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Seed development and its relationship to fruit structure in species of Bromelioideae (Bromeliaceae) with fleshy fruits

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In Bromeliaceae, fruit type and seed morphology have been used to distinguish the subfamilies. We studied seed and fruit development of three species of Bromelioideae (*Aechmea bromeliifolia*, *Billbergia distachia* and *Neoregelia bahiana*) relating seed characters to fruit structure. *Aechmea bromeliifolia* has few ovules per locule inserted within the apical portion of the ovary and the seeds are larger, with a long chalazal appendage, growing towards the fruit base. In *B. distachia* and *N. bahiana*, the ovules are numerous and subapically to centrally inserted in the ovary; the seeds are smaller, with a radial disposition, and the chalazal appendages are short (*B. distachia*) or absent (*N. bahiana*). The chalazal appendages grow during seed development, and thus their presence/length may be related to the number of ovules/seeds per locule and to the disposition of the ovules inside the locules. The fruits are berries, and juiciness is promoted by mesocarp cells and by substances secreted inside the locules by the placental obturator at later stages of fruit development. These fruit and seed features are strategies for zoochory and provide evidence that each species studied is dispersed by a different type of animal.

ADDITIONAL KEYWORDS: chalazal appendage – fruit anatomy – mucilaginous seed coat – secretory obturator – seed anatomy – septal nectary.

INTRODUCTION

Bromeliaceae are widely distributed in the Neotropics (Smith, 1934; Smith & Downs, 1974) and are an early-diverging lineage in Poales (Linder & Rudall, 2005; Givnish *et al.*, 2010, 2018; Bouchenak-Khelladi, Muasya & Linder, 2014). The family emerges sister to Typhaceae, together forming the first-diverging lineage (Bouchenak-Khelladi *et al.*, 2014) or, with Typhaceae and Rapateaceae, as successive lineages sister to the remaining Poales (Givnish *et al.*, 2010, 2018). Bromeliaceae are divided into eight subfamilies, including the late-diverging Bromelioideae (Givnish *et al.*, 2007). In Bromeliaceae, the ovary may be inferior, half-inferior or superior and the fruits are usually dry capsules, with winged or plumose seeds (Smith & Till, 1998; Benzing, 2000). In Bromelioideae, the fruits are berries with a fleshy pericarp, and the seeds have a mucilaginous sarcotesta, with or without appendages (Smith & Downs, 1974, 1979; Smith & Till, 1998; Benzing, 2000).

Although the morphological and anatomical characters of the fruits and seeds are relevant for the taxonomy of Bromeliaceae (Smith & Downs, 1974; Fagundes & Mariath, 2010; Magalhães & Mariath, 2012; Santos-Silva, Mastroberti & Mariath, 2015), there have been few studies on seed and fruit development of Bromelioideae, and these studies were based on small sample sizes, relative to the high species richness of the subfamily and their ecological and ornamental importance (Benzing, 2000).

Sajo, Prychid & Rudall (2004a) studied ovule development in 12 species currently circumscribed in subfamilies Bromelioideae (*Aechmea alba* Mez, *A. conglomerata* Baker, *A. fosteriana* L.B.Sm., *Orthophytum vagans* M.B.Foster and *Quesnelia edmundoi* L.B.Sm.), Brocchinioideae, Pitcairnioideae, Puyoideae and Tillandsioideae, showing that most

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(including *A. conglomerata* and *A. fosteriana*) possess an appendage at the chalazal end, which is variable in shape and size. According to Sajo *et al.* (2004a), the ovule integuments are poorly developed in the species of Bromelioideae and the seed coat is gelatinous. Although the ovules of Bromelioideae have been described as usually lacking chalazal appendages (Smith & Downs, 1974; Smith & Till, 1998; Benzing, 2000), such structures have also been observed in *Aechmea strobilacea* L.B.Sm. and *A. pitcairnioides* Mez (Rauh & Barthlott, 1982), and in *Billbergia nutans* H.H.Wendl. ex Regel (Fagundes & Mariath, 2014). In the last-named species, the length of the chalazal appendage exhibits intraspecific variation, and therefore this character is not useful for taxonomy (Fagundes & Mariath, 2014). The chalazal appendages develop late in the ovule (Fagundes & Mariath, 2014; Kuhn *et al.*, 2016), indicating that they are probably related to functional aspects of the seed.

In Rapateaceae the seeds of some species also have chalazal appendages, which were described as having a dermal origin (Venturelli & Bouman, 1988). Multiple chalazal appendages were observed in *Catopsis* Griseb. (Tillandsioideae) with dermal and subdermal origin (Sajo *et al.*, 2004a; Kuhn *et al.*, 2016). In the remaining Bromeliaceae studied, seeds with an appendage have a single one, which is also dermal and subdermal in origin (Sajo *et al.*, 2004a; Kuhn *et al.*, 2016). The ovules in Bromeliaceae have a two-layered inner integument in most of the species studied, whereas the number of cell layers of the outer integument is variable among the genera (Nogueira *et al.*, 2015; Kuhn *et al.*, 2016), deserving particular attention in future studies of seed ontogeny. Despite the uniform appearance of mature seeds in Bromelioideae, but considering the wide morphological variation in the ovules, we hypothesize that the ontogenetic processes during seed development may be distinct among their species. In addition, the number of ovules and their disposition and the available area inside the ovary locules may be related to the presence or absence and length of seed appendages, modulating the final shape of seeds.

Recently, Thadeo, Hampilos & Stevenson (2015) studied the ontogeny of fleshy fruits in the monocotyledons, including a species of Bromeliaceae [*Aechmea aquilega* (Salisb.) Griseb.; Bromelioideae] and identified three basic pathways for fleshiness based on the anatomy and structure of pericarp: true berries with a uniform pericarp, typical drupes with an endocarp differentiated by the presence of stony pyrenes, and specialized drupes involving mesocarp and endocarp in the differentiation of the stony pyrenes. These different patterns do not follow a single pathway during fruit evolution, indicating that fleshiness appeared multiple times during the evolution of the monocots, probably as a functional convergence (Thadeo *et al.*, 2015).

The fleshy fruits of Bromelioideae are probably dispersed by animals (Benzing, 2000), but only a few ecological studies have evaluated fruit and seed dispersion in the subfamily and how their structure is related to dispersers. In this context, we aimed to analyse comparatively the development of seeds and fruits in three species of Bromelioideae. The taxa were selected based on having different fruit and seed morphology, including seeds with long appendages [*Aechmea bromeliifolia* (Rudge) Baker ex Benth. & Hook.f. var. *albobracteata* Philcox], seeds with short appendages [*Billbergia distachia* (Vell.) Mez] and seeds without appendages [*Neoregelia bahiana* (Ule) L.B.Sm.]. Seed development was related to fruit structure and the features of both seeds and fruits were related to dispersal modes.

MATERIAL AND METHODS

PLANT MATERIAL

Flowers at anthesis and fruits at different developmental stages of *A. bromeliifolia* var. *albobracteata*, *B. distachia* and *N. bahiana* (Fig. 1) were collected from natural populations, fixed in FAA 50 (37% formaldehyde, acetic acid glacial, 50% ethanol, 1:1:18, by vol.; Johansen, 1940) and stored in 50–70% ethanol for analysis using light microscopy, scanning electron microscopy (SEM) and stereomicroscopy. Voucher specimens were deposited at Herbarium Rioclarense (HRCB) of the Instituto de Biociências, Universidade Estadual Paulista. The voucher data and collection sites are detailed in Table 1. The taxa were chosen because of their differences in fruit size and position of the placentae, in addition to the presence/length of the seed chalazal appendages. Such differences are also given in Table 1.

METHODOLOGY

Anatomical studies were performed using flowers at anthesis and fruits at different developmental stages embedded in paraffin. Mature seeds were also embedded in (2-hydroxyethyl)-methacrylate (Leica Historesin Embedding Kit) (Gerrits & Smid, 1983) due to their hardness. Transverse and longitudinal sections 6–10 μm thick were cut using a rotary microtome (Leica, RM2065). The sections obtained from the samples embedded in paraffin were stained with Astrablau-safranin (Bukatsch, 1972), whereas the sections from the samples embedded in historesin were stained with periodic acid-Schiff's reagent (PAS) and toluidine blue (O'Brien, Feder & McCully, 1964; Feder & O'Brien, 1968; Baum, 2008). All the sections

