

Morphoanatomy and ontogeny of fruit in Bromeliaceae species¹

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RESUMO – (Morfoanatomia e ontogenia de fruto em espécies de Bromeliaceae). O presente estudo objetivou apresentar um panorama acerca da estrutura de frutos em Bromeliaceae, devido à extrema escassez de estudos nessa área; revelando a morfologia, anatomia e ontogenia dos frutos de seis espécies pertencentes a seis gêneros distintos, representativos de três subfamílias. Todas as espécies estudadas apresentam ovário tricarpelar e trilobular, com obturador na região da placenta. *Dyckia maritima*, *Pitcairnia flammea*, *Tillandsia aeranthes* e *Vriesea carinata* possuem frutos do tipo cápsula (septicida ou biscida), caracterizados pela presença de macrosclereídes no exocarpo ou endocarpo, fibras na região ventral dos carpelos, seis linhas de deiscência e três feixes vasculares por carpelo. *Aechmea calyculata* e *Billbergia nutans* possuem frutos do tipo baga, com exocarpo e hipoderme como camadas mecânicas e muitos feixes vasculares por carpelo. Diversos caracteres úteis para a taxonomia de Bromeliaceae foram destacados, sendo os frutos ótimas ferramentas para a delimitação, principalmente, de gêneros e subfamílias. Em relação às cápsulas de Bromeliaceae, as macrosclereídes são firmadas como estruturas marcantes do pericarpo e a anatomia do fruto está intimamente relacionada ao tipo de deiscência. Os espessamentos de parede celular em “U” demonstram um caráter comum na família e passam a constar como estruturas ocorrentes também em frutos de monocotiledôneas.

Palavras-chave: anatomia, Bromeliaceae, desenvolvimento, ovário, pericarpo

ABSTRACT – (Morphoanatomy and ontogeny of fruit in Bromeliaceae species). This study aims to give an overall view of fruit structure in Bromeliaceae, since these studies are extremely scarce in this family, showing the morphology, anatomy and ontogeny of fruits of six species belonging to six different genera, representing three subfamilies. All species studied have a tricarpellary and trilobular ovary, with an obturator covering the placental region. *Dyckia maritima*, *Pitcairnia flammea*, *Tillandsia aeranthes* and *Vriesea carinata* have capsular fruits (septicidal or biscidal), characterized mainly by the presence of macrosclereids in the exocarp or endocarp, fibers in the ventral region of the carpels, six dehiscence lines and three vascular bundles in each carpel. *Aechmea calyculata* and *Billbergia nutans* have fruits classified as berries, presenting exocarp and hypodermis as mechanical layers and many vascular bundles in each carpel. Many useful characters for taxonomy of Bromeliaceae were revealed, so fruits are excellent tools for delimitation of genera and subfamilies. In relation to Bromeliaceae capsules, macrosclereids are conspicuous structures of the pericarp and fruit anatomy is greatly related to dehiscence type. The U-shaped cell wall thickenings are a very common character in this family and can be identified as structures also occurring in monocot fruits.

Key words: anatomy, Bromeliaceae, development, ovary, pericarp

Introduction

Bromeliaceae currently covers 58 genera and 3172 species (Luther 2008). The family is included in the order Poales (APG II 2003) and has a typically Neotropical geographic distribution (Smith & Downs 1974). Its representatives are epiphytic, rupicolous or terricolous herbs, whose fruits are classified as capsules or berries (Benzing 2000).

Although the monophyletic origin of this family has been confirmed (Ranker *et al.* 1990; Horres *et al.* 2000; Barfuss *et al.* 2005), the origins, phylogenetic relations and circumscriptions of the subfamilies, genera and species are as yet little known, and, above all, there is much difficulty in delimiting genera and species. Therefore, the infrafamilial relations of Bromeliaceae have been the target of several investigations, but the phylogeny of the group is still being intensely discussed, with significant differences among the authors.

Traditionally, Bromeliaceae is divided into three subfamilies: Pitcairnioideae, Tillandsioideae and Bromelioideae, distinguished especially by the morphology of flowers, fruits and seeds (Smith & Downs 1974). However, Givnish *et al.* (2007) proposed a new arrangement for the family based on molecular data, increasing the number of subfamilies to eight (Brocchinioideae, Lindmanioideae, Tillandsioideae, Hechtioideae, Navioideae, Pitcairnioideae, Puyoideae and Bromelioideae). This new classification

became more natural, so that the recognized subfamilies are monophyletic.

As for fruit morphology and anatomy, several studies have shown that they are used to delimit or resolve taxonomic problems among species, genera, tribes and even monotypical families, such as Tobe *et al.* (1992), Smitt *et al.* (1995), Doweld (1998), Decraene *et al.* (2000), Doweld (2001) and Moon & Hong (2006). Moreover, this type of investigation is essential to help understand the true origin of the fruits of angiosperms and to contribute to making their classification more uniform. As the reproductive organ responsible for seed dispersal, through morphoanatomical studies, the fruit helps us to understand how the species are dispersed and these are important data for proposals to conserve biodiversity.

Despite what has been presented about the importance of structural studies in fruit, these are few in comparison to those on other plant organs, and very few angiosperm families have a representative number of publications in this field, as in the case of Fabaceae and Anacardiaceae. In contrast to these, only two anatomical studies of fruit were recorded for Bromeliaceae, in *Ananas comosus* (Okimoto 1948) and *Tillandsia tricholepis* (Morra *et al.* 2002).

Therefore, the purpose of this study is to characterize and compare the morphoanatomy and ontogeny of the fruit of six species of Bromeliaceae – *Aechmea calyculata* and

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Billbergia nutans (Bromelioideae), *Dyckia maritima* and *Pitcairnia flammea* (Pitcairnioideae), *Tillandsia aeranthos* and *Vriesea carinata* (Tillandsioideae) – corresponding to the three subfamilies that occur in the state of Rio Grande do Sul, presenting an overview of the structure of fruits in Bromeliaceae and correlating the data to the current phylogenies.

Materials and methods

The botanical material was obtained partly from the Bromeliaceae Collection in the Botanical Garden of Porto Alegre, under the following numbers: *Aechmea calyculata*, BROM 00022, BROM 00252, BROM 00393; *Billbergia nutans*, BROM 00106, BROM 00189, BROM 00739; *Pitcairnia flammea*, BROM 00140, BROM 00324, BROM 00335. The other species were collected in the counties of Porto Alegre, Viamão, Dom Pedro de Alcântara and Caraá, in Rio Grande do Sul, and the exsiccatae deposited in the ICN Herbarium of the Federal University of Rio Grande do Sul under numbers: *Dyckia maritima*, ICN 188806; *Tillandsia aeranthos*, ICN 188807; *Vriesea carinata*, ICN 144794.

The collection involved flowers and fruits at different stages of development, from three or more individuals. It should be pointed out that the flowers in pre-anthesis are different from floral buds because they have fully developed floral verticillates. The material was dissected, and only the ovaries and fruits were preserved and fixed in glutaraldehyde 1% and formaldehyde 4% in sodium phosphate buffer 0.1M, pH 7.2 (McDowell & Trump 1976).

In order to prepare transverse, longitudinal and paradermal histological sections, the samples were passed through a sodium phosphate buffer 0.1M, pH 7.2 (Gabriel 1982), and later dehydrated in an ethylic series until they were included in hydroxyethylmethacrylate (Gerrits & Smid 1983). After this, the sections were made, 4 µm thick, in a Zeiss rotation microtome HM 340 E, and stained with Toluidine Blue O 0.05%, pH 4.4 (O'Brien & McCully 1981). A few histochemical tests were performed, such as Lugol for starch (Johansen 1940), Hydrochloric Acid 10% for calcium oxalate (Chamberlain 1932), Phenol for silica (Johansen 1940) and Ruthenium Red for pectins (Johansen 1940). The analyses and photomicrographs of the histological material were done under bright field optical microscopy, using a Leica DM R microscope.

For scanning electron microscopy (SEM), the material was dehydrated in an ascending acetone series and then submitted to drying by the critical point method (Gersterberger & Leins 1978) using BAL-TEC CPD 030 equipment. After this, the samples were mounted on aluminum stubs and sputter coated with platinum in the BAL-TEC SCD 050 metalizing apparatus. The observations and electromicrographic record were performed in a JEOL 6060 microscope, under 10kV.

The terminology used is according to Roth (1977), for the definition of layers of pericarp, Spjut (1994) and Barroso *et al.* (1999), for the classification of the type of fruit and type of dehiscence.

The following developmental stages were established to describe most of the species: I – undifferentiated ovary of floral buds; II – ovary and young fruit from flowers in pre-anthesis, anthesis and post-anthesis; III – mature fruit and, when appropriate, dry fruit. The description of *Vriesea carinata* follows the stages: I – undifferentiated ovary of floral buds; II – ovary from flowers in pre-anthesis; III – ovary and young fruit from flowers in anthesis and post-anthesis; IV – mature, dry fruit. In species in which the ovary wall is fused to non-carpellary tissues, the characterization of the ovary and fruit results from the topographic analysis of the tissues.

