* constitutes one more feature that distinguishes this species from the others of the genus *Banisteriopsis*. Additional analyses of the floral vasculature of other species of *Diplopterys* would indicate if this is a characteristic of the genus, or just a species level variation.

Although there are many distinctive differences among the three *Banisteriopsis* species and *D. pubipetala*, they have some common features. All four species have schizocarpic fruits formed by three samaras linked to the torus. One hypothesis concerning the evolution of the fruits of Malpighiaceae proposes that the ancestral species of the family probably had a non-winged fruit and that the wing arose at the base of the family clade, but was subsequently lost in many lineages (Davis *et al.* 2001). This loss appears in some species of *Diplopterys* that demonstrate a very reduced dorsal wing (Gates 1982). As such, the presence of wings on the fruits of the four studied species is considered an ancestral trait of the Malpighiaceae. Besides this, the large variation in the inclination angle of the samaras may be an interesting feature, but this angle has not been emphasized in the literature and more detailed studies are needed to verify how this characteristic varies among the different winged genera of Malpighiaceae.

In comparing the different species studied here, it was observed that the exocarp and the outer mesocarp are similar in all of them, as is the presence of a reasonably thick layer of flattened cells that are elongated in many directions in the median portion of the pericarp. The presence of such a sclerenchymatic layer in the mesocarp has been reported for two other Malpighiaceae, *Malpighia emarginata* DC. (Laskowski & Bautista 2000) and *Byrsonima intermedia* A.Juss. (Souto & Oliveira 2005); these species have drupoid fruits, in which the lignified pyrene is composed of cell layers derived from both the mesocarp and the endocarp. *Lophantera lactescens* Ducke (Paoli 1997) also has schizocarpic fruits, but they are formed by three cocci in which the mesocarp is entirely parenchymatous and the endocarp is woody. Although the published literature emphasizes large variations concerning the consistency and dehiscence in Malpighiaceae fruits (Anderson 1979; Barroso *et al.* 1999), the occurrence of a lignified layer in the fruits appears to be a constant feature of this family.

Another anatomical feature that highlights is the intense proliferation of the endocarp of *D. pubipetala*, forming an abundant aerenchyma tissue that collapses in the mature pericarp. This tissue forms a seed cushion, and it has an important role in seed development. According to Roth (1977), fruits that become lignified before the seeds are

fully grown often form a soft tissue that can be compressed to accommodate the developing seed. Seed cushions are common in the Leguminosae (Martins & Oliveira 2001; Pietrobom & Oliveira 2004; Nakamura & Oliveira 2005), and are always associated with dry pericarps. No previous reference of the existence of this structure in the Malpighiaceae was found, which can be explained by the dearth of anatomical and ontogenetic studies of dry fruits, especially samaras in this family.

The unicellular non-glandular trichomes encountered in the species analyzed here have been reported in the literature as a typical characteristic of the Malpighiaceae. They occur on stems, leaves, flowers, and fruits (Metcalfe & Chalk 1950; Judd *et al.* 1999), and can sometimes take on a verrucose aspect as seen in this work. According to Metcalfe & Chalk (1950), these trichomes can even be called malpighiaceae due to their wide occurrence in this family. Judd *et al.* (1999) pointed out that these trichomes can take on T, V, or Y forms, with either similar or unequal arms. The occurrence of multicellular non-glandular trichomes is generally uncommon in the family, although they were observed on the ovaries and fruits of all four species examined here.

In arid environments with high herbivory pressure, such as in the *cerrado*, appropriate defense strategies are very important for plants. All of the species of this work have many cells containing phenolic substances, which are considered to be efficient in defense against herbivory (Swain 1979). Additionally, the ovarian epidermis and the exocarp of the species evaluated have large quantities of the malpighiaceae trichomes, which tend to reduce water loss, another important aspect in the savanna. A significant presence of phenolic substances and of non-glandular trichomes was also observed in fruits of *Byrsonima intermedia*, another Malpighiaceae typical of the *cerrado* (Souto & Oliveira 2005). Abundant phenolic substances have also been noted in the pericarps of woody plants from other families encountered in this biome, such as Leguminosae (Paiva & Oliveira 2004; Paiva *et al.* 2008) and Styracaceae (Julio & Oliveira 2007), which reinforces the defensive role of these substances in woody savanna plants.