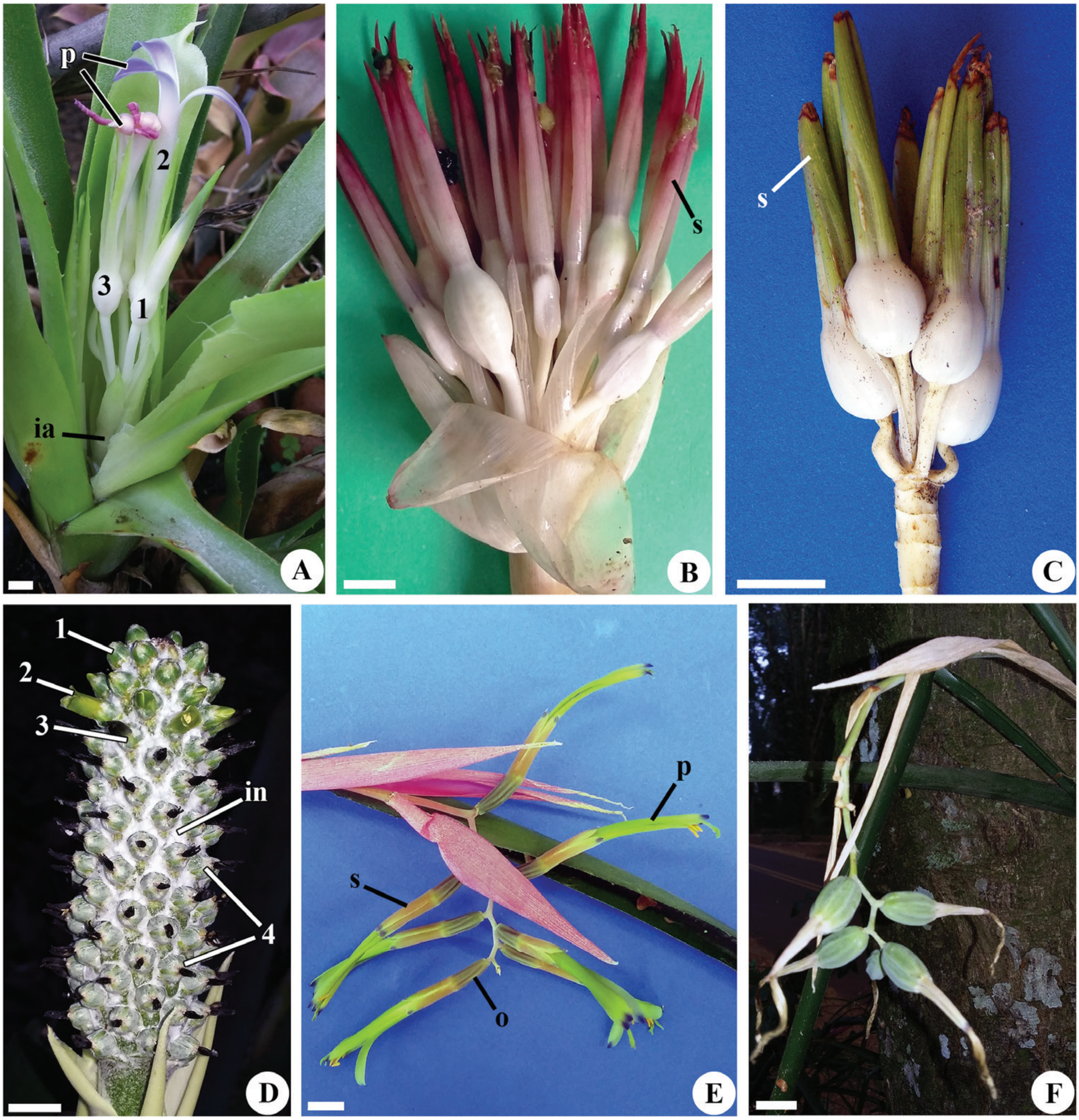

Figure 1. Morphology of flowers and fruits. A–C, *Neoregelia bahiana* (A, C, Mucugê, Bahia; B, Serra do Cipó, Minas Gerais) with flowers at pre-anthesis, anthesis and post-anthesis (A), young fruits at different developmental stages (B), and mature fruits (C). D, *Aechmea bromeliifolia* var. *albobracteata* with flowers and fruits at different developmental stages, from floral buds on the inflorescence apex to mature fruits on the base. E–F, *Billbergia distachia* with flowers at anthesis and post-anthesis (E) and mature fruits (F). 1, flower at pre-anthesis; 2, flower at anthesis; 3, flower at post-anthesis; 4, developing fruits; ia, inflorescence axis; in, indumentum; o, ovary; p, petal; s, sepal. Scale bars: 1 cm.

were mounted on permanent slides using Entellan (Merck). Images were obtained with a microscope equipped with a camera, using the software LAS (Leica Application Suite 4.0.0). Histochemical tests were performed with hydrochloric acid and glacial acetic acid solutions for calcium oxalate detection (Chamberlain, 1932) and phenol for silica compounds (Johansen, 1940).

Table 1. Morphological characters of fruits and seeds, collection sites and herbarium records of the species studied

	Aechmea bromeliifolia var. *albobracteata*	*Billbergia distachia*	*Neoregelia bahiana*
Fruit size (length × width, cm)	1.0 × 0.5	3.5 × 0.8	4.5 × 0.8
Placentation	Axile; in the apical portion of the ovary	Axile; in the subapical–central portion of the ovary	Axile; in the subapical–central portion of the ovary
Seed coat	Mucilaginous, with a long appendage	Mucilaginous, with a short appendage	Mucilaginous, without appendage
Collection site	Cerrado; Rio Claro, SP, Brazil	Cerrado; Rio Claro, SP, Brazil	Campos rupestres; Serra do Cipó, MG and Mucugê, BA, Brazil
Life form	Epiphytic, rupicolous, terricolous	Epiphytic	Rupicolous
Vouchers	*K. R. Silva 90*	*K. R. Silva 91*	*K. R. Silva & K. O. Nardi 75; M. V. Dantas-Queiroz et al. 129*

For SEM (Zeiss, Σigma VP), seeds and fruits were dehydrated in 70% ethanol and immersed in formaldehyde-dimethylacetal for 24 h (Gerstberger & Leins, 1978). After critical-point drying in CO_2 (CPD 030 – Bal-Tec AG, Balzers), the samples were coated with gold (SCD 050 – Bal-Tec AG, Balzers), and the results were documented using the Zeiss SmartSEM software. Images of mature fruits and seeds were taken using a stereomicroscope (Axio, Zoom.V16) equipped with a camera (Axiocam 506) and ZEN 2.1 (blue edition) software.

RESULTS

MORPHOLOGY

Among the species studied, only *N. bahiana* bears a short inflorescence axis (penduncle) nested in the leaf rosette and the inflorescence maturation is centripetal (Fig. 1A). In this species the ovaries and fruits are white (Fig. 1A–C). Individuals collected in the Serra do Cipó, Minas Gerais, bear more fruits, with persistent reddish sepals (Fig. 1B), than those from Mucugê, Bahia, which bear few fruits, with persistent green sepals (Fig. 1C). The inflorescence axis is long, thickened and with congested flowers and fruits in *A. bromeliifolia* (Fig. 1D) and thin with sparse flowers and fruits in *B. distachia* (Fig. 1E, F). In both these species, inflorescence maturation is acropetal (Fig. 1D–F). In *A. bromeliifolia* the fruits, floral bracts and inflorescence axes are covered by a dense and whitish indument (peltate trichomes or scales) (Figs 1D, 2A). *Billbergia distachia* has green fruits with a reflective surface (Fig. 1F), nine conspicuous longitudinal ribs and a sinuous shape in transverse section (Fig. 3A).

OVARY AND OVULE STRUCTURE

In the three studied species, the ovary is inferior (Figs 1A, E, 2A–D, F, H), tricarpellate and trilocular (Figs 2I, 3A, B), with interlocular septal nectaries and ovules inserted on axile placentae (Fig. 3A–C). The placenta is apical in *A. bromeliifolia* (Fig. 2A–C) and subapical to central in *B. distachia* (Fig. 2D, E) and *N. bahiana* (Fig. 2F, H). In *A. bromeliifolia* there are about ten ovules per locule (Figs 2A, 3D) that grow towards the fruit base during seed development (Fig. 2B, C). In the other two species there are more ovules per locule and the ovules are smaller (Figs 2D, E, 3B, C), growing radially during seed development (Fig. 2F–I). The chalazal end of mature ovules and seeds of *N. bahiana* has a rounded shape, without an appendage (Figs 3C, I, 4A). In the other two species the ovules/seeds have a chalazal appendage, which vary in size; they are long-acuminate in *A. bromeliifolia* (Figs 3D, 4D) and short and rounded in *B. distachia* (Figs 3F, H, 4B, C).

OVULE ANATOMY AND SEED DEVELOPMENT

In the three species studied the mature ovules are bitegmic and anatropous with their micropyles facing the placenta (Figs 3D–F, 4A–E). The micropyle is delimited by the inner integument in *A. bromeliifolia* (Figs 3E, 4D) and *B. distachia* (Figs 3F, 4C) or by the inner and outer integuments in *N. bahiana* (Fig. 4A, E). The outer integument has four cell layers in

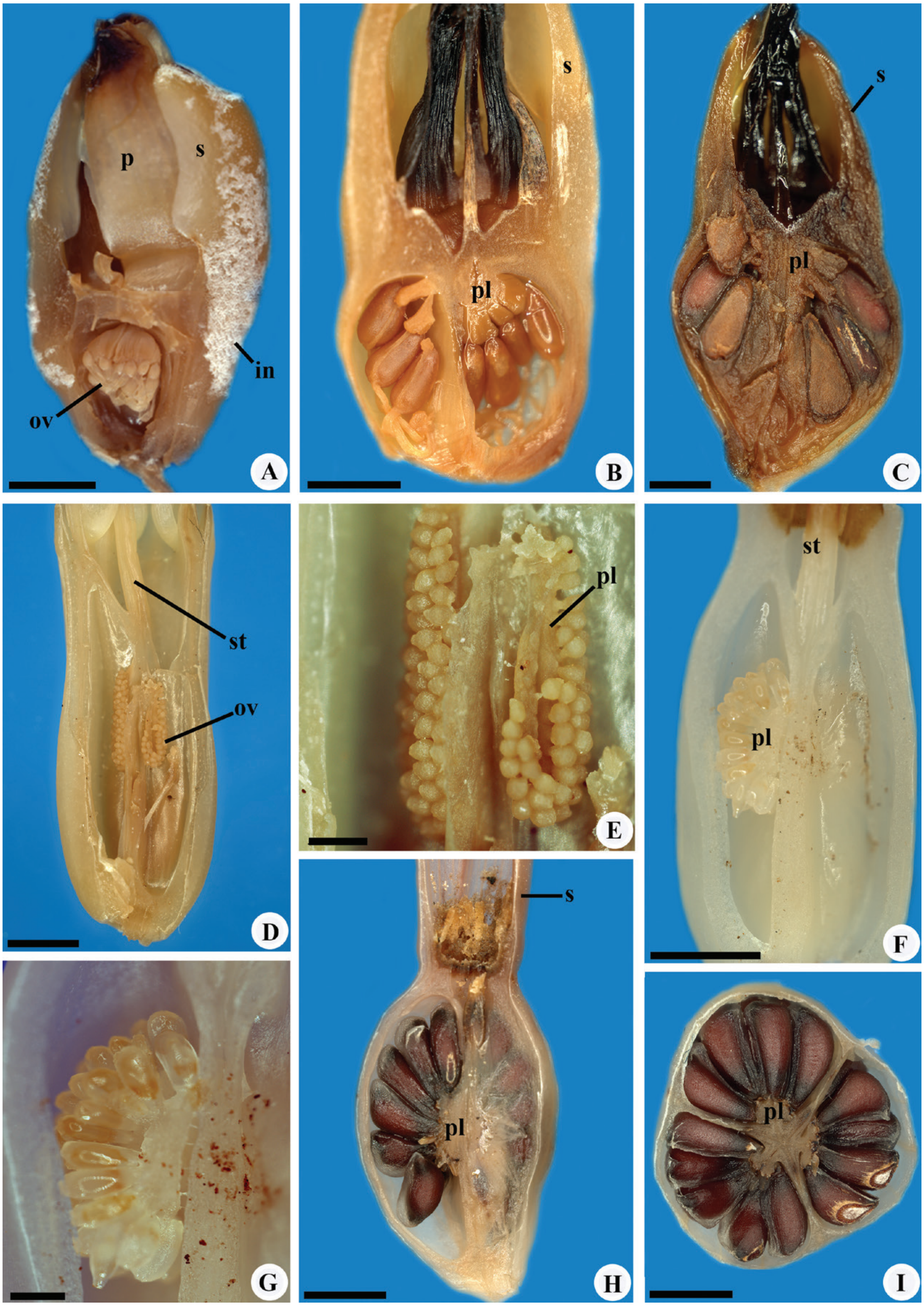

Figure 2. Ovary and fruit structure in longitudinal (A–H) and transverse (I) sections. A–C, *Aechmea bromeliifolia* var. *albobracteata*. D–E, *Billbergia distachia*. F–I, *Neoregelia bahiana*; the samples were stored in 70% ethanol. A, D, general aspect of the mature ovary, in a flower at anthesis. B, F, general aspect of the young fruit. C, H–I, general aspect of the mature fruit. E, G, detail of the ovules and developing seeds, respectively. in, indumentum; ov, ovules; p, petal; pl, placenta; s, sepal; st, style. Scale bars: A–F, I, 2 mm; G–H, 0.5 mm.