Results

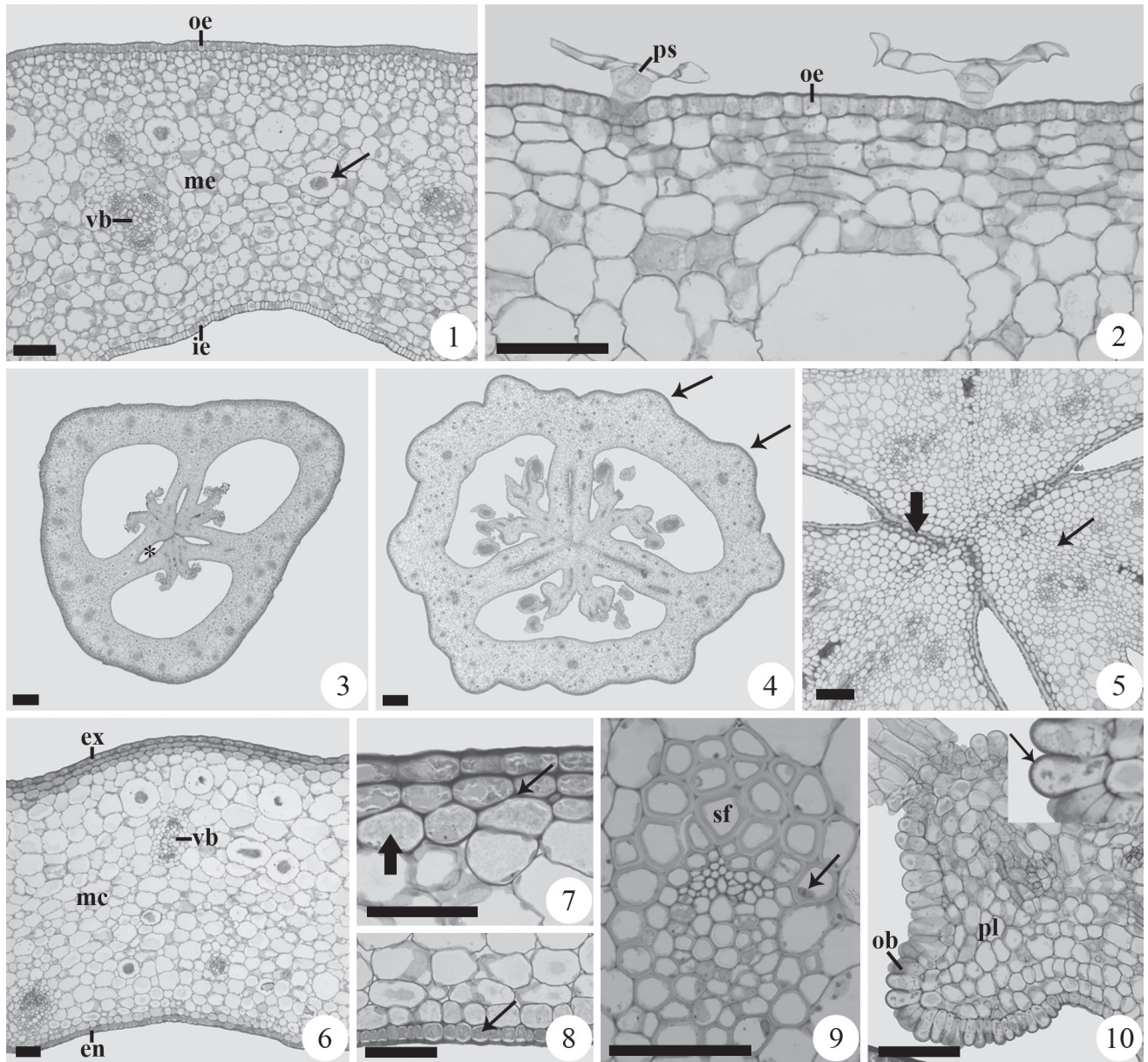
1. Bromelioideae species

Stage I – The ovary is tricarpeal and trilobular, with the presence of an obturator in the placental region, continuing the inner epidermis, and septal nectaries (not discussed in this study). These characters are common to the other

species studied. The ovary is inferior with the carpels completely fused, which present ramifications of the septal nectary in their lateral regions. The ovary wall is fused to non-carpellary tissues, so that they cannot be distinguished. The epidermises are unistratified with rectangular cells in section, while the mesophyll is parenchymatous, pluristratified and composed predominantly of isodiametric cells (Fig. 1); this can also be seen in the other species analyzed. The epidermises have anticlinally elongated cells (Fig. 1-2). In *Aechmea calyculata*, the outer epidermis has cells with dense cytoplasmic content and spherical silica bodies, as well as peltate scales distributed irregularly on the epidermal surface (Fig. 2). The mesophyll is composed of 18-22 cell layers, following a gradient with a decreasing cell volume from the central region to the one close to the peripheries (Fig. 1). Idioblasts containing calcium oxalate raphides frequently occur in the mesophyll. They occur in all carpel regions in *B. nutans* and in the dorsal and lateral regions of the carpels in *A. calyculata* (Fig. 1). The fusion of adjacent carpels along their entire laterals characterizes syncarpy as total, and the complete fusion of tissues, without distinguishing the limit between the carpels, characterizes it as true (Fig. 3-4). Each carpel presents many vascular bundles (Fig. 3-4) – about 15 in *A. calyculata* and 25 in *B. nutans*. In *Billbergia nutans*, the ovary has an outer surface with ribs, always nine of them (Fig. 4). In the central region of the ovary, there are six distinct lines: three in the septal region, extending only to the nectariferous region, and three ventral sutures of the carpels (Fig. 5). The obturator has elongated cells with dome-shaped outer periclinal faces. Starch grains are present in the ovary wall and in the non-carpellary tissues, a trait common to the other species studied. In this stage, anticlinal cell divisions are observed in the mesophyll, in a longitudinal plane.

Stage II – The ovary, and then the fruit (Fig. 3-4), undergo changes referring to cell enlargement and cell differentiation. In the fruit wall as a whole, there is an accumulation of pectins inside the cells (Fig. 6-8) and cell enlargement in a tangential direction (Fig. 6). The exocarp and adjacent cell layers (4-5 layers in *A. calyculata* and 2-3 layers in *B. nutans*) have little thickened cell walls, just as the outer periclinal wall of the endocarp (Fig. 7-8). In the exocarp a differentiation of stomata occurs with equal cell wall thickenings in the periclinal walls of the guard cells, which are elevated compared to the other exocarp cells in *A. calyculata*, and at the same level of the other epidermal cells in *B. nutans*. In *A. calyculata*, fibers are differentiated around the vascular bundles, forming a bundle sheath (Fig. 9). The obturator cells differentiate forming labyrinths of wall ingrowths (Fig. 10).

Stage III – The mature fruit is formed by the pericarp, derived from the ovary wall, and by non-carpellary tissues which are wrapped around the ovary (Fig. 11, 14-15, 21-22).



Figures 1-10. Ovary and developing fruit of Bromelioideae, mostly in transverse section. 1. Ovary wall with idioblasts in the mesophyll (arrow) of *A. calyculata*. 2. Outer epidermis with peltate scales (longitudinal section) of *A. calyculata*. 3-4. General view of developing fruit of *A. calyculata* (asterisk: ramification of the septal nectary) and *B. nutans* (arrows: ribs), respectively. 5. Six different rows of cells in the central region of the fruit of *A. calyculata*, with three ventral sutures (arrow) and three lines in the septal region (broad arrow). 6. Fruit wall of *B. nutans*. 7. Exocarp and hypodermal cell layers of *B. nutans*, showing cell wall thickening (arrow) and the pectic content of the cells (broad arrow). 8. Endocarp of *B. nutans*, with pectic cell content (arrow). 9. Vascular bundle wrapped in metabolically active bundle sheath fibers (arrow: nucleus) of *A. calyculata*. 10. Obturator in the placental region of *A. calyculata*, with detail (arrow: cell wall labyrinth). en: endocarp, ex: exocarp, ic: inner epidermis, mc: mesocarp, me: mesophyll, ob: obturator, oe: outer epidermis, pl: placenta, ps: peltate scale, sf: bundle sheath fibers, vb: vascular bundle. Bars= 50 μ m (Fig. 1-2, 5-10); 200 μ m (Fig. 3-4).

In *A. calyculata*, the fruit is formed also by the calyx (Fig. 11-13) and is identified as an ellipsoid armed berry, hairy, about 0.9 cm long and dark-purple color, with persistent bract, corolla, gynoecium and androecium. The sepals are fused over about 1/3 of their length, and the apices of the free portions form caudate, rigid projections.