Another interesting characteristic is the frequent occurrence of druses. These crystals are apparently common in the ovaries and fruits of the Malpighiaceae, and have also been reported for *Janusia guaranitica* (Lorenzo 1981), *Lophantera lactescens* (Paoli 1997), and *Malpighia emarginata* (Laskowski & Bautista 1999), indicating that these inclusions constitute a common trait of the Malpighiaceae.

In spite of the fact that there are no other published descriptions available concerning the anatomy and ontogeny of winged fruits among the Malpighiaceae, the structure of the wings of *D. pubipetala*, and of the three species of *Banisteriopsis*, was observed to be similar to that of other species with winged fruits, such as *Tipuana tipu* (Benth.) Kuntze (Martins & Oliveira 2001), *Pterocarpus violaceus*

Vogel (Nakamura & Oliveira 2005) and *Centrolobium tomentosum* Guill. ex Benth. (Oliveira *et al.* 2007), three species of Leguminosae. As winged fruits are associated with anemochory, the presence of an ample structure of low density is very interesting. These characteristics are made possible by the presence of aerenchyma tissue with abundant intercellular spaces filled only with air that increases fruit volume without adding significant weight.

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References

- Anderson, W.R. 1979. Floral conservatism in neotropical Malpighiaceae. **Biotropica** **11**: 219-223.
- Anderson, W.R. 1990. The origin of the Malpighiaceae: the evidence from morphology. **Memoirs of the New York Botanical Garden** **64**: 210-224.
- Anderson, W.R. & Davis, C.C. 2006. Expansion of *Diplopterys* at the expense of *Banisteriopsis* (Malpighiaceae). **Harvard Papers in Botany** **11**: 1-16.
- Barroso, G.M.; Morim, M.P.; Peixoto, A.L. & Ichaso, C.L.F. 1999. **Frutos e sementes: morfologia aplicada à sistemática de dicotiledôneas**. Viçosa, Editora UFV.
- Bukatsch, F. 1972. Bemerkungen zur doppelfärbung Astrablau-Safranin. **Mikrokosmos** **61**: 255.
- Davis, C.C.; Anderson, W.R. & Donoghue, M.J. 2001. Phylogeny of Malpighiaceae: evidence from chloroplast *ndhF* e *trnL-F* nucleotide sequences. **American Journal of Botany** **88**: 1830-1846.
- Davis, C.C. & Anderson, W.R. 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. **American Journal of Botany** **97**: 2031-2048.
- Dop, P. & Gautié, A. 1928. **Manual de technique botanique**. 2 ed. Paris, J. Lamane.
- Gates, B. 1982. *Banisteriopsis*, *Diplopterys* (Malpighiaceae). **Flora Neotropica Monographs** **30**: 1-236.
- Jensen, W.A. 1962. **Botanical histochemistry: principles and practice**. San Francisco, W. H. Freeman.
- Johansen, D.A. 1940. **Plant microtechnique**. New York, McGraw-Hill Book.
- Judd, W.S.; Campbell, C.S.; Kellogg, E.A. & Stevens, P.F. 1999. **Plant Systematics: a phylogenetic approach**. Sunderland, Sinauer Associates.
- Julio, P.G.S. & Oliveira, D.M.T. 2007. Morfoanatomia e ontogênese do fruto e semente de *Styrax camporum* Pohl. (Styracaceae), espécie do cerrado do Estado de São Paulo. **Revista Brasileira de Botânica** **30**: 189-203.