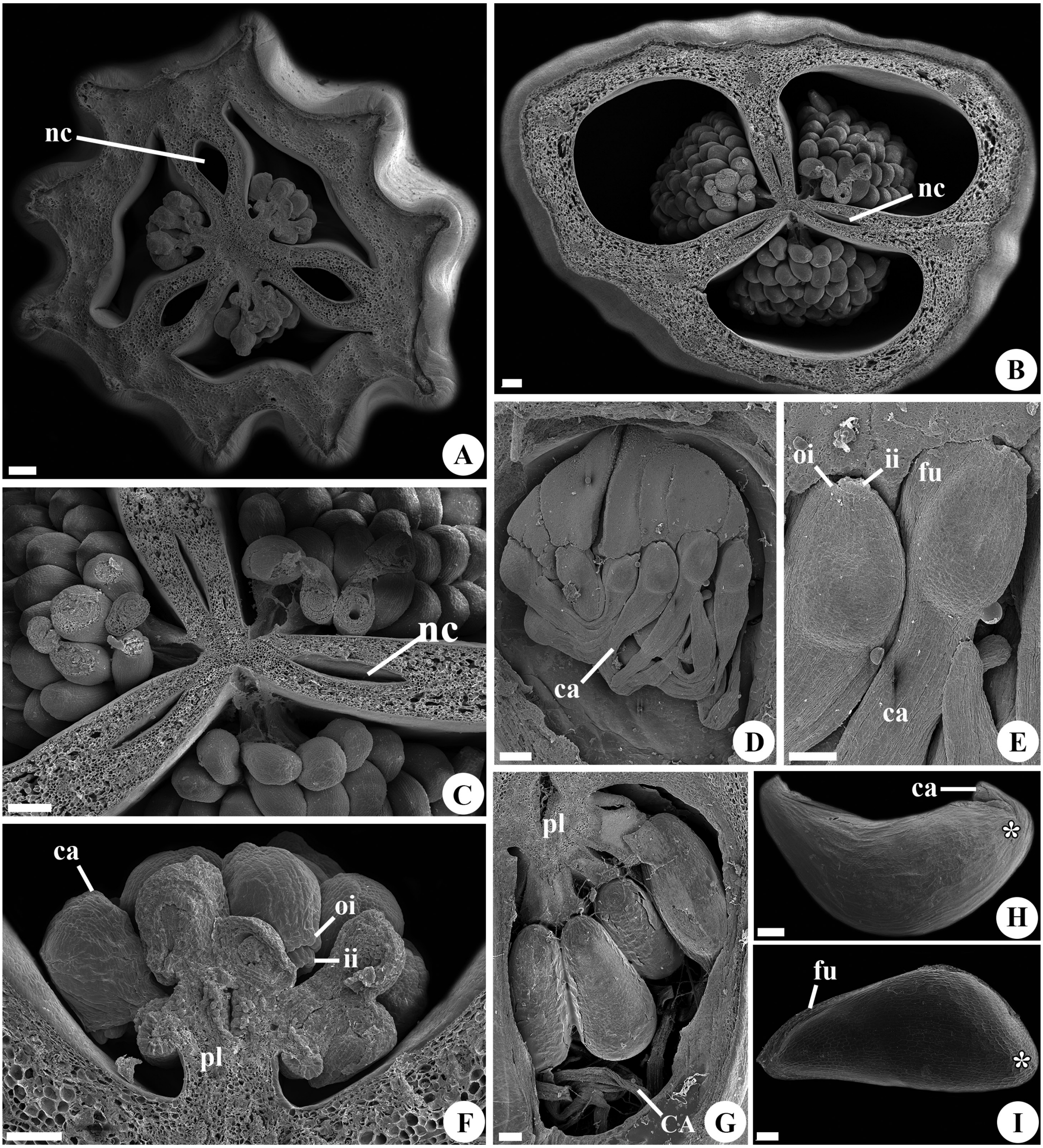

Figure 3. Ovaries in transverse (A–C, F) and longitudinal sections (D–E), fruit in longitudinal section (G) and seed morphology (H–I) under SEM. A, F, H, *Billbergia distachia*. B–C, I, *Neoregelia bahiana*. D–E, G, *Aechmea bromeliifolia* var. *albobracteata*. A–B, general aspect of the ovary, showing the axile placentation and the interlocular nectariferous channels. C, detail of the nectariferous channels and locules with numerous ovules. D–F, detail of the anatropous and bitegmic ovules with chalazal appendages. G, mature seeds with long appendages and inserted apically in the locule. H–I, mature seeds with short appendages or without appendages, respectively. ca, chalazal appendage; fu, funicle; ii, inner integument; nc, nectariferous channels; oi, outer integument; pl, placenta; *, chalazal end. Scale bars: A–D, F–I, 200 µm; E, 100 µm.

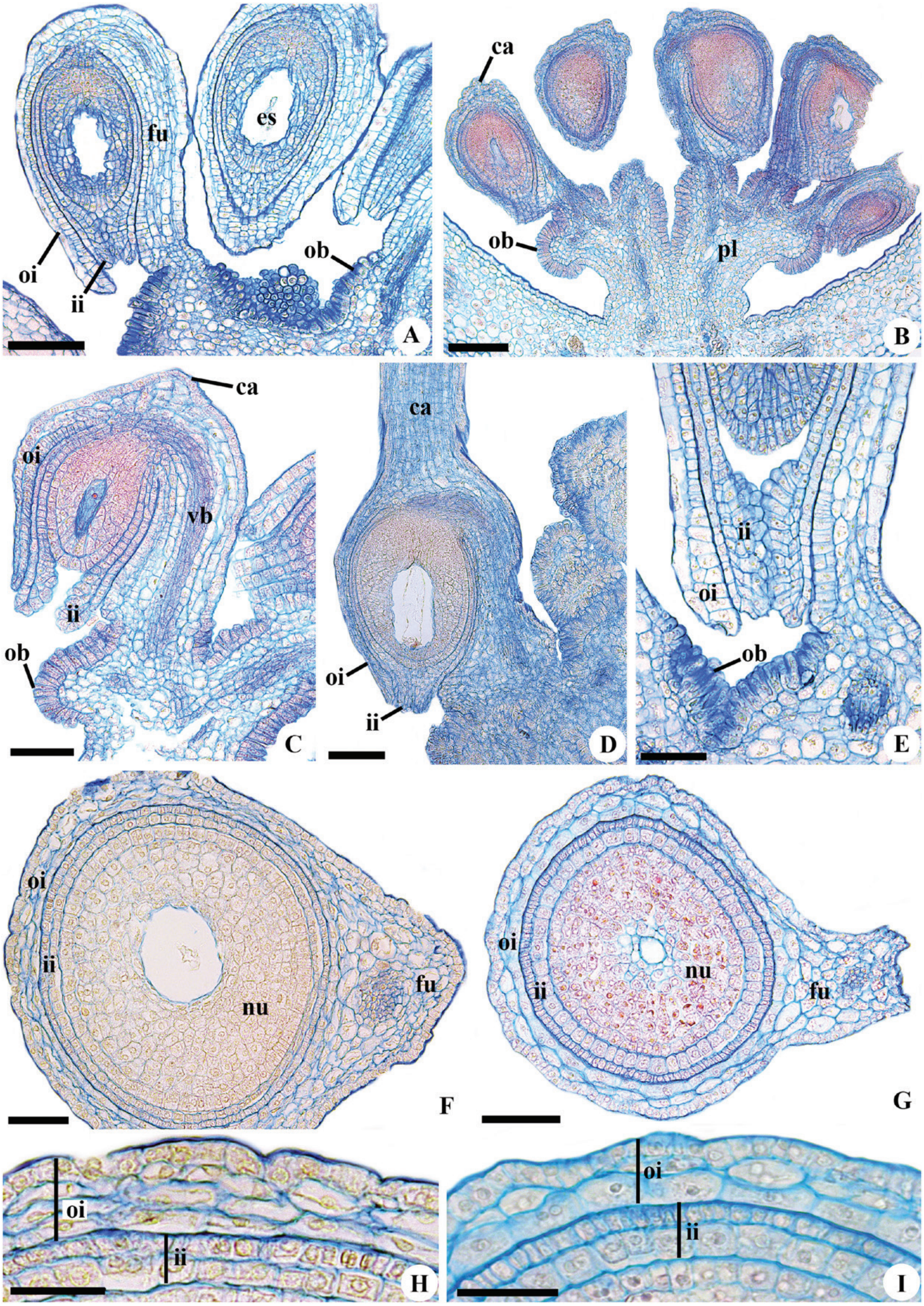

Figure 4. Mature ovules (with mature embryo sac) in median longitudinal (A–E) and transverse sections (F–I) stained with Astrablau-safranin. A, E, *Neoregelia bahiana*. B–C, G–I, *Billbergia distachia*. D, F, H, *Aechmea bromeliifolia* var. *albobracteata*. A–D, F–G, general aspect of the ovules. E, detail of the micropyle. H–I, details showing the number of layers of the integuments. ca, chalazal appendage; es, embryo sac; fu, funicle; ii, inner integument; nu, nucellus; ob, obturator; oi, outer integument; pl, placenta; vb, vascular bundle. Scale bars: A–B, D, 100 µm; C, E–H, 50 µm; I, 25 µm.

A. bromeliifolia (Fig. 4F, H) and two or three cell layers in *B. distachia* (Fig. 4G, I) and *N. bahiana* (Fig. 4E). The inner integument is composed of two cell layers in the three species (Fig. 4F–I), except at the micropylar region where there are three to four layers, the innermost of which is composed of papillose cells (Fig. 4C, E). In *A. bromeliifolia* and *B. distachia*, the appendage is a prolongation of the chalazal tissues, of dermal and subdermal origin (Fig. 4C, D). It consists of several layers of parenchymatous cells in *A. bromeliifolia* (Fig. 4D).