The exocarp is represented by sclereids with U-shaped cell wall thickenings, with thickened anticlinal and inner periclinal walls; on the other hand, the sclereids of the

hypodermis have cell walls thickened along their length, with conspicuous pits (Fig. 16). The stomata are elevated in relation to the surface, superposed on the subsidiary cells. Both the exocarp and the endocarp have longitudinally elongated cells, and the former has sinuous cell walls and the latter has straight cell walls (Fig. 17-18). In the region from the calyx, the fruit wall is thicker, with a greater number of idioblasts and about 15 vascular bundles in each region corresponding to a sepal (Fig. 12-13). Furthermore, the cell

walls of the inner epidermis become thicker only in this region. In the sepal cohesion zone, there is a colenchymatous tissue with pectic cell content, close to the inner epidermis (Fig. 13). Throughout the fruit, the peltate scales are formed by an asymmetrical shield, non-organized into distinct disc and wing, with rather elongated extremities (Fig. 19-20), by 3-4 stalk cells and two basal cells, with the nearer cells from the exocarp and hypodermis disposed radially, forming a projection. Compounds are deposited inside the exocarp, hypodermis and endocarp cells, and they make the fruit dark-purple, almost black (Fig. 16-18).

In *B. nutans*, the fruit (Fig. 21) is an ovaloid, greenish berry, about 1.5 cm long, with a persistent calyx, corolla, androecium and gynoecium. Bundle sheath fibers are differentiated in the mesocarp (Fig. 22). Both the exocarp and the endocarp have longitudinally elongated cells with straight cell walls. The endocarp has an irregular pattern, in cell shape and in cell distribution, which follows only a tendency to longitudinal disposition (Fig. 23-26).

In both species, the mesocarp maintains the same number of cell layers and the same pattern of cell volume gradient and the 2-3 cell layers adjacent to the exocarp acquire thicker walls (Fig. 16, 23), which constitute a hypodermis.

The sclerenchymatous tissues are metabolically active due to the presence of cellular organelles, including the nucleus, inside the cells (Fig. 9). The stomata are located in exocarp depressions with the guard cells superposed on the under-arching subsidiary cells, and distributed longitudinally in relation to the fruit axis. The number of starch grains is reduced during development, until they disappear completely at fruit maturity. The ontogenesis of the fruit occurs from the apex towards the base and results in their growth in length and diameter, and this is mainly a consequence of the increased fruit perimeter, and not so much of pericarp thickness. The characteristics mentioned can also be seen in the other species studied.

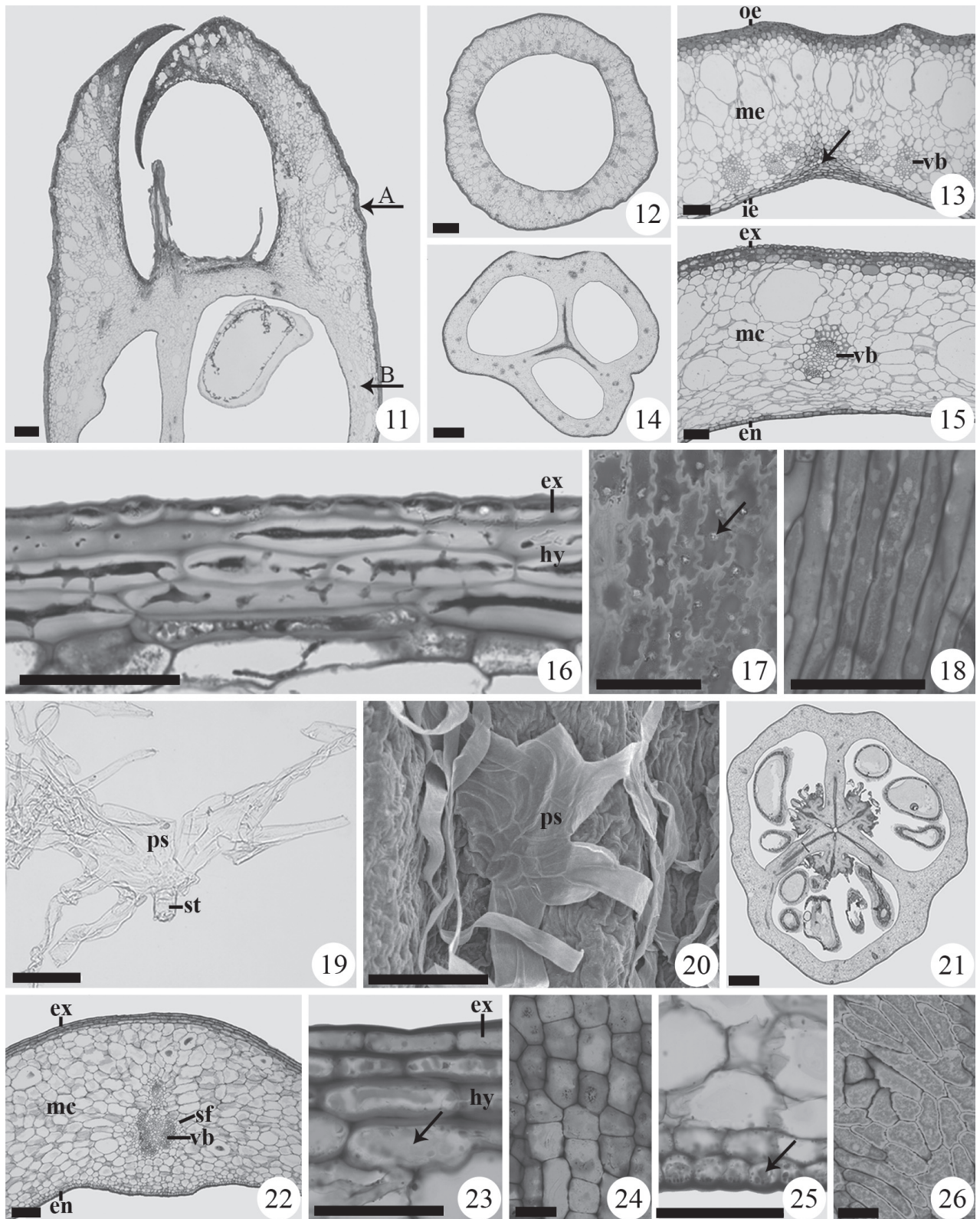
2. Pitcairnioideae and Tillandsioideae species

Stage I – The ovary is superior, except for *Pitcairnia flammea*, which has a semi-inferior ovary, with the inferior region fused to non-carpellary tissues. Tissue fusion between the carpels is not complete, since it is possible to distinguish the united epidermises in the lateral region of the carpels, which characterizes syncarpy of the species as being false (Fig. 27-28). In *Dyckia maritima* and *Pitcairnia flammea*, the carpels are fused to each other only in the ventral regions (Fig. 27), while in *Tillandsia aeranthis* and *Vriesea carinata*, the carpels are fused over the length of their lateral regions (Fig. 28). In this way, syncarpy is characterized as partial in *D. maritima* and *P. flammea*, because it is restricted to the ventral regions, and total in *T. aeranthis* and *V. carinata*. The ovary wall consists of two epidermises with elongated cells with dense content, the outer epidermis is in the anticlinal direction and the inner one in the periclinal

direction, in transverse section (Fig. 29). In *P. flammea*, the cell content is denser in the inner epidermis. In *T. aeranthis*, the cell content is quite dense compared to the other species. The mesophyll is composed of 6-8 cell layers in *D. maritima* and *T. aeranthis* (Fig. 29), of 8-10 cell layers in *P. flammea* and of 12-14 cell layers in *V. carinata*. Six dehiscence lines are clearly seen, each formed by two rows of cells that are minuscule compared to the other carpellary tissues. Of these, three are located in the lateral region of the carpels (septa) and are characterized by the continuation of the outer epidermises of adjacent carpels (Fig. 30-31) and the other three are located in the ventral region of the carpels (placentation zone) constituting the ventral sutures (Fig. 30). In the mesophyll there are idioblasts containing calcium oxalate raphides, except for *V. carinata*. Their location varies in the carpel according to species – occurring in the lateral and ventral regions in *D. maritima*, in the lateral and dorsal regions in *P. flammea* and in the ventral region in *T. aeranthis*. Three vascular bundles are identified per carpel, a main one in the dorsal region and two of a smaller caliber in the ventral region (Fig. 29-30). Cell divisions occur in the mesophyll of *D. maritima*, *P. flammea* and *T. aeranthis*, mostly in the anticlinal direction. The obturator is formed by elongated cells with dome-shaped outer periclinal faces in *D. maritima* and *P. flammea*. On the other hand, in *T. aeranthis*, the obturator cells undergo periclinal divisions, becoming very elongated and forming one to two strata (Fig. 32), while in *V. carinata* the obturator is represented by elongated cells.

Stage II – This stage only occurs in *V. carinata*. Cell divisions are found in the mesophyll, mainly in the anticlinal direction, and there is a clear, more intense proliferation in the inner region of this tissue (Fig. 33).