- Laskowski, L.E. & Bautista, D. 1999. Características anatómicas de la flor del semeruco (*Malpighia emarginata* DC.). **Ernstia** **9**: 19-36.
- Laskowski, L.E. & Bautista, D. 2000. Características anatómicas y desarrollo del fruto del semeruco (*Malpighia emarginata* DC.). **Ernstia** **10**: 105-115.
- Lorenzo, E. 1981. Sobre la inflorescencia, morfología floral y embriología de *Janusia guaranítica* (Malpighiaceae). **Kurtziana** **14**: 101-124.
- Mamede, M.C.H. 1993. Estudo comparativo de flores casmógamas, cleistógamas e de frutos de *Camarea affinis* St.-Hil. (Malpighiaceae). **Acta Botanica Brasílica** **7**: 21-31.
- Martins, M.A.G. & Oliveira, D.M.T. 2001. Morfo-anatomia e ontogênese do fruto e da semente de *Tipuana tipu* (Benth.) O. Kuntze (Fabaceae: Faboideae). **Revista Brasileira de Botânica** **24**: 109-121.
- Metcalfe, C.R. & Chalk, L. 1950. **Anatomy of dicotyledons**. 2 ed. Oxford, Clarendon Press.
- Nakamura, A.T. & Oliveira, D.M.T. 2005. Morfoanatomia e ontogênese da sâmara de *Pterocarpus violaceus* Vogel (Fabaceae: Faboideae). **Revista Brasileira de Botânica** **28**: 375-387.
- O'Brien, T.P.; Feder, N. & McCully, M.E. 1964. Polychromatic staining of plant cell walls by toluidine blue O. **Protoplasma** **59**: 368-373.
- Oliveira, D.M.T.; Siqueira, A.C.N. & Nakamura, A.T. 2007. Anatomia e ontogênese da sâmara de *Centrolobium tomentosum* Guill. ex Benth. (Leguminosae: Papilionoideae). **Rodriguesia** **58**: 231-247.
- Paiva, E.A.S. & Oliveira, D.M.T. 2004. Ontogenesis of the fruit pulp layer of *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae: Caesalpinioideae). **Australian Journal of Botany** **52**: 677-683.
- Paiva, E.A.S.; Oliveira, D.M.T. & Machado, S.R. 2008. Anatomy and ontogeny of the pericarp of *Pterodon emarginatus* Vogel (Fabaceae, Faboideae), with emphasis on secretory ducts. **Anais da Academia Brasileira de Ciências** **80**: 455-465.
- Paiva, E.A.S.; Pinho, S.Z. & Oliveira, D.M.T. 2011. Large plant samples: how to process for GMA embedding? Pp. 37-49. In: Chiarini-Garcia, H. & Melo, R.C.N. (Eds.). **Light microscopy: methods and protocols**. Methods in Molecular Biology, vol. 689. New York, Springer/Humana Press.
- Paoli, A.A.S. 1997. Morfo-anatomia de frutos e sementes de *Lophantera lactescens* Ducke (Malpighiaceae). **Revista Brasileira de Sementes** **19**: 238-244.
- Pietrobon, R.C.V. & Oliveira, D.M.T. 2004. Morfoanatomia e ontogênese do pericarpo de *Schizolobium parahyba* (Vell.) Blake (Fabaceae, Caesalpinioideae). **Revista Brasileira de Botânica** **27**: 767-779.
- Roth, I. 1977. **Fruits of Angiosperms**. Handbuch der Pflanzenanatomie. Berlin, Gebrüder Borntraeger.
- Sass, J.E. 1951. **Botanical microtechnique**. Ames, Iowa State University.
- Souto, L.S. & Oliveira, D.M.T. 2005. Morfoanatomia e ontogênese do fruto e semente de *Byrsonima intermedia* A.Juss. (Malpighiaceae). **Revista Brasileira de Botânica** **28**: 697-712.
- Swain, T. 1979. Tanins and lignins. In: Rosenthal, G.A. & Janzen, D.H. (Eds.). **Herbivores: their interaction with secondary plant metabolites**. New York, Academic Press.