During seed development, the ovules elongate longitudinally, allowing growth of the embryo and the formation of endosperm (Fig. 5A). The seed coat differentiates before embryo development (Fig. 5A), and all cell layers of both outer and inner integuments are persistent, forming the seed coat, which is thus composed of testa and tegmen (Fig. 5C–F). The testa (exotesta, mesotesta and endotesta) and the funicle and the chalazal appendage of *A. bromeliifolia* and *B. distachia* become mucilaginous (Fig. 5D–G), with a positive reaction to PAS reagent indicating the presence of polysaccharides. In transverse section, its cells with cellulosic and thin walls enlarge and elongate periclinally, whereas cells of the tegmen (exotegmen and endotegmen) elongate anticlinally and have thickened walls (Fig. 5D–F). Cells of the exotegmen have slightly thickened walls in *A. bromeliifolia* and *B. distachia* (Fig. 5E), but pronounced thickened anticlinal walls in *N. bahiana* (Fig. 5F, H). Cells of the endotegmen have U-shaped thickened walls in transverse section (Fig. 5F) and store phenolic compounds (Fig. 5F, I) in all three species.

After fertilization, the primary endosperm cell undergoes divisions, forming several nuclei during the early stages of embryo development (Fig. 5B) and the cell walls are formed later. The outermost layer of the endosperm is differentiated into the aleurone layer (Fig. 5D–F, asterisk). The nucellus is completely obliterated during the development of the endosperm and embryo (Fig. 5D–F). The embryo is differentiated into the cotyledon and hypocotyl-radicular axis (Fig. 5C, J) and in mature seeds the embryo is about one-third of the seed length in *N. bahiana*, one-half in *B. distachia* and almost the same as the seed length in *A. bromeliifolia* (Fig. 5C).

OVARY ANATOMY

In the inferior ovary of the three species studied the interlocular septal nectaries form wide channels inside the septa at the fertile zone of the ovary, where the ovules are attached to the placentae (Figs 3A, 6A–D). These channels are lined by secretory cells and extend towards the ovary apex (Fig. 6E, F), releasing the nectar at the style base (Fig. 6G). In the style, three canals are lined by the pollen tube transmitting tissue (Fig. 6G).

The inferior ovary wall is composed of a single-layered epidermis and a multilayered parenchymatous tissue (Figs 6F, 7A, B). The epidermal cells are smaller than the mesophyll cells (Fig. 7A, B) and contain silica bodies in the outer epidermis in *A. bromeliifolia*, which persist in the fruit (Fig. 7C, D). In this species, stomata and scales occur from the apex to middle third of the ovary (Fig. 6A); the scales have an asymmetrical shield with elongated wing, one or two cells in the stalk and two basal cells (Fig. 7F). Stomata are also present in *B. distachia* (Fig. 7G), throughout the ovary and fruit. In *N. bahiana* no stomata or scales were observed (Fig. 7B). In all three species, the inner epidermis has cells with outer periclinal walls slightly thickened by cellulose (Fig. 7J); this wall thickening remains in the fruits. At the placentae, the epidermal cells are elongated and secretory, representing an obturator (Fig. 4A–E). The parenchymatous tissue contains idioblasts with calcium oxalate raphides and cells with starch grains (Fig. 7G, H). The starch grains are conspicuous in cells close to the vascular bundles (Fig. 7H). The parenchymatous cells facing the inner epidermis have larger intercellular spaces than those facing the outer epidermis (Fig. 7A, B). Several vascular bundles occur in the ovary wall (Fig. 6B), more than nine in *A. bromeliifolia* and >21 in *B. distachia* and *N. bahiana*. Compound vascular bundles were observed facing the septae (Figs 6B, 7H).

FRUIT DEVELOPMENT

During fruit development, structural changes occur in the ovary wall cells to form the pericarp, but there is no periclinal cell division, and thus the number of cell layers is maintained (Fig. 7B, E). Growth of pericarp is due to enlargement of its cells.

In *A. bromeliifolia*, cells of the exocarp (derivative of the outermost layer of the inferior ovary) acquires U-shaped wall thickenings in young (Fig. 7C) and mature fruits (Fig. 7D) and, in mature fruits, the cell walls of three or four subepidermal layers are also thickened (Fig. 7D). In this later stage of development, scale cells are obliterated and we can see only their insertion sites (Fig. 7D, arrowhead). In the mature fruits of *B. distachia* and *N. bahiana,* a slight thickening occurs in cell walls of the exocarp and in one or two subepidermal layers (Fig. 7E). In addition, wall thickenings in cells of the vascular bundles sheath also occur during fruit development in the three species, but this thickening is more pronounced in *A. bromeliifolia* (Fig. 7I). As the fruit develops, the raphides decrease, as do the starch grains (Fig. 7D, E).

During fruit development the obturator cells undergo physiological changes, exhibiting a dense cytoplasm in the young and mature fruits (Figs 8A, B, 9B).

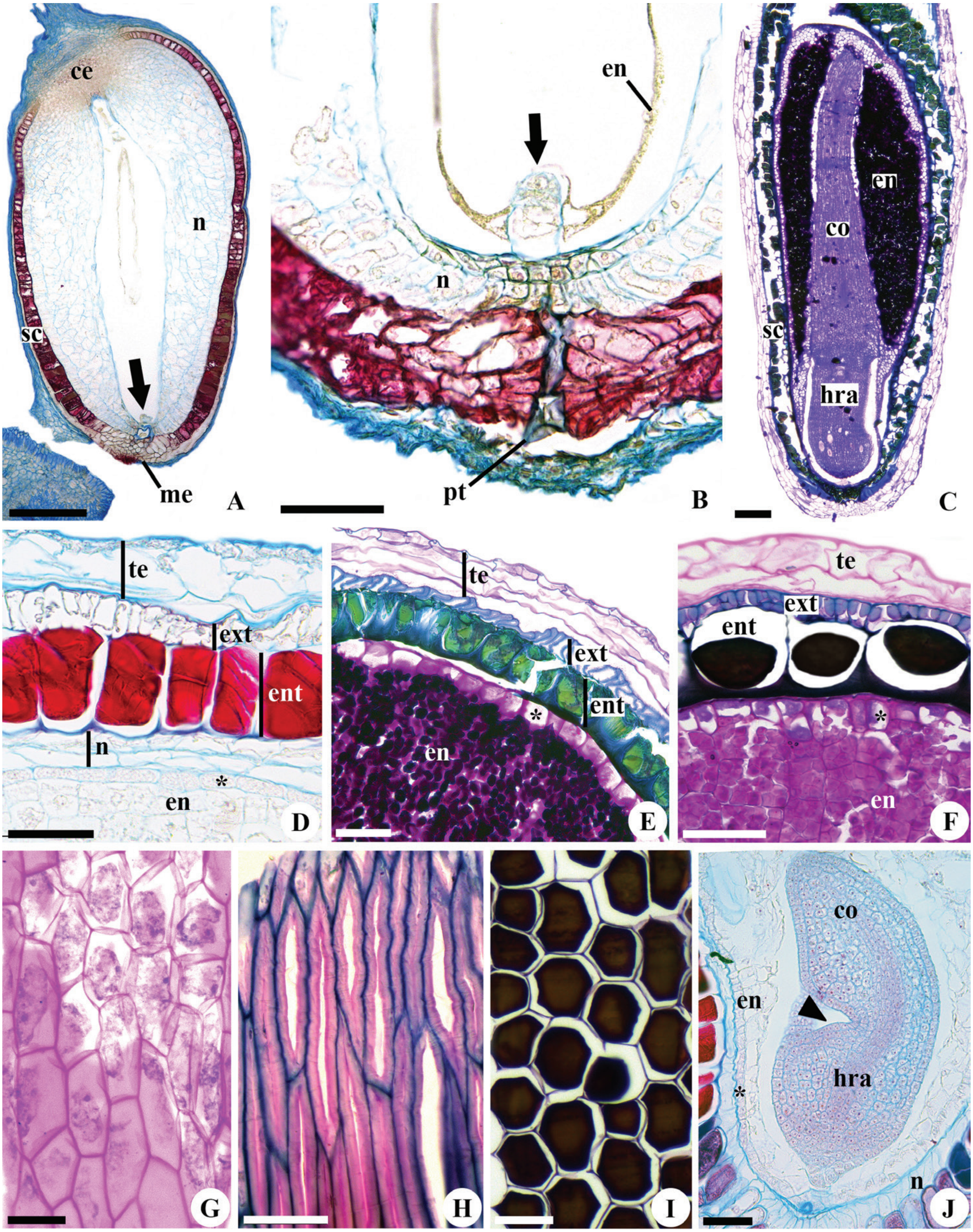

Figure 5. Seeds in longitudinal (A–C, G–J) and transverse sections (D–F) stained with Astrablau-safranin (A–B, D, J) or PAS and toluidine blue (C, E–I). A–C, *Aechmea bromeliifolia* var. *albobracteata*. D–E, J, *Billbergia distachia*. F–I, *Neoregelia*

In later stages of fruit development, these cells secrete substances inside the locules (Figs 8D, 9B, E).

In the septal nectaries, the secretory epidermal cells and adjacent cells undergo wall thickening during fruit development (Figs 8A–D; 9B). This wall thickening increases in the mature fruits (Fig. 9C, E), especially in *A. bromeliifolia* (Fig. 9D). In *N. bahiana*, after pollination the cells underneath the secretory cells of the septal nectaries undergo periclinal divisions and their walls become increasingly lignified (Fig. 9F).

DISCUSSION

Seed development and its relationship to fruit structure

In Bromelioideae, the number and size of ovules are variable. The higher number of ovules in *B. distachia* and *N. bahiana* is related to their smaller size, whereas in *A. bromeliifolia* there are fewer, larger ovules. According to Fagundes & Mariath (2014), the presence of a small number of ovules per carpel is probably a plesiomorphic condition in Bromeliaceae. Because the three species studied here belong to the core bromelioids, a late-diverging clade of Bromelioideae (Schulte, Barfuss & Zizka, 2009), the presence of a few, large ovules in the ovary of *A. bromeliifolia* may be interpreted as a reversal.

The presence/length of the seed chalazal appendages may be also related to the number and size of ovules and their disposition inside the ovary. In *B. distachia* and *N. bahiana*, for example, the numerous and small ovules are subapically to centrally inserted in the ovary and the seed chalazal appendages are short or absent, respectively. This relationship has also been observed in other species of Bromelioideae (Sajo *et al.*, 2004a) and among individuals of different populations of the same species, in which the length of the seed chalazal appendage varied according to the number and size of ovules/seeds, being short to long, as described for *B. nutans* (Fagundes & Mariath, 2014).