Stages III (*V. carinata*) and II (other species) – In this stage (Fig. 34), cell volume increases in the mesocarp and cell elongation occurs, in the anticlinal direction of a transverse section, in the exocarp of *D. maritima* (Fig. 35) and in the endocarp of *P. flammea*, *T. aeranthis* and *V. carinata* (Fig. 36). In *D. maritima*, there is deposition of cell wall in the outer periclinal walls of the exocarp (Fig. 35, 37) and the accumulation of mucilage in the vacuole of cells located around the stomata, in the exocarp and in some mesocarp cells (Fig. 37-38). In *P. flammea*, the exocarp cells become subtly elongated in the periclinal direction, in transverse section. In *D. maritima* and *P. flammea*, stomata appear in the exocarp, elevated compared to the epidermal surface (Fig. 38-40), whose guard cells develop equally thickened periclinal walls, in the former species, and inner periclinal walls thicker than the outer ones in the latter species. In the mesocarp, an increased quantity of idioblasts with raphides is found in *D. maritima* and *P. flammea*, which are strongly associated with the placenta in *D. maritima* (Fig. 41). The obturator (Fig. 41) develops a labyrinth of wall ingrowths in the outer periclinal face of its cells. In *V. carinata*, the



Figures 11-26. Mature fruit of Bromelioideae, mostly in transverse section. 11-20. *A. calyculata*. 11. General view of the fruit, in longitudinal section, showing the portion from the calyx (arrow A) and from the ovary and non-carpellary tissues (arrow B). 12. General view of portion A. 13. Detail of the fruit wall (arrow: cohesion tissue of the sepals). 14. General view of portion B. 15. Detail of the fruit wall. 16. Exocarp and hypodermis. 17. Exocarp, in paradermal section, with silica bodies (arrow). 18. Endocarp, in paradermal section. 19-20. Peltate scale, in optical microscopy and SEM, respectively. 21-26. *B. nutans*. 21. General view of mature fruit. 22. Fruit wall. 23. Exocarp, hypodermis and other mesocarp cells (arrow: pectic cell content). 24. Exocarp, in paradermal section. 25. Endocarp with pectic cell content (arrow). 26. Endocarp, in paradermal section. en: endocarp, ex: exocarp, hy: hypodermis, ie: inner epidermis, mc: mesocarp, me: mesophyll, oe: outer epidermis, ps: peltate scale, sf: bundle sheath fibers, st: stalk, vb: vascular bundle. Bars= 50 μ m (Fig. 15-20, 23-26); 100 μ m (Fig. 13, 22); 200 μ m (Fig. 11); 400 μ m (Fig. 12, 14, 21).

obturator cells divide, becoming rather elongated and forming uni- to bistratified tissue. The outer surface of the fruit presents a smooth cuticular pattern except for *P. flammea*, which has a striate pattern (Fig. 42-43).

Stages IV (*V. carinata*) and III (other species) – The pericarp reaches maturity by differentiation of the sclerenchymatous tissues (Fig. 44-46, 51-53, 58-60, 66-67).

In *D. maritima*, the mature fruit (Fig. 44) has an ovaloid shape, about 0.5 cm long, brown color and with a persistent calyx and corolla. In this stage, the mucilage deposited in the cells close to the stomata disappears. In the dorsal vascular bundle, close to the phloem, and between the fibers, some radially aligned cells remain parenchymatous, forming a dehiscence zone (Fig. 45). The exocarp cells acquire very thick walls and their shape is radially elongated in the transversal direction, and are characterized as macrosclereids (Fig. 45, 47-48). This tissue is unistratified over most of its length, and is pluristratified in restricted regions. The endocarp is constituted by sclereids with not very thick cell walls, which are tangentially elongated in the transverse direction (Fig. 45, 49-50).

In *P. flammea*, the fruit (Fig. 51) is characterized by its elongated, conical shape, length of about 0.9 cm and dark-green color, with persistent calyx, corolla, gynoecium and androecium. The exocarp cells are characterized by the softly elongated shape in the longitudinal direction, with a subtle deposition of cell wall on their outer periclinal faces (Fig. 52, 54-55). As in some mesocarp cells, the exocarp has a pectic cell content (Fig. 54-55). The endocarp cells acquire very thick and conspicuous U-shaped cell walls, and they have a radially and tangentially elongated shape in the transverse direction (Fig. 52, 56-57), being classified as macrosclereids.

In *T. aeranthis* and *V. carinata*, the mature fruit (Fig. 58, 66) is elongated, dark-green, with persistent bract and calyx. In the former species, the fruit has a cylindrical shape and is about 2 cm long, while in the latter the fruit is obovoid and about 3 cm long. Both the exocarp and endocarp cells undergo deposition of U-shaped cell wall (Fig. 59, 61, 63, 67-68, 70). The conspicuous endocarp cells are characterized as macrosclereids, which show the lamellation of their walls as a reflex of deposition (Fig. 65). As to shape, the exocarp sclereids are longitudinally elongated (Fig. 61-62, 68-69) while those of the endocarp are radially and tangentially elongated in the transverse direction (Fig. 63-64, 70-71).

The mesocarp is homogeneous in *D. maritima* and *T. aeranthis* (Fig. 45, 59), and it is divided into two parenchymatous regions in *P. flammea*, the outer one with larger cells and the inner one with smaller cells (Fig. 52), and following a gradient of cell volume in *V. carinata*, which diminishes from the center towards the exocarp and endocarp (Fig. 67). There are 8-10 mesocarp cell layers in *D. maritima* and *T. aeranthis*, 9-11 in *P. flammea* and 14-16 in *V. carinata*. In the mesocarp, a subtle thickening occurs in the outer periclinal walls of the cell layer adjacent to the exocarp, characterized as hypodermis (Fig. 45, 61, 68),

except for *P. flammea*. Around the dorsal vascular bundle, fibers which form the bundle sheath are differentiated. These fibers also form bundle sheath extensions in *D. maritima* (Fig. 45) and a bundle sheath extension towards the endocarp in *P. flammea* (Fig. 53). In the ventral region of the carpels, a great amount of fibers appear among the ventral vascular bundles and the dehiscence lines (Fig. 46). In *T. aeranthis*, these fibers are inconspicuous due to their scarcity and to the thin layer of cell wall deposited (Fig. 60). In this stage, the quantity of idioblasts containing raphides in the mesocarp diminishes in *D. maritima* or even disappears in *P. flammea* and *T. aeranthis*. Furthermore, a cell compression is found in the outer mesocarp, comprising around 1-2 cell layers in *T. aeranthis* (Fig. 59) and three layers in *V. carinata* (Fig. 67).

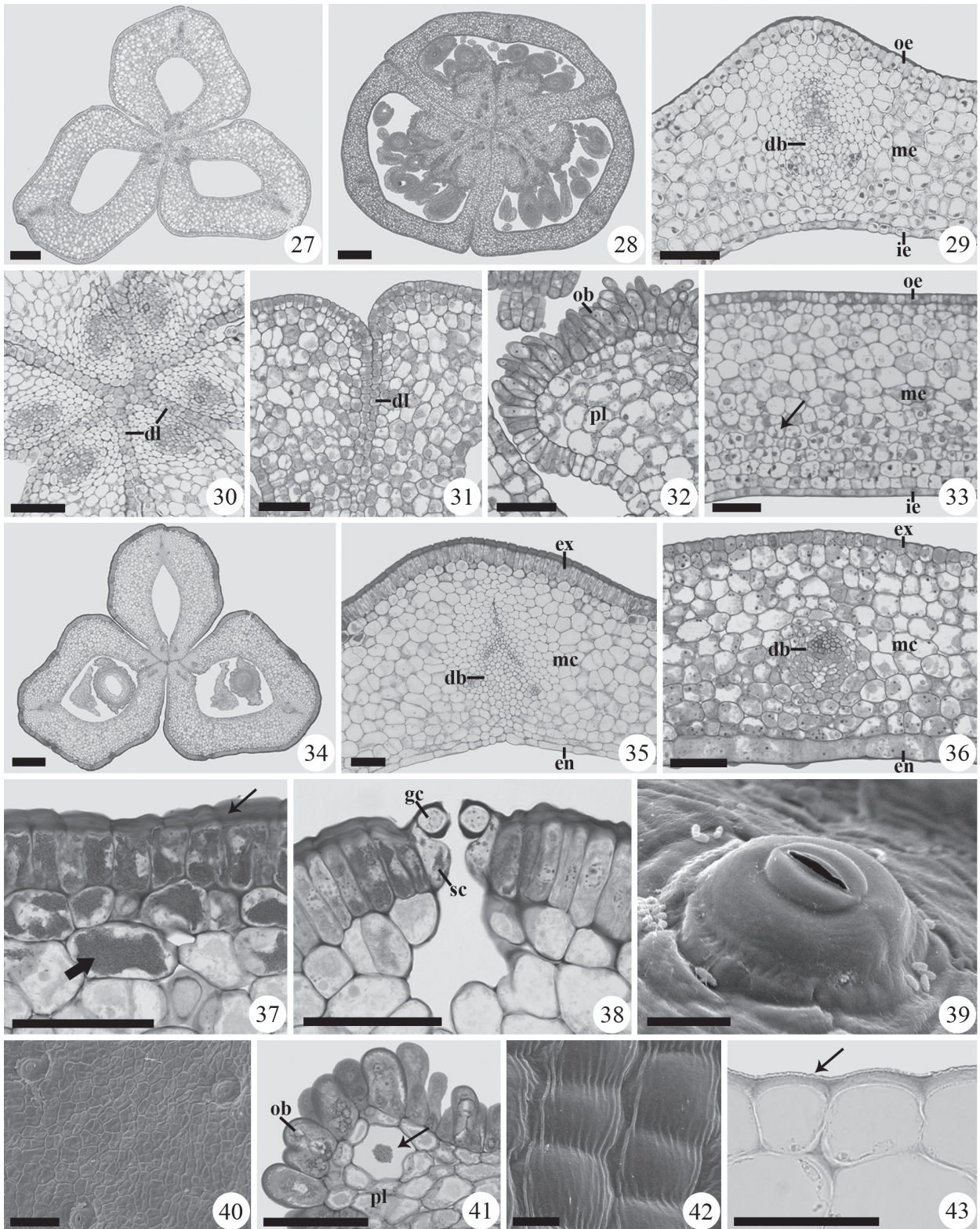
The dry fruit basically maintains the same structure as the fruit when it was fleshy, and only a marked compression of some cell layers of the mesocarp is outstanding. After dehydration, dehiscence of the fruit takes place from the apex to the base, as a result of the concomitant rupture of the six dehiscence lines in the central region of the fruit, and besides this, of the dorsal vascular bundle in its middle region in *D. maritima*, where the parenchymatous cells are located in a radial row. In *D. maritima*, the capsule opens into six erect valves, each one corresponding to half a carpel, and the septal openings occur until the base and the openings of the locules occur over 2/3 of the fruit length. Therefore, the capsule is classified as biscidal – because of the combination of septicidal dehiscence with loculicidal. In *P. flammea*, the capsule is of the septicidal type, with openings occurring over 2/3 of its length, showing three erect valves originating in each of the carpels, so that the valves remain joined at their apexes due to the persistent style. In *T. aeranthis* and *V. carinata*, the capsule opens until its base in three valves which are completely folded back, and it is classified as septicidal.