In *A. bromeliifolia*, the few ovules are inserted in the apical portion of the ovary; a larger space is available for development of the seeds, which have long-acuminate chalazal appendages that grow towards the fruit base, as observed in other species of *Aechmea* Ruiz & Pav. (Sajo *et al.*, 2004a). It should be emphasized that this relationship applies only to species of Bromelioideae, in which the seeds have a mucilaginous coat and the fruits are fleshy. The same does not occur in species of the other subfamilies, with plumose or flattened seeds and capsular fruits.

In species of *Tillandsia* L. (Tillandsioideae), for example, there are many ovules per locule with distinct chalazal appendages (Sajo *et al.*, 2004a), but these appendages are involved with the flight apparatus. Species of Pitcairnioideae *s.l.* may also exhibit several ovules per locule, but the seeds are flattened, with caudate appendages or without appendages (Smith & Downs, 1974; Varadarajan & Gilmartin, 1988; Sajo *et al.*, 2004a). All these variations indicate distinct ontogenetic processes, and they are relevant for establishing homologies for a better understanding of the evolution of morphological traits of seeds in Bromeliaceae and their relationship to dispersal and the ability to colonize different substrates (Smith, 1934; Benzing, 2000).

Seed coat development in Bromelioideae

Although some studies on the anatomy of seeds of Bromeliaceae have been performed (e.g. Müller, 1895; Szidat, 1922; Smith & Downs, 1974; Varadarajan & Gilmartin, 1988; Smith & Till, 1998; Benzing, 2000; Palací, Brown & Tuthill, 2004; Magalhães & Mariath, 2012; Prado *et al.*, 2014), there is little information regarding their ontogeny and this information is restricted to seeds of species of Tillandsioideae, describing the formation of the flight apparatus (Szidat, 1922; Palací *et al.*, 2004).

In Tillandsioideae, the seeds are formed in dehiscent capsules, whereas the mucilaginous seeds of Bromelioideae are formed in fleshy and indehiscent fruits (Smith & Downs, 1974; Smith & Till, 1998; Benzing, 2000). The mucilaginous nature of the seeds of Bromelioideae is observed in later stages of seed development and is probably related to fruit dispersal by animals (Benzing, 2000).

In the literature, mature seeds of Bromelioideae have been described as usually unappendaged (Smith & Downs, 1974; Smith & Till, 1998; Benzing, 2000), although chalazal appendages are present in the ovules of many species (Smith & Downs, 1979; Sajo *et al.*, 2004a; Fagundes & Mariath, 2014). This is because the mucilaginous coat, including the chalazal appendage, may degenerate inside the fruits, leading in part to their juiciness (Szidat, 1922; Smith & Downs, 1974;

bahiana. A, C, general aspect of the young and mature seeds, respectively. B, detail at the micropylar end of a young seed showing the embryo and endosperm development. D–F, details of young (D) and mature (E–F) seeds, showing the development of the seed coat and endosperm. G–I, detail of the testa, exotegmen and endotegmen in the mature seed, respectively. J, detail of the differentiated embryo in a young seed. Arrows, proembryo; arrowhead, shoot apical meristem; ce, chalazal end; co, cotyledon; en, endosperm; ent, endotegmen; ext, exotegmen; hra, hypocotyl–radicular axis; me, micropylar end; n, nucellus; pt, pollen tube; sc, seed coat; te, testa; *, aleurone layer. Scale bars: A, C, 200 µm; B, D–J, 50 µm.

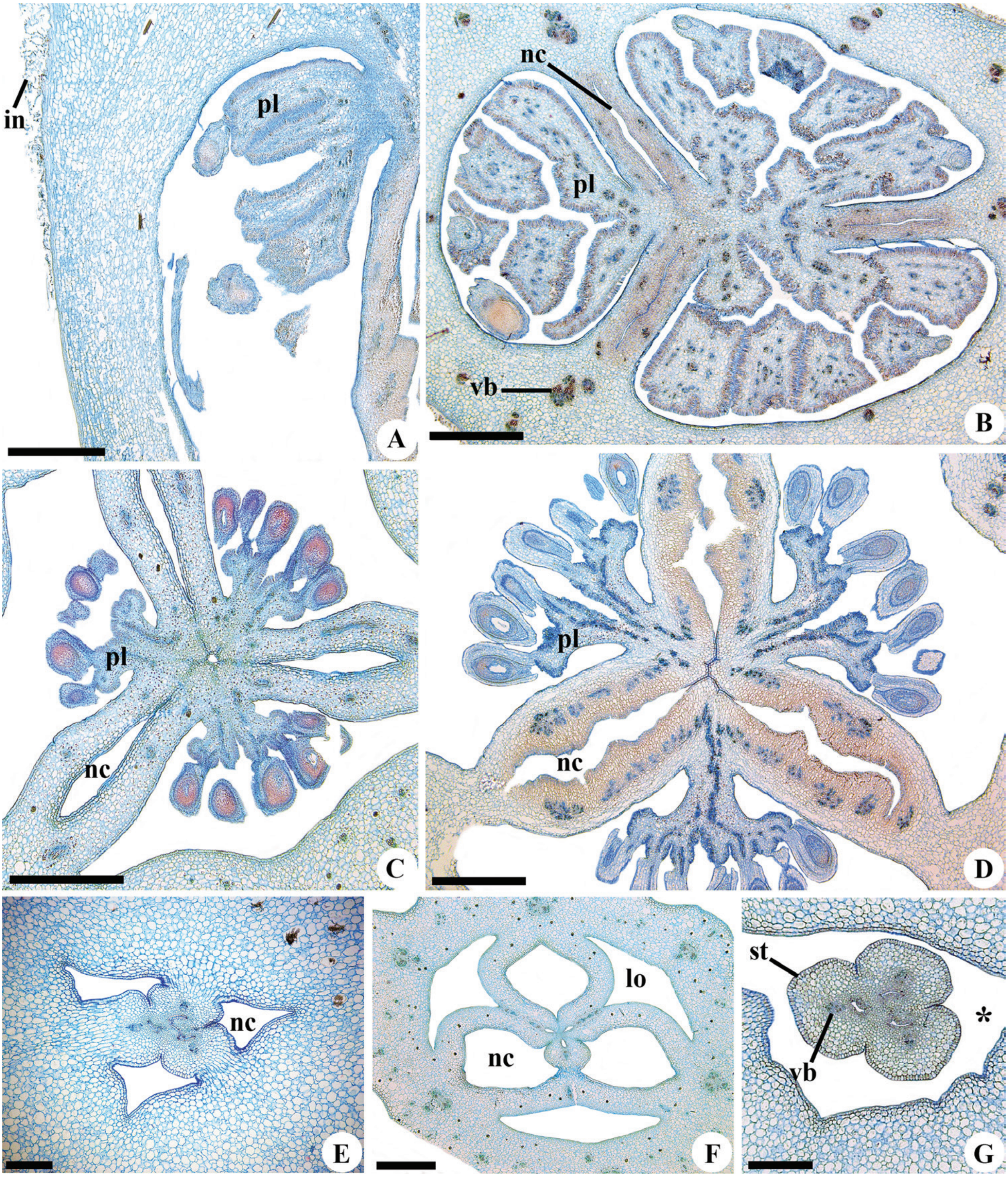

Figure 6. Ovaries showing the placentation and position of the nectariferous channels in longitudinal (A) and transverse sections (B–G) stained with Astrablau-safranin. A–B, E, G, *Aechmea bromeliifolia* var. *albobracteata*. C, F, *Billbergia distachia*. D, *Neoregelia bahiana*. A, detail of a locule showing the apical position of the placentae. B–D, general aspect of the ovary at apical (B) and subapical (C–D) portions, showing the axile placentation and the interlocular nectariferous channels. E–G, apical portion of the ovary showing the divergence of nectariferous channels to the style base. in, indumentum; lo, locule; nc, nectariferous channels; pl, placenta; st, style; vb, vascular bundle; *, site where the nectar is released. Scale bars: A–F, 500 µm; G, 200 µm.

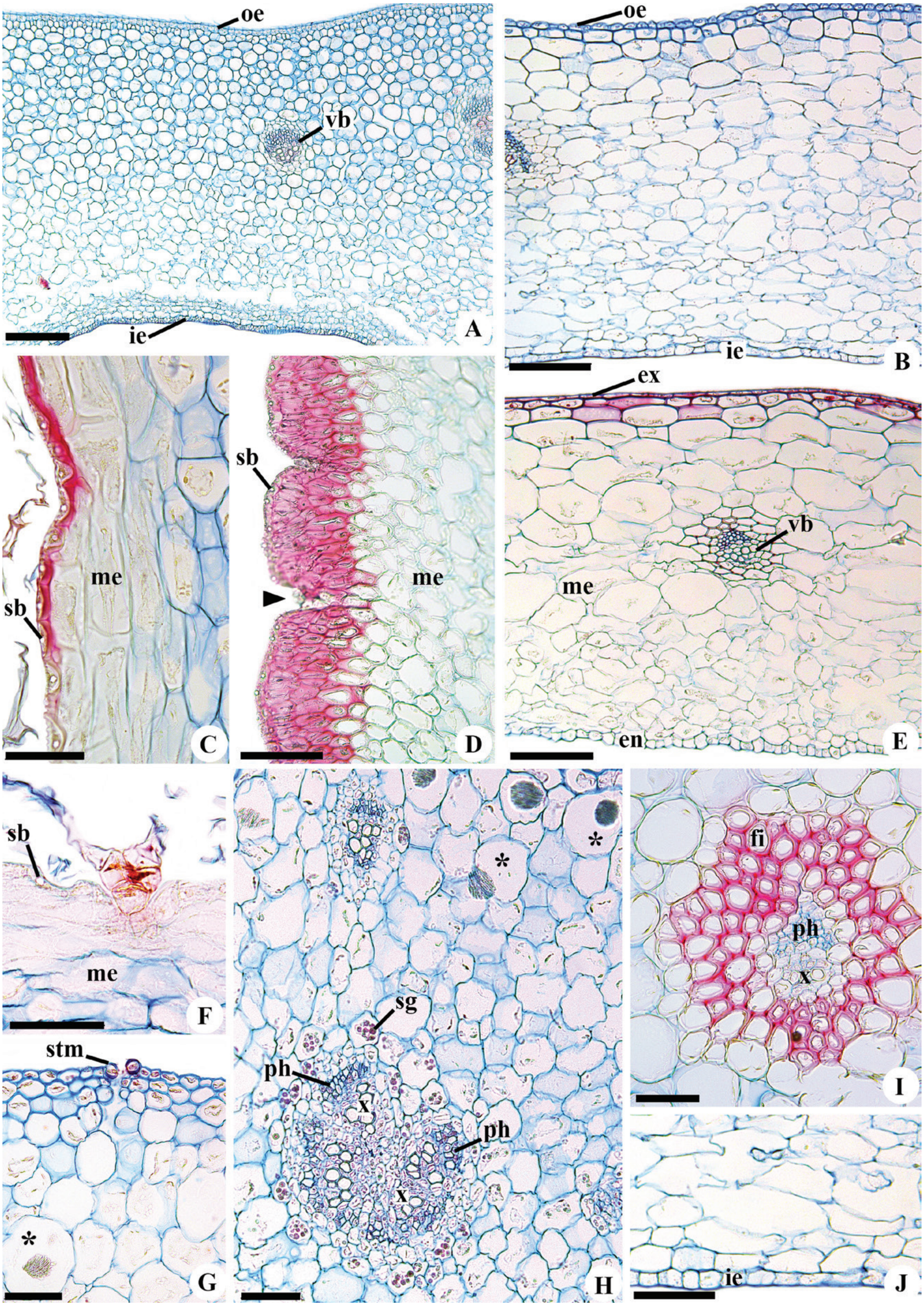

Figure 7. Details of ovaries and fruits in transverse (A–B, D–E, G–J) and longitudinal sections (C, F) stained with Astrablau-safranin. A, C–D, F, I, *Aechmea bromeliifolia* var. *albobracteata*. B, E, J, *Neoregelia bahiana*. G–H, *Billbergia distachia*. A–B,

Benzing, 2000), in combination with the substances stored by mesocarp cells. In this case the seed coat in mature seeds is constituted only by the tegmen.

We demonstrate here that the mucilaginous and fleshy tissue of the seed coat results from the development of the outer integument forming the testa, corroborating the term sarcotesta (Corner, 1976) used to describe the seeds of Bromelioideae (Smith & Till, 1998). Thus, our results add new information regarding the development of seeds in Bromeliaceae, indicating distinct ontogenetic processes in Bromelioideae compared to Tillandsioideae. In the former, the number of layers in the outer integument does not increase during seed development, but its cells enlarge by the accumulation of compounds, resulting in the mucilaginous testa. The same occurs in cells of the chalazal appendages, which also constitute the testa. Thus, the sarcotesta in Bromelioideae is characterized by a multilayered mucilaginous tissue with or without chalazal appendages, varying in size when present.

Seeds in Tillandsioideae also have appendages, which may be chalazal and/or micropylar (Szidat, 1922; Palací *et al.*, 2004; Magalhães & Mariath, 2012). They are usually single, consisting of several cell layers with dermal and subdermal origin (Fagundes & Mariath, 2014). Only in *Catopsis*, multiple appendages with dermal and subdermal origin (hairy structures) occur in the chalazal end of the ovule as an apomorphy of the genus, forming the flight apparatus of the seeds (Palací *et al.*, 2004; Sajo *et al.*, 2004a; Kuhn *et al.*, 2016). In the other species of Tillandsoideae, the flight apparatus is formed by the elongation and splintering of testa cells, resulting in numerous long unicellular hairs (Palací *et al.*, 2004; Magalhães & Mariath, 2012). In this context, the mucilaginous chalazal appendages of the seeds of Bromelioideae are homologous only to the single chalazal appendage found in Tillandsioideae. Although they have the same ontogenetic origin, they are anatomically distinct at seed maturity, because their features evolved in response to different dispersal mechanisms.

Considerations on the evolution of fleshy fruits in Bromeliaceae

The species studied belong to the eu-bromelioid clade, a group well supported by molecular data (Schulte *et al.*, 2009). The differences here observed in inflorescence and fruit morphology illustrate the variations found in Bromelioideae (Smith & Downs, 1974; Smith & Till, 1998; Benzing, 2000).

In the three species studied, enlargement of the cells of the mesocarp may be identified as the major contributor to fruit juiciness. There is also a mechanical hypodermis, which results from thickening of the cell walls of the subepidermal layers adjacent to the exocarp. Enlargement of the cells of the mesocarp and the presence of a mechanical tissue have also been observed in the fleshy fruits of other species of Bromelioideae (Okimoto, 1948; Fagundes & Mariath, 2010).

The indehiscent fruits of Bromelioideae, classified as berries, are a common feature of this subfamily (Smith & Downs, 1974; Smith & Till, 1998; Benzing, 2000; Fagundes & Mariath, 2010). Their anatomy and morphology are defined by the inferior position of the ovary. Such fruits are formed by carpellary and non-carpellary tissues, as reported previously for *Aechmea calyculata* (É.Morren) Baker and *B. nutans* (Fagundes & Mariath, 2010) and evidenced by the presence of a great number of vascular bundles supplying sepals, petals and stamens in the inferior ovary wall. For Pitcairnioideae and Tillandsioideae, which have superior ovaries and capsular fruits, only three vascular bundles per carpel were reported, one dorsal and two ventral (Fagundes & Mariath, 2010; Santos-Silva *et al.*, 2015). The species studied here show three vascular bundles in the style, which correspond to the dorsal vascular bundle of each carpel.

Sajo, Rudall & Prychid (2004b), studying the floral anatomy of Bromeliaceae, pointed out that hypogyny could have resulted from one or more reversals in the family. However, considering the updated phylogenetic context of Bromeliaceae (Givnish *et al.*, 2007), in which Bromelioideae appear as a late-diverging lineage, their epigynous flowers, giving rise to fleshy and indehiscent fruits, have most probably evolved from hypogynous flowers that form dry, capsule-like fruits, as found in the early-diverging subfamilies.

The fleshiness of fruits in monocots has appeared multiple times during their evolution (Thadeo *et al.*, 2015), but in Bromeliaceae this condition is a synapomorphy of Bromelioideae. Thadeo *et al.* (2015) classified the fruits of *Aechmea aquilega* as true berries

general aspect of the ovary wall. C–D, differentiation of the exocarp and mesocarp in young and mature fruits, respectively. E, general aspect of the pericarp in a mature fruit. F, scale on the pericarp in a young fruit. G, arrangement of cells of the outer epidermis and parenchyma in the ovary. H, detail of the ovary wall showing idioblasts with raphides, cells with starch grains, and a compound vascular bundle. I, collateral vascular bundle in the pericarp surrounded by thick-walled cells. J, detail showing the cells of the inner epidermis and parenchyma in the ovary. Arrowhead, site where a scale was inserted; en, endocarp; ex, exocarp; fi, fibres; ie, inner epidermis; me, mesocarp; oe, outer epidermis; ph, phloem; sb, silica body; sg, starch grains; stm, stomata; vb, vascular bundle; x, xylem; *, idioblast. Scale bars: A–B, D–E, 100 µm; C, F–J, 50 µm.

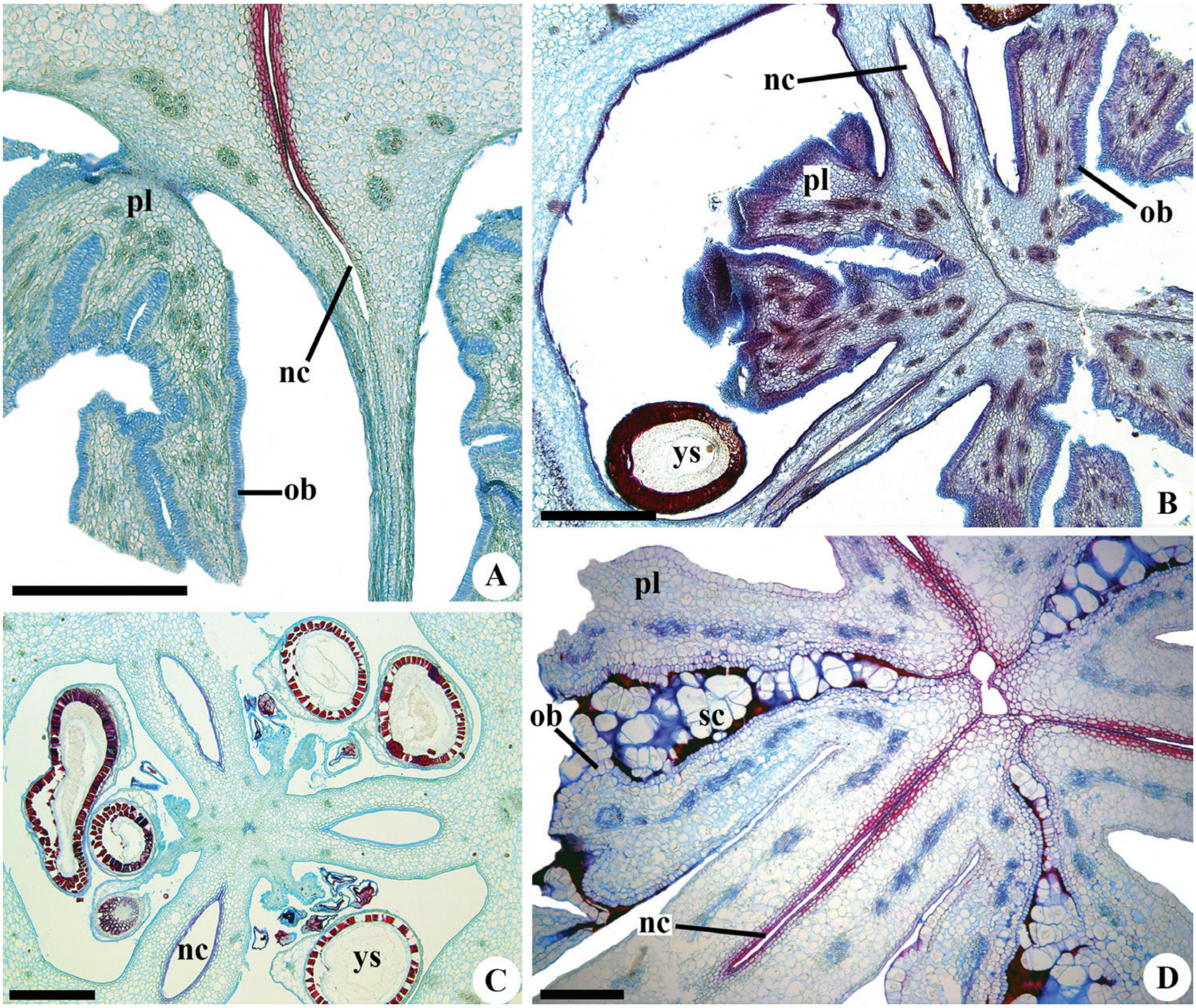

Figure 8. Fruits showing the placentation and position of the nectariferous channels in longitudinal (A) and transverse sections (B–D) stained with Astrablau-safranin. A–B, *Aechmea bromeliifolia* var. *albobracteata*. C, *Billbergia distachia*. D, *Neoregelia bahiana*. A, detail of a young fruit showing the apical position of the placenta and nectariferous channels. B–C, detail of the central region of young fruits, showing the axile placentation and the interlocular nectariferous channels. D, detail of the central region of a mature fruit with substances secreted by the obturator. nc, nectariferous channel; ob, obturator; pl, placenta; sc, secretion; ys, young seed. Scale bars: A–C, 500 µm; D, 200 µm.

because their pericarp is uniform. It is different from the species studied here, in which the pericarp comprises an exocarp and part of the mesocarp with thick-walled cells (with lignified walls in *A. bromeliifolia*). In the other two species of Bromelioideae studied, and in *A. calyculata* and *B. nutans*, the pericarp also has cells with thickened walls in the exocarp and subepidermal layers adjacent to the exocarp (Fagundes & Mariath, 2010), having a heterogeneous aspect. Therefore, the uniform aspect of the pericarp observed in *A. aquilega* (Thadeo *et al.*, 2015) is not a pattern for Bromelioideae, but we agree with the classification of the fruits as true berries due to the absence of a differentiated hardened endocarp forming stony pyrenes.