The morphological and anatomical characters referring to the ovary and fruit of all species analyzed are compiled in Tab. 1, in order to summarize and compare the data presented in this study.

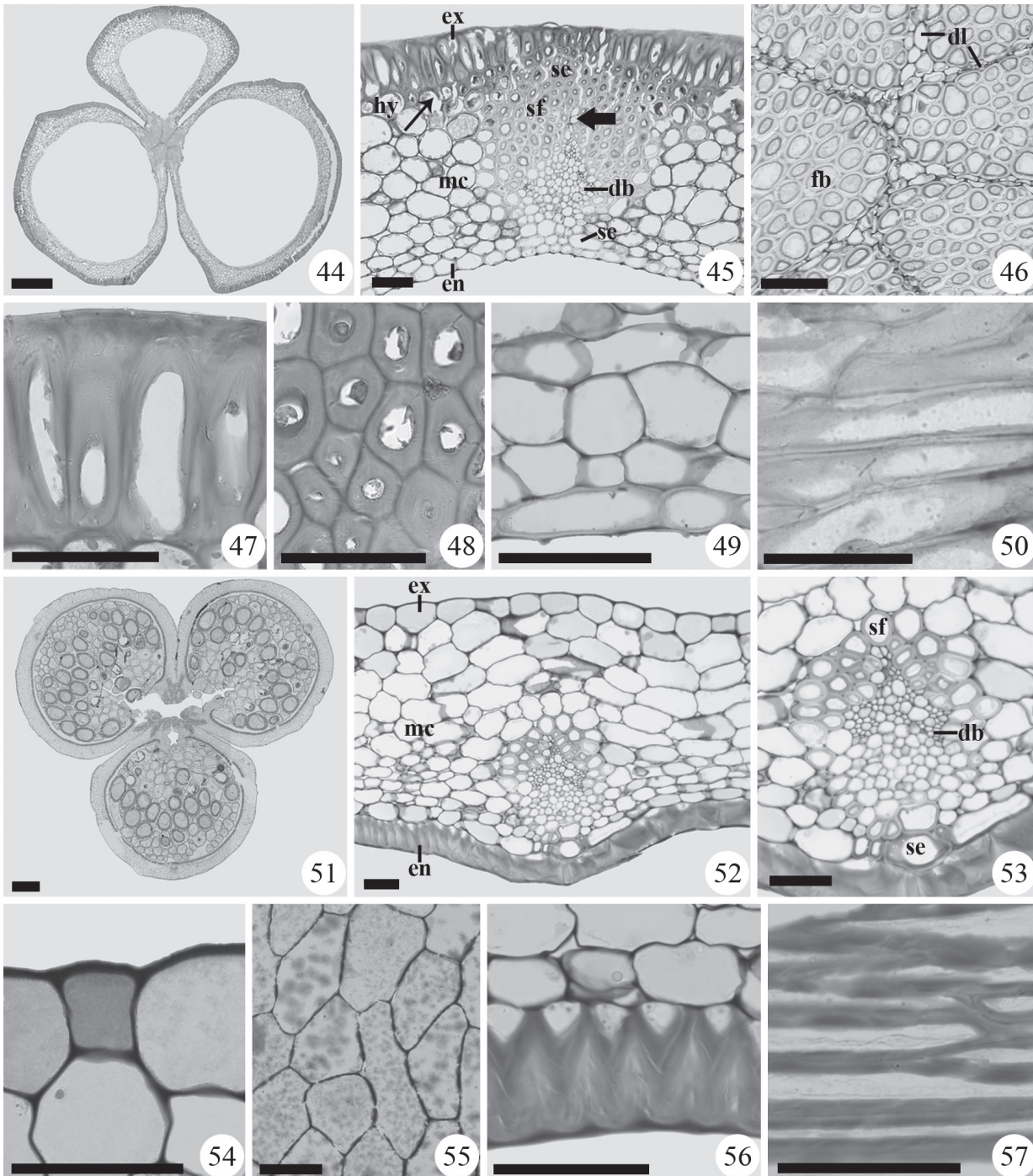
Discussion

Many investigators have emphasized that pericarp growth already begins in the floral bud, mainly by cell multiplication, and it stops gradually until anthesis (Nitsch 1953). In the ontogenesis of the infrutescence of *Ananas comosus*, cell division is apparently complete before the flower opens, so that the increase in tissue volume is derived from cell enlargement (Okimoto 1948), which also occurs in all species analyzed in this study.

As for carpel fusion, considering the syncarpic context of the family, the gynoecium can be characterized as partially or totally syncarpic, concerning the union of part or the whole length of the carpel sides. Furthermore, syncarpic can be defined as false or true (Lersten 2004) if the



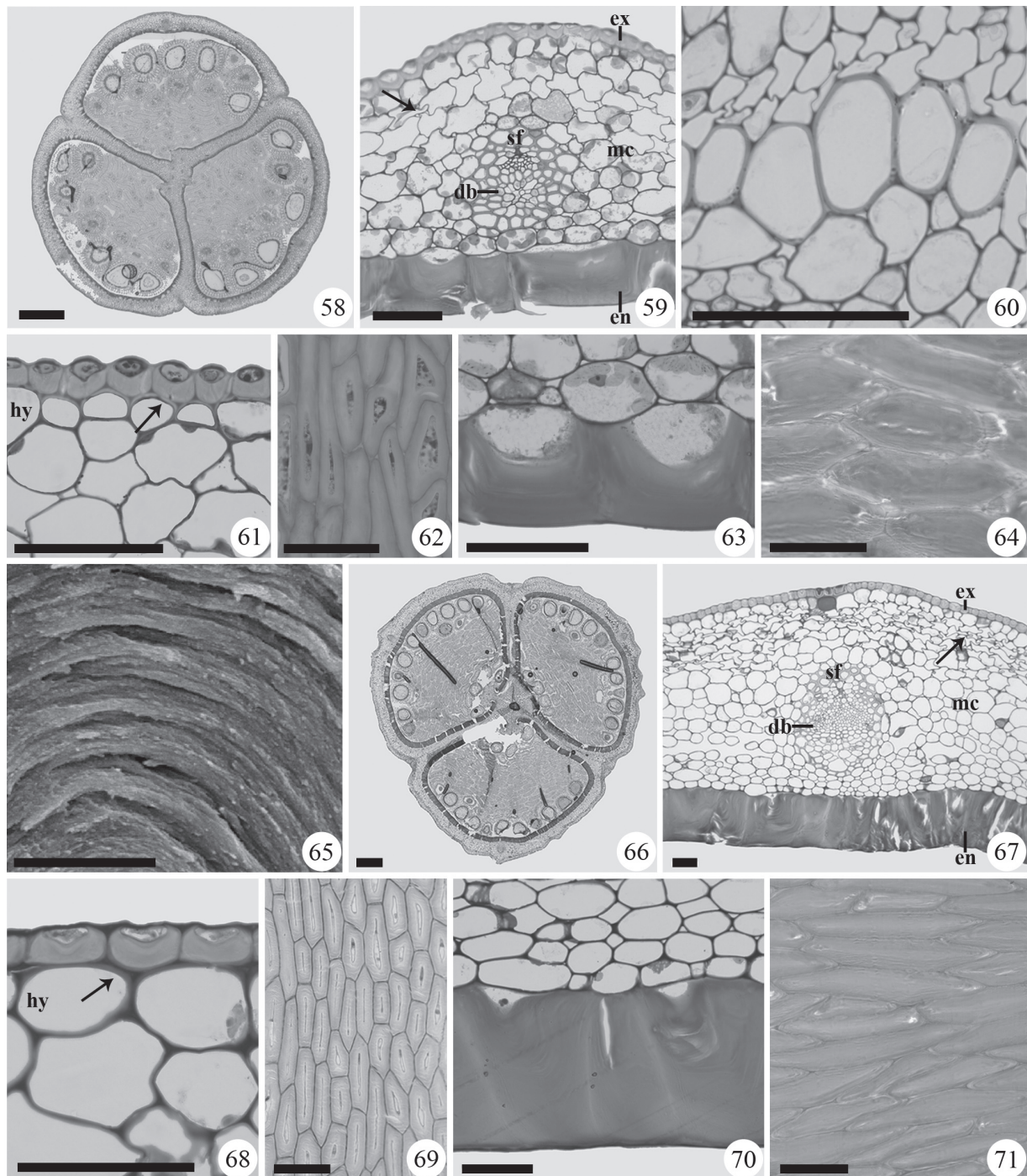
Figures 27-43. Ovary and developing fruit of Pitcairnioideae and Tillandsioideae, mostly in transverse section. 27-28. General view of ovary of *D. maritima* and *T. aeranthis*, respectively. 29. Ovary wall of *D. maritima*. 30. Six dehiscence lines of *D. maritima*. 31. Fusion region between two carpels of *T. aeranthis*, showing one of the dehiscence lines. 32. Obturator of *T. aeranthis*. 33. Developing ovary wall of *V. carinata*, with cell divisions (arrow). 34. General view of the developing fruit of *D. maritima*. 35-36. Wall of developing fruit of *D. maritima* and *T. aeranthis*, respectively. 37. Exocarp of *D. maritima*, with thick outer periclinal wall (arrow) and mucilaginous content, present also in some mesocarp cells (broad arrow). 38. Stoma of *D. maritima*. 39-40. Stomata of *D. maritima*, in SEM. 41. Obturator and idioblast in the placenta (arrow) of *D. maritima*. 42. Striate surface of the fruit of *P. flammea*, in SEM. 43. Striate surface (arrow) of *P. flammea*. db: dorsal vascular bundle, dl: dehiscence line, en: endocarp, ex: exocarp, gc: guard cell, ie: inner epidermis, mc: mesocarp, me: mesophyll, ob: obturator, oe: outer epidermis, pl: placenta, sc: subsidiary cell. Bars= 10 μ m (Fig. 39, 42); 50 μ m (Fig. 29-33, 35-38, 40-41, 43); 100 μ m (Fig. 27); 200 μ m (Fig. 28, 34).