Fagundes & Mariath (2010) discussed evolutionary trends in fruit morphology of Bromeliaceae and described structural differences in fruit anatomy and dehiscence processes among species of Tillandsioideae and Pitcairnioideae. In all of these studied species, capsule dehiscence occurs by the formation of dehiscence lines in the septa and/or locules (Fagundes & Mariath, 2010; Santos-Silva *et al.*, 2015), which are not present in the fleshy fruits of Bromelioideae.

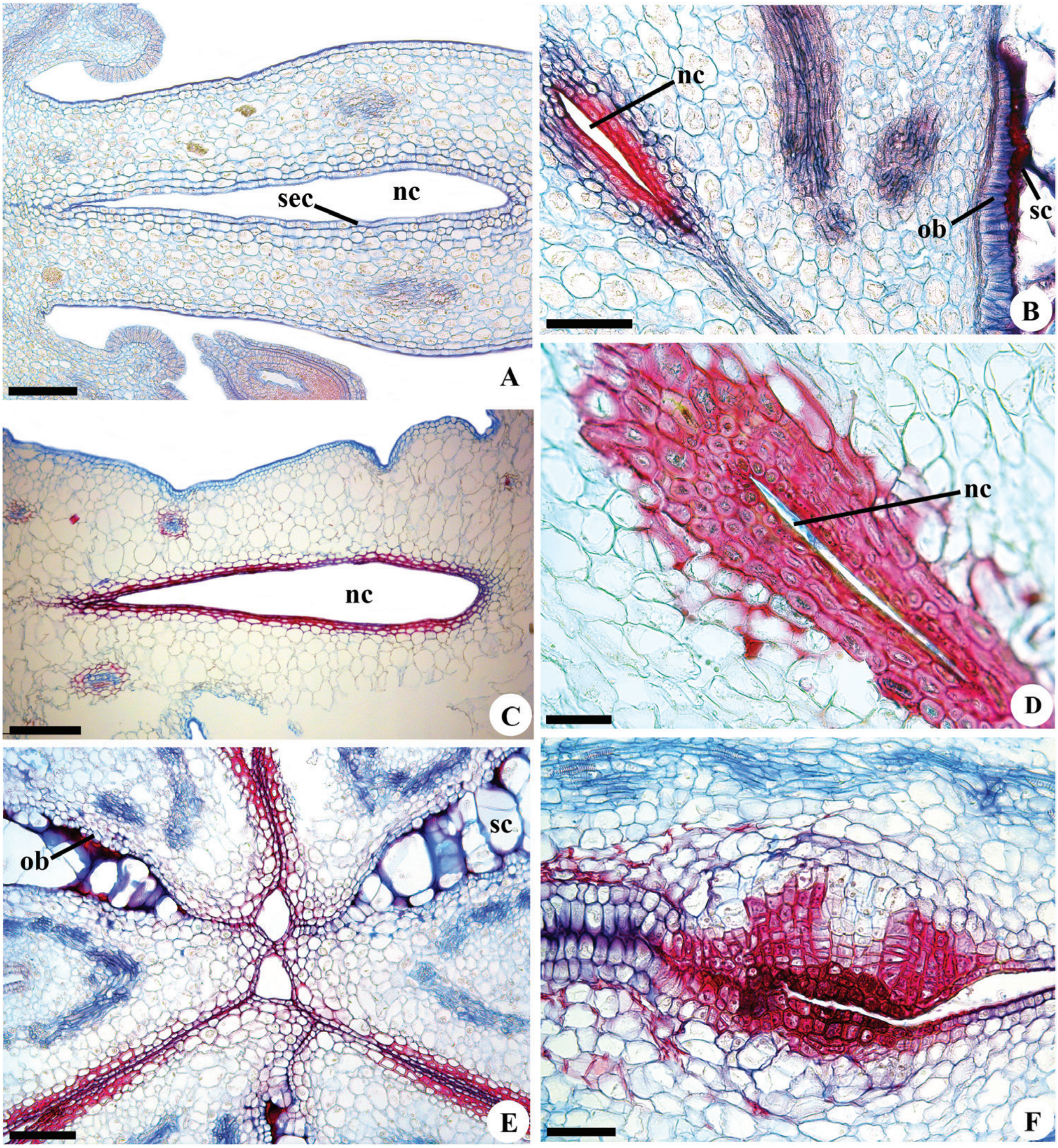

Figure 9. Structural changes in the septal nectaries during fruit development, in transverse sections stained with Astrablau-safranin. A, C, *Billbergia distachia*. B, D, *Aechmea bromeliifolia* var. *albobracteata*. E–F, *Neoregelia bahiana*. A–B, detail of the septal nectary in an ovary and young fruit, respectively. C–D, detail of the septal nectary in mature fruits. E, detail of the central region of a mature fruit with substances secreted by the obturator. F, detail of the septal nectary in a mature fruit. nc, nectariferous channel; ob, obturator; sc, secretion; sec, secretory epidermal cells. Scale bars: A–B, E, 100 μm; C, 200 μm; D, F, 50 μm.

The lines of cells forming the septal nectariferous tissue in the species studied here and also in other species of Bromelioideae (Sajo *et al.*, 2004b; Fagundes & Mariath, 2010) are homologous to the dehiscence lines in the septa of capsules. The septal nectaries are a common feature in Bromeliaceae and, according to Sajo *et al.* (2004b), they are interlocular in Bromelioideae and infralocular in the other subfamilies. Therefore, the shift from dry to fleshy fruits in Bromeliaceae may be related to an increase in the extent of the septal nectaries.

It should be emphasized that the dehiscent fruits of Tillandsioideae have conspicuous lignified cells in the exocarp, mesocarp and endocarp, and in Pitcairnioideae the lignified cells occur in the exocarp or in the endocarp and mesocarp (Fagundes & Mariath, 2010; Santos-Silva *et al.*, 2015). In the fruits of Bromelioideae only the exocarp and some subepidermal layers have lignified cells in some species (Fagundes & Mariath, 2010; our data), and thus the reduction of sclerenchymatous strata may be related to indehiscence.

FUNCTIONAL ASPECTS OF THE FLESHY FRUIT TRAITS

The juiciness of the fruits of Bromelioideae is related to dispersal by animals (Benzing, 2000) and is given by the enlargement of mesocarp cells during fruit development, as observed here and in other species of Bromelioideae (Fagundes & Mariath, 2010). In addition, the placental obturator may lead to an increase in juiciness. The presence of an obturator is a common feature in Bromeliaceae, reported for species in different subfamilies (Sajo *et al.*, 2004a; Fagundes & Mariath, 2010). During fruit development, structural changes such as elongation or cell division have been reported for obturator cells (Fagundes & Mariath, 2010), indicating they are functional in the fruits. In this context, our results add new information to this tissue functionality, showing that it secretes substances inside the locules of developing fruits, increasing their juiciness.

During fruit development the secretory cells of the septal nectaries also undergo structural and physiological changes. In the apical part of the fruits the walls of the nectariferous secretory cells undergo thickening, and we suggest that these thick-walled cells promote a mechanical barrier against the establishment of pathogens inside the fruits. The development of a multilayered, partly sclerified tissue underneath the secretory cells of the septal nectaries observed in all mature fruits of *N. bahiana* is a diagnostic feature of this species and is reported here for the first time for fruits of Bromeliaceae.

In the three species studied here, the quantity of raphides and starch grains declines with fruit maturity, demonstrating that calcium and carbohydrates are mobilized during fruit development. The function of the raphide-containing idioblasts for calcium storage has been reported by Fagundes & Mariath (2010), who also observed a decrease in the quantity of raphides in the mature fruit and interpreted that they were mobilized for fruit development or redirected to other structures such as the seeds. The decreasing number of starch grains occurs during enlargement of the mesocarp cells and has also been reported for other species of Bromelioideae such as *Ananas comosus* (L.) Merril (Okimoto, 1948; Smith & Harris, 1995), *A. calyculata* and *B. nutans* (Fagundes & Mariath, 2010). The presence of starch grains close to the vascular bundles, as observed here, facilitates the transport of the mobilized carbohydrates throughout the fruit.

HYPOTHESES ON FRUIT DISPERSAL

For species that occur on rocky outcrops such as *N. bahiana* (with a restricted distribution in the campos rupestres/rocky fields of the Espinhaço Mountain Range in the states of Bahia and Minas Gerais, Brazil) (Versieux *et al.*, 2008; Forzza *et al.*, 2015; Silva, Versieux & Oriani, 2018), lizards are probable dispersers; they may search for these plants as a food and water supply, because the water-tank formed by the leaf rosette and containing the infructescence grows near the ground. This hypothesis is based on the fact that the lizards consume fleshy fruits of different types, size and coloration, and that saurochory is common in islands or mainland areas from a wide latitudinal range (Valido & Olesen, 2007). Studies on the occurrence and distribution of lizards have been carried out for different areas of the Espinhaço Range, indicating that these reptiles (including endemic species) are common in campos rupestres (Rodrigues, 1987; Cassimiro & Rodrigues, 2009; de Carvalho, de Brito & Fernandes, 2013; Cruz *et al.*, 2014). The lizards may transport viable seeds from the mother-plant to another site, such as the cracks found on the rocky substrate, where humidity is greater, increasing the chances of survival and establishment of the seedlings. The mucilaginous coat of the seeds may also contribute to the food mass to be consumed and may also protect the seeds while passing through the digestive tract of the dispersers. Fruit dispersal by lizards has also been observed in species of Cactaceae with fleshy fruits and mucilaginous seeds, which grow in arid regions such as the rocky outcrops (e.g. Taylor, 1991; Taylor & Zappi, 2004; Gomes, Quirino & Machado, 2013; Gomes *et al.*, 2016, 2017; Koski, Valadares & Barreto-Lima, 2018).