Figures 44-57. Mature fruit of Pitcairnioideae, mostly in transverse section. 44-50. *D. maritima*. 44. General view of mature fruit. 45. Fruit wall, showing thickening of hypodermis cell wall (arrow) and dehiscence zone (broad arrow). 46. Dehiscence lines wrapped in fibers in the central region of the fruit. 47. Exocarp. 48. Exocarp, in paradermal section. 49. Endocarp. 50. Endocarp, in paradermal section. 51-57. *P. flammaea*. 51. General view of the mature fruit. 52. Fruit wall. 53. Dorsal vascular bundle. 54. Exocarp. 55. Exocarp, in paradermal section. 56. Endocarp. 57. Endocarp, in paradermal section. db: dorsal vascular bundle, dl: dehiscence line, en: endocarp, ex: exocarp, fb: fibers, hy: hypodermis, mc: mesocarp, se: bundle sheath extension, sf: bundle sheath fibers. Bars= 50 μ m (Fig. 45-50, 52-57); 400 μ m (Fig. 44, 51).

fusion is superficial or not, distinguishing the tissue limit between carpels. In this study, the terms “false” and “true” have a morphological connotation of tissue distinction between carpels, and should not be confounded with the functional significance of the syncarpy studied by Carr & Carr (1961), which distinguishes the “pseudosyncarpic”

gynoecium from the “eusyncarpic” one as regards the fertilization process. The study performed by Sajo *et al.* (2004a) pointed to the degree of fusion of the carpels only as total or partial, finding that the representatives of Brocchinioideae, Tillandsioideae and Bromelioideae present a totally syncarpic gynoecium and those of



Figures 58-71. Mature fruit of Tillandsioideae, mostly in transverse section. 58-65. *T. aeranthis*. 58. General view of mature fruit. 59. Fruit wall, showing the compressed cell layers (arrow). 60. Fibers. 61. Exocarp and hypodermis with subtle cell wall thickening (arrow). 62. Exocarp, in paradermal section. 63. Endocarp. 64. Endocarp, in paradermal section. 65. Cell wall lamellation of the endocarp, in SEM. 66-71. *V. carinata*. 66. General view of mature fruit. 67. Fruit wall, with compressed cell layers (arrow). 68. Exocarp and hypodermis, the latter with unequal cell wall thickening (arrow). 69. Exocarp, in paradermal section. 70. Endocarp. 71. Endocarp, in paradermal section. db: dorsal vascular bundle, en: endocarp, ex: exocarp, hy: hypodermis, mc: mesocarp, sf: bundle sheath fibers. Bars= 5 μ m (Fig. 65); 50 μ m (Fig. 59-64, 67-71); 400 μ m (Fig. 58, 66).

Pitcairnioideae and Puyoideae a partially apocarpic one, just as had been identified for *Puya spathacea* (Puyoideae), as partially syncarpic, by Kulkarni & Pai (1982).

In this study, the species presented different degrees of carpel fusion, typical of each subfamily, which agrees with the data of Sajo *et al.* (2004a), but adds important information

regarding the identification of the following types of syncarpy: false and total, in Tillandsioideae; false and partial, in Pitcairnioideae; true and total, in Bromelioideae. Thus, the syncarpy character is highly relevant for group taxonomy in delimiting the subfamilies, so that, considering the new phylogeny of the family (Givnish *et al.* 2007), its detailed

Table 1. Comparison of morphoanatomical characters involving the ovary and mature fruit among the six species studied. DE: dehiscence, EN: endocarp, EX: exocarp, MC: mesocarp, VA: vascularization, (t): in the transverse direction.

Subfamily	Bromelioideae		Pitcairnioideae		Tillandsioideae		
Character/Species	<i>Aechmea calyculata</i> (E. Morren) Baker	<i>Billbergia nutans</i> H. Wendl. ex Regel	<i>Dyckia maritima</i> Baker	<i>Pitcairnia flammea</i> Lindl.	<i>Tillandsia aeranthos</i> (Loisel.) L. B. Sm.	<i>Vriesea carinata</i> Wawra	
Ovary							
Position	Inferior	Inferior	Superior	Semi-inferior	Superior	Superior	
Sincarpy	Total and True	Total and True	Partial and False	Partial and False	Total and False	Total and False	
Outer epidermis	Unistratified	Unistratified	Unistratified	Unistratified	Unistratified	Unistratified	
N° strata	18-22	18-22	6-8	8-10	6-8	12-14	
Mesophyll	Idioblasts with raphides	Present (dorsal and lateral regions)	Present (all regions)	Present (lateral and ventral regions)	Present (lateral and dorsal regions)	Present (ventral region)	Absent
Inner epidermis	Unistratified	Unistratified	Unistratified	Unistratified	Unistratified	Unistratified	
Vascular bundles/Carpel	Many (about 15)	Many (about 25)	Three	Three	Three	Three	
Cell shape	Elongated	Elongated	Elongated	Elongated	Very elongated	Very elongated	
Obturator	N° strata	One	One	One	One	One to two	One to two
Fruit							
Type	Berry	Berry	Capsule	Capsule	Capsule	Capsule	
Shape	Elipsoid	Ovaloid	Ovaloid	Elongated and Conical	Elongated and Cylindrical	Elongated and Obovoid	
Color	Dark-purple	Green	Brown	Dark-green	Dark-green	Dark-green	
Length (cm)	0,9	1,5	0,5	0,9	2	3	
Cell type and shape	Sclereids longitudinally elongated	Cells longitudinally elongated	Macrosclereids radially elongated (t)	Cells longitudinally elongated	Sclereids longitudinally elongated	Sclereids longitudinally elongated	
Cell wall	U-shaped	Uniform	Uniform	Thick outer periclinal wall	U-shaped	U-shaped	
EX	Stomata	Present, elevated in relation to the surface	Present, at the same level of the surface	Present, elevated in relation to the surface	Present, elevated in relation to the surface	Absent	Absent
Trichomes	Present	Absent	Absent	Absent	Absent	Absent	
Surface	Smooth	Smooth	Smooth	Striate	Smooth	Smooth	
N° strata	18-22	18-22	8-10	9-11	8-10	14-16	
Hypodermis	2-3 cell layers	2-3 cell layers	One cell layer	Absent	One cell layer	One cell layer	
MC	Ventral region	Parenchymatous cells	Parenchymatous cells	Fibers in great quantity, with very thick cell walls	Fibers in great quantity, with very thick cell walls	Fibers in little quantity, with not very thick cell walls	Fibers in great quantity, with very thick cell walls
VA	Dorsal vascular bundle	Bundle sheath fibers	Bundle sheath fibers	Bundle sheath fibers, with sheath extensions	Bundle sheath fibers, with sheath extension towards the endocarp	Bundle sheath fibers	Bundle sheath fibers
EN	Cell type and shape	Cells longitudinally elongated	Cells longitudinally elongated	Sclereids tangentially elongated (t)	Macrosclereids radially and tangentially elongated (t)	Macrosclereids radially and tangentially elongated (t)	Macrosclereids radially and tangentially elongated (t)
Cell wall	Thick outer periclinal wall	Thick outer periclinal wall	Uniform	U-shaped	U-shaped	U-shaped	
Type	--	--	Biscidal	Septicidal	Septicidal	Septicidal	
N° valves	--	--	Six	Three	Three	Three	
DE	Disposition of valves	--	--	Erect	Erect, joined at their apexes	Folded back	Folded back

verification may help understand the evolutionary process undergone by the reproductive structures.

The obturator is a type of transmitting tissue which is differentiated in the carpel margins and is closely associated with the micropyle of the ovules (Tilton & Horner 1980). Sajo *et al.* (2004b) cited the presence of this tissue in the placental region, but did not describe it. In this study, the morphology of the obturator was verified in the family, and in Bromelioideae and Pitcairnioideae the cells are not very elongated, with a dome-shaped, outer periclinal face, and in Tillandsioideae, the cells are very elongated, forming uni- or bistratified tissue. In the six species studied, the development of cell wall labyrinth was observed in the outer periclinal face of the cells of this tissue. These descriptions and comparisons are completely new for Bromeliaceae, showing the common occurrence of this tissue in its different taxa, and the existence of morphological variability of the character.

The definition of pericarp layers varies according to the author's interpretation. Roth (1977) says that the delimitation of the exocarp, mesocarp and endocarp can be *lato* or *stricto sensu*. In *lato sensu*, exocarp and endocarp can be composed by their respective epidermises and subepidermal tissues, while in *stricto sensu*, both layers originate only from their epidermises. In this study a *stricto sensu* definition was adopted, since it represents the separation of the tissues from the point of view of their origin.

In this study the fruit of *Aechmea calyculata* and *Billbergia nutans* was classified as a berry, which consists of an indehiscent fruit with a pericarp that is not differentiated internally by a rigid endocarp, according to the concept of Spjut (1994). Some authors classify the fleshy fruit originating from an inferior ovary (sometimes superior, with hypanthium) as pomaceous fruit, which are divided into specific subtypes of families or genera (Hertel 1959; Souza 2006). Considering that the fruits of the species analyzed here, as well as all Bromelioideae, originate in the inferior ovary, and that, in the category of the pomaceous fruits, they cannot be classified within their subtypes, it would be plausible to create a subtype that is representative of these fruits. For this, however, it would be necessary to perform the anatomical study of a wide number of species which would represent that particular subfamily more completely. Therefore, this study follows the traditional classification for Bromelioideae fruits, considered berries.

The dry fruit of the remaining species was determined as being of the capsule type, i.e., a simple fruit, dry or fleshy, from a superior or inferior ovary, bicarpellary to pluricarpellary, pluri-ovulate and dehiscent through longitudinal slits (Souza 2006). The capsules are classified based on their dehiscence pattern, which is defined as the exposure or release of seeds through a naturally produced opening in the pericarp (Spjut 1994).

The U-shaped cell wall thickenings, a marked characteristic in the leaf structure of Bromeliaceae (Braga 1977; Aoyama & Sajo 2003; Proença & Sajo 2004; Proença

& Sajo 2007), result from the thickening of the inner periclinal wall and of both the anticlinal walls, showing conspicuous stratification, and they have already been recorded in cells of the epicarp or endocarp of species of Asteraceae, Caryophyllaceae, Lamiaceae, Ranunculaceae, Boraginaceae, Valerianaceae and Plumbaginaceae, all eudicotyledons (Roth 1977). This pattern of thickening was detected in the exocarp and/or endocarp of four of the species examined in this study, showing that it is not exclusive to eudicotyledon fruits.

A well developed hypodermis, adjacent to the exocarp, is characteristic of many fruits, including most berries already studied (Roth 1977), and it can be observed in berries of *Aechmea calyculata* and *Billbergia nutans*, which presented 2-3 cell layers in their hypodermis. *Dyckia maritima*, *Tillandsia aeranthos* and *Vriesea carinata* also presented a hypodermis in their fruits, but it was composed only of a single inconspicuous cell layer, and the cell wall thickenings were limited to the outer periclinal walls.

Different forms of sclereids occur most commonly in the fleshy tissue of berries and drupes, while fibers prevail in the endocarp of drupes, in nuts and in capsules, and play an important role in the dehiscence mechanism of the latter (Roth 1977). This pattern was also identified in the fruits of Bromeliaceae, with the presence of sclereids in the mesocarp of berries and fibers in the mesocarp of capsules.

In the species with capsular fruits, besides the fibers in the mesocarp, sclereids occur in the endocarp, and sometimes in the exocarp. These sclereids, characterized as such by their shape, differential thickening of the cell wall and type of pit, are of different sizes and shapes; some of them are not very elongated, and others are very elongated, similar to fibers in a paradermal section, as in the endocarp of *Pitcairnia flammea*. In this case, the macrosclereids can be considered fibriform.

The stomata are characterized, in the vegetative organs, by guard cells that are usually located below the epidermal surface in the genera *Aechmea* and *Billbergia* and elevated in the genus *Dyckia* (Tomlinson 1969). Other evaluations confirmed the patterns of *Billbergia* and *Dyckia* (Proença & Sajo 2007), but differed concerning the genus *Aechmea*, revealing stomata located at the same level (Aoyama & Sajo 2003) or at a level lower than that of the epidermal cells (Proença & Sajo 2004). In this study, it was found that stomata are elevated in relation to the surface in *D. maritima*, corroborating the data from the literature for the genus; and elevated for *A. calyculata*, and, at the same level, for *B. nutans*, which goes against the existing data for the genera. In all species, the stomata are located in individual furrows, as described by Proença & Sajo (2007) for some species, and they have guard cells above the under-arching subsidiary cells, as found by Tomlinson (1969). The orientation of the stomata in the leaves of monocotyledons is often parallel to the longer axis of the organ (Croxdale 1998), a pattern which coincides with the fruits of all species analyzed here.

According to Tomlinson (1969), peltate scales similar

to those present in the leaves may occur in inflorescence axes and in reproductive organs. In relation to structure, in Bromelioideae, these trichomes normally have two basal cells and a shield never organized into distinct disc and wing, and in the genus *Aechmea* there are 3-4 or more stalk cells. According to Braga (1977), the peltate scales of *Aechmea* have a rounded shield, regular or irregular. In this study, the fruit of *A. calyculata* presented peltate scales that agreed with the descriptions above, except for their asymmetrical and extremely irregular shield – very similar to the starred scales common to the genera *Cottendorfia* and *Navia* (Robinson 1969; Tomlinson 1969). In this sense, Strehl (1983) remarks that there is very great variation of scale morphology in Bromelioideae, and there are forms similar to those of the Pitcairnioideae and Tillandsioideae.

According to Benzing (2000), besides the known functions of water and nutrient absorption from the atmosphere, reflection of solar radiation and reduction of transpiration, the peltate trichomes can also attract dispersers, due to the formation of dense indumenta which reflect the dim light from the fleshy fruits. Roth (1977) ascribes to trichomes in fruits the roles of mechanical protection and reducing transpiration, when lost at fruit maturity, or of facilitating dispersal by wind or by animals, when they persist in the mature fruit. Considering the permanence of the peltate scales until the complete development of the fruit and the dispersal mechanism of subfamily Bromelioideae – which includes *Aechmea calyculata* – determined as zoochory, possibly the trichomes of this species are related to the attraction of dispersers. For *Aechmea*, there are no records of chiropterachory, which reduces the probability of the reflection of dim light acting as an attractant to the disperser. In this way, if the peltate scales are morphological adaptations to the disperser animal, the mechanism by which attraction truly occurs is not known.

Secondary sculpture of the fruit surface, which refers to the micro-ornamentation of the cuticle, is striate in *Pitcairnia flammea* and smooth in the rest of the species. In a taxonomic context, the cuticular sculptures can constitute excellent diagnostic characters, but their significance for delimitation of categories above the species level is very limited. As an adaptation to the environment, it is assumed that the surface area augmented by the sculpture increases the energy exchange with the colder surrounding air, controlling the temperature of the surface under insolation (Barthlott 1981). Considering that *P. flammea* is a heliophilous species (Reitz 1983) and that the respective genus does not have the CAM character (Crayn *et al.* 2004), typical of many bromeliads from arid environments, it is possible that the secondary sculpture observed helps to control temperature.

According to Fahn & Zohary (1955), two factors are necessary for dehiscence: the presence of crossed sclerenchymatous tissues and/or crossed cellulose micelles; the presence of a separation tissue. The morphology of all capsules studied confirmed the presence of these structures

through the occurrence of the dehiscence lines and fibers and sclereids at different orientations.

In loculicidal capsules, the mechanical layers responsible for fruit opening generally reside in the region of the endocarp, the mesocarp, or in both, as shown in representatives of Melianthaceae (Doweld 2001), Pedaliaceae (Day 2000), Meliaceae (Pinto *et al.* 2003), Bignoniaceae (Souza *et al.* 2005) and Malvaceae (Souza 2006). In *Dyckia maritima*, on the contrary, the main mechanical tissues are located in the exocarp and in the mesocarp. This differentiated disposition of the tissues can be the result of the combined occurrence of loculicidal dehiscence with the septicial.

In this study, the term septicial was used according to authors Spjut (1994) and Barroso *et al.* (1999), which includes openings in two distinct regions (septa and ventral sutures), since it was considered more appropriate to adopt the concept of dehiscence from the ecological point of view, which presupposes the opening of the locule followed by exposure of the seeds. However, from this point of view, the term septicial, in its etymology (septi = septa; cidal = opening), does not reflect its real concept completely.

As for the structure of the septicial capsules, in *Prestonia coalita* (Apocynaceae) Gomes (2008) observed the presence of the sclerenchymatous exocarp with inverted U-shaped cell walls, mesocarp with a ring of longitudinal fibers and endocarp with groups of longitudinal fibers adjacent to the dehiscence zone. This structural pattern shares similarities with the septicial capsules of Bromeliaceae described in this study, which have an exocarp and endocarp, or only the endocarp, with U-shaped cell wall thickening and longitudinal fibers close to the dehiscence lines.

Morra *et al.* (2002) found that the septicial capsule of *Tillandsia tricholepis* presents an exocarp with thin cell walls, mesocarp with 2-3 cell layers and endocarp with cells similar to fibrosclereids, with thickened and lignified anticlinal walls and inner periclinal wall. In this study, the mature pericarp of *Tillandsia aeranthis* was characterized as having an exocarp with unequally thickened cell walls, in a U-shape, a mesocarp with 8-10 cell layers and endocarp constituted of macrosclereids, also with U-shaped cell walls. In this way, there is a clear interspecific variation of characters relative to the mature fruit, and this type of comparison and finding have never occurred before for the family.

In a phylogenetic context, dry and dehiscent fruits are considered to be the most primitive, drupes and berries originate from capsules due to the loss of dehiscence, and the ventricidal and septicial types of dehiscence are the most basal among all the existing ones (Roth 1977). In the recent phylogeny performed for Bromeliaceae, Tillandsioideae diverges earlier, with the later rise of Pitcairnioideae and, finally, Bromelioideae (Givnish *et al.* 2007). Considering the traditional morphological characterization of the fruits of Bromeliaceae (Smith & Downs 1974), the morphoanatomical detailing of this study proved that the capsules of *Tillandsia aeranthis* and *Vriesea*

carinata (Tillandsioideae) are septicidal, the capsule of *Pitcairnia flammea* (Pitcairnioideae) is septicidal, but with a differentiated shape and opening from the previous ones, and that of *Dyckia maritima* (Pitcairnioideae) is biscidal. These data corroborate the evolutionary considerations mentioned previously, since Tillandsioideae presents traits considered basal, Pitcairnioideae shows new traits, such as the loculicidal dehiscence, besides the septicidal one, and Bromelioideae presents the fruit structure as a berry, derived and differentiated from the previous ones (capsules).

As well as Tillandsioideae and Pitcairnioideae, all the other subfamilies that were not analyzed in this study present fruit of the capsule type, which probably is a plesiomorphy of the family, taking into account a parsimonious view of the types of fruits, in the light of the phylogeny of Givnish *et al.* (2007), which positions Bromelioideae (with baccate fruits) as the latest-divergent group.

Considering the issues presented, this study shows that the probable evolution of the fruits from dehiscent capsules to indehiscent berries in Bromeliaceae occurred through the reduction of sclerenchymatous strata. Of the three subfamilies analyzed, Tillandsioideae presented capsules with conspicuous sclerenchymatous strata in the exocarp, endocarp and mesocarp, Pitcairnioideae presented capsules with these strata in the exocarp or endocarp and in the mesocarp, and finally, Bromelioideae presented berries with rigid strata in the exocarp and hypodermis. Furthermore, the two species of Bromelioideae showed the existence of cell rows similar to the dehiscence lines of the other species, which certainly constitute relicts of the dehiscence apparatus. This tendency towards the reduction of the sclerenchymatous strata, leading to fruit indehiscence, was also shown in Fabaceae in the study by Fahn & Zohary (1955).

Based on the morphological and anatomical characters of this study, the species that differed most among themselves within the same subfamily were *D. maritima* and *P. flammea*. This reflects the current taxonomic reviews which position the genera *Dyckia* and *Pitcairnia* in two distinct tribes – Dyckieae and Pitcairnieae, respectively (Robinson & Taylor 1999). The similarity between *A. calyculata* and *B. nutans* is appropriate to the phylogeny of Faria *et al.* (2004), in which the clade where the former species is inserted is positioned as the sister group of the clade which includes *Billbergia*. On the other hand, the great similarity between *T. aeranthos* and *V. carinata* agrees with the close phylogenetic relationship between the respective genera, inserted in a same clade within Tillandsioideae (Givnish *et al.* 2007). Among the subfamilies, the morphological approach of Tillandsioideae and Pitcairnioideae, in which the capsular fruit character is retained, as already discussed, is clearly perceived.

In relation to the chemical compounds, there are many raphides in all parts of the plant in Bromeliaceae (Tomlinson 1969) and they occur in many idioblasts in the infrutescence of *Ananas comosus*, in the carpellary and non-carpellary tissues (Okimoto 1948). In this study, the calcium oxalate

raphides occur in the ovary, non-carpellary tissues and/or fruits of all species, except in *V. carinata*, expressing distribution patterns differentiated according to species. In this case, it is believed that the presence of raphides is related mainly to the calcium storage function which can be used for the ontogenesis of the fruit itself or redirected to other structures such as the obturator, ovule and seed, considering the association of raphides with the placenta in some species and their decline or disappearance in the mature fruit. The starch grains are easily identified in the carpellary and non-carpellary tissues of *Ananas comosus*, and they become less conspicuous as the cells expand, and disappear when the fruit matures (Okimoto 1948; Smith & Harris 1995), a pattern which is also observed in all species analyzed here. The silica bodies, detected in the exocarp of *Aechmea calyculata* in this study, are very common in Bromeliaceae, and almost invariably occur as spherical bodies in the epidermis of leaves and stems (Tomlinson 1969).

The mucilages, complex polymers of high molecular weight, may serve as a food reserve or for water retention in general cases (Fahn 1979). In *Ziziphus* species (Rhamnaceae), it was found that mucilage seems not to benefit water relations in the tissues, and may be a significant source of carbohydrates and solutes for survival during dry periods (Clifford *et al.* 2002). *Dyckia maritima* is a xerophyte that contains mucilage in the cells around the stomata of the developing fruit, which would point to a role in water retention. However, *Dyckia* presents the CAM character (Crayn *et al.* 2004), which is already an efficient strategy against desiccation. Thus, it is assumed that it would not be necessary to have another mechanism against water loss, and mucilage in *D. maritima* may play a reserve role, as described for *Ziziphus* spp.

Based on the morphoanatomical description of fruit ontogenesis, the present study enabled the identification of characters that are important to distinguish subfamilies and genera, and, with the help of literature, to delimit species. The following characters of the ovary and fruit stand out as useful in the taxonomy of Bromeliaceae: type of syncarpy; position of the ovary; shape of cells and number of cell strata of the obturator; type, shape, size and color of the fruit; shape, size and cell wall thickening of the exocarp and endocarp; number of cell layers of the mesocarp; quantity and cell wall thickening of the fibers that border the dehiscence lines; number of vascular bundles; presence of sheath extension in the vascular bundles; presence of hypodermis, and also the number of cell layers, cell shape and cell wall thickening; presence of trichomes in the exocarp; presence of stomata in the exocarp; type of dehiscence; disposition of the valves.

As general conclusions of the study, it is emphasized that fruits are considered excellent tools to delimit mainly the genera and subfamilies of Bromeliaceae. The anatomy of the dehiscent fruit is closely correlated with the type of dehiscence, since capsules with the same opening mechanisms proved more similar to each other than capsules

of the same taxonomic level. The presence of characters common to the family was detected, such as calcium oxalate raphides, silica bodies, U-shaped cell wall thickenings, guard cells superposed on the under-arching subsidiary cells and peltate scales. The U-shaped cell wall thickenings, previously reported only for eudicotyledon fruits, begin to be considered structures also belonging to monocotyledon fruits. And, finally, macrosclereids are considered marked structures of the pericarp of Bromeliaceae capsules.

As for the evolution of the fruits in the family, the characters observed reproduce the information about the phylogeny of fruits in angiosperms, so that the most basal subfamily presents characters treated as basal for fruits and so on; the differences and similarities among the taxa studied reflect the taxonomic reviews and current phylogenies of Bromeliaceae; the evolution of the fruits shows a tendency to indehiscence; and, adding the data of this study to known morphological information, the capsule character is presumably plesiomorphic.

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