For the other two species studied, in which the inflorescence axis is long and the fruits are exposed, we hypothesize that the main dispersers are flying animals. The fruits of *A. bromeliifolia* are more distinct, especially regarding the greater thickness of the cells of the pericarp, including the exocarp and outermost layers of the mesocarp. The same feature was observed in *A. calyculata* (Fagundes & Mariath, 2010) and may be related to the organization of the flowers and fruits, which are partly sunken in a thickened axis. The wall thickness of the pericarp cells promotes a greater rigidity, protecting such congested fruits. Another feature that distinguishes the fruits of *Aechmea* spp. is the presence of scales and silica bodies in the exocarp, which may give additional protection to the fruits. Benzing (2000) pointed out that peltate scales on the ovaries/fruits may act in pollination and/or seed dispersal, as may occur in *A. bromeliifolia*. The fruit features of *Aechmea* spp. indicate they are dispersed by birds, because the fruits are scentless and generally coloured (red, white, blue or brown) (Benzing, 2012), besides having the protective structures previously cited, which may preserve the seeds while the birds remove the fruits from the infructescence with their beaks. The birds may also consume the mucilaginous seed appendages, which are long in *A. bromeliifolia*. The colour of the peduncular bracts of *A. bromeliifolia* (white in *A. bromeliifolia* var. *albobracteata* and pinkish in *A. bromeliifolia* var. *bromeliifolia*) (Gelli de Faria, Wendt & Brown, 2010) may act in disperser attraction as well. Long-distance dispersal by birds may be responsible for the wide distribution of this species, which occurs from Central America to Argentina, growing on different substrates and exhibiting different life forms (Table 1; Gelli de Faria *et al.*, 2010; Forzza *et al.*, 2015).

Bats have been reported as the main dispersers of fruits in *Billbergia* Thunb., due to the presence of scales in the pericarp of some species, such as in *B. robert-readii* E.Gross & Rauh, or by the reflective surfaces of the fruits (Benzing, 2012), such as in *B. distachia*; both these characteristics increase the brightness of the fruits at night by reflecting dim light (Benzing, 2000, 2012). In addition, fruits of *Billbergia* are large, with an odour reminiscent of rotten fruit (Benzing, 2012), favouring dispersal by bats. The mucilaginous seed coat with a short appendage may be a protective barrier to ensure safe passage of seeds through the digestive tract of the bats. Dispersal by bats may promote a relatively wide distribution of species among forest environments given that *B. distachia* occurs through the south-eastern and southern regions of Brazil in forested cerrado and in the Atlantic Forest (Forzza *et al.*, 2015).

According to Smith & Till (1998), seed dispersal by ants may also occur in Bromeliaceae, probably in previously damaged fruits and with the seeds exposed, as the compounds of the mucilaginous seed coat may attract these insects. Therefore, studies evaluating the composition of the mucilaginous seed coat cells are important to establish relationships with possible dispersers.

We highlight the fact that all the dispersal hypotheses cited above were made based on the fruit features of each species studied, but field observations and experiments are necessary to confirm them. Our results also open avenues for future studies on the development of fleshy fruits in other species and genera of Bromelioideae in a comparative approach to better understand their ecological and evolutionary aspects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Baum S. 2008. The PAS reaction for staining cell walls. *CSH Protocols* **3:** 1–3.

Benzing DH. 2000. *Bromeliaceae: profile of an adaptative radiation.* New York: Cambridge University Press.

Benzing DH. 2012. *Air plants: epiphytes and aerial gardens*. Ithaca: Comstock Publishing Associates & Cornell University Press.

Bouchenak-Khelladi Y, **Muasya AM**, **Linder HP. 2014.** A revised evolutionary history of Poales: origins and diversification. *Botanical Journal of the Linnean Society* **175:** 4–16.

Bukatsch F. 1972. Bermerkungen zur Doppelfärbung Astrablau-Safranin. *Mikrokosmos* **61:** 255.

de Carvalho ALG, **de Brito MR**, **Fernandes DS. 2013.** Biogeography of the lizard genus *Tropidurus* Wied-Neuwied, 1825 (Squamata: Tropiduridae): distribution, endemism, and area relationships in South America. *PLoS One* **8:** e59736.

Cassimiro J, **Rodrigues MT. 2009.** A new species of lizard genus *Gymnodactylus* Spix, 1825 (Squamata: Gekkota: Phyllodactylidae) from Serra do Sincorá, northeastern Brazil, and the status of *G. carvalhoi* Vanzolini, 2005. *Zootaxa* **2008:** 38–52.

Chamberlain CJ. 1932. *Methods in plant histology*. Chicago: The University of Chicago Press.

Corner EJH. 1976. *The seeds of dicotyledons*. Cambridge: Cambridge University Press.

Cruz AJR, **Drummond LO**, **Lucena VD**, **Magalhães AP**, **Braga CAC**, **Rolin JM**, **Pires MRS. 2014.** Lizard fauna (Squamata: Sauria) from Serra do Ouro Branco, southern Espinhaço Range, Minas Gerais, Brazil. *Check List* **10:** 1290–1299.

Fagundes NF, **Mariath JEA. 2010.** Morphoanatomy and ontogeny of fruit in Bromeliaceae species. *Acta Botanica Brasilica* **24:** 765–779.

Fagundes NF, **Mariath JEA. 2014.** Ovule ontogeny in *Billbergia nutans* in the evolutionary context of Bromeliaceae (Poales). *Plant Systematics and Evolution* **300:** 1323–1336.

Feder N, **O'Brien TP. 1968.** Plant microtechnique: some principles and new methods. *American Journal of Botany* **55:** 123–142.

Forzza RC, **Costa A**, **Siqueira Filho JA**, **Martinelli G**, **Monteiro RF**, **Santos-Silva F**, **Saraiva DP**, **Paixão-Souza B**, **Louzada RB**, **Versieux L. 2015.** Bromeliaceae in *Lista de espécies da flora do Brasil*. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB5771. (date last accessed, 4 November 2019).

Gelli de Faria AP, **Wendt T**, **Brown GK. 2010.** A revision of *Aechmea* subgenus *Macrochordion* (Bromeliaceae) based on phenetic analyses of morphological variation. *Botanical Journal of the Linnean Society* **162:** 1–27.

Gerrits PO, **Smid L. 1983.** A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *Journal of Microscopy* **132:** 81–85.

Gerstberger P, **Leins P. 1978.** Rasterelektronenmikroskopische untersuchungen an Blütenknospen von *Physalis philadelphia* (Solanaceae). *Berichte der Deutschen Botanischen Gesellschaft* **91:** 381–387.

Givnish TJ, **Ames M**, **McNeal JR**, **McKain MR**, **Steele PR**, **dePamphilis CW**, **Graham SW**, **Pires JC**, **Stevenson DW**, **Zomlefer WB**, **Briggs BG**, **Duvall MR**, **Moore MJ**, **Heaney JM**, **Soltis DE**, **Soltis PS**, **Thiele K**, **Leebens-Mack JH. 2010.** Assembling the tree of the monocotyledons: plastome sequence phylogeny and evolution of Poales. *Annals of the Missouri Botanical Garden* **97:** 584–616.

Givnish TJ, **Millam KC**, **Berry PE**, **Sytsma KJ. 2007.** Phylogeny, adaptive radiation and historical biogeography of Bromeliaceae inferred from *ndhF* sequence data. *Aliso* **23:** 3–26.

Givnish TJ, **Zuluaga A**, **Spalink D**, **Soto Gomez M**, **Lam VKY**, **Saarela JM**, **Sass C**, **Iles WJD**, **de Sousa DJL**, **Leebens-Mack J**, **Chris Pires J**, **Zomlefer WB**, **Gandolfo MA**, **Davis JI**, **Stevenson DW**, **dePamphilis C**, **Specht CD**, **Graham SW**, **Barrett CF**, **Ané C. 2018.** Monocot plastid phylogenomics, timeline, net rates of species diversification, the power of multi-gene analyses, and a functional model for the origin of monocots. *American Journal of Botany* **105:** 1888–1910.

Gomes VGN, **Quirino ZGM**, **Machado IC. 2013.** Pollination and seed dispersal of *Melocactus ernestii* Vaupel subsp. *ernestii* (Cactaceae) by lizards: an example of double mutualism. *Plant Biology* **16:** 315–322.

Gomes VGN, **Meiado MV**, **Quirino ZGM**, **Araújo HFP**, **Machado IC. 2017.** Synchronous fruiting and common seed dispersers of two endemic columnar cacti in the Caatinga, a dry forest in Brazil. *Plant Ecology* **218:** 1325–1338.

Gomes VGN, **Meiado MV**, **Quirino ZGM**, **Machado IC. 2016.** Seed removal by lizards and effect of gut passage on germination in a columnar cactus of the Caatinga, a tropical dry forest in Brazil. *Journal of Arid Environments* **135:** 85–89.

Johansen DA. 1940. *Plant microtechnique*. New York: McGraw Hill.

Koski DA, **Valadares RT**, **Barreto-Lima AF. 2018.** Fruits in the lizard's menu: consumption of *Coleocephalocereus fluminensis* (Cactaceae) by *Tropidurus torquatus* (Tropiduridae). *Herpetology Notes* **11:** 853–855.

Kuhn SA, **Nogueira FM**, **Fagundes NF**, **Mariath JEA. 2016.** Morphoanatomy of the ovary and ovule in Bromeliaceae subfamily Tillandsioideae and its systematic relevance. *Botanical Journal of the Linnean Society* **181:** 343–361.

Linder HP, **Rudall PJ. 2005.** Evolutionary history of Poales. *Annual Review of Ecology Evolution and Systematics* **36:** 107–124.

Magalhães RI, **Mariath JEA. 2012.** Seed morphoanatomy and its systematic relevance to Tillandsioideae (Bromeliaceae). *Plant Systematics and Evolution* **298:** 1881–1895.

Müller F. 1895. Die Keimung einiger Bromeliaceen. *Berichte der Deutschen Botanischen Gesellschaft* **13:** 175–182.

Nogueira FM, **Fagundes NF**, **Kuhn SA**, **Fregonezi JN**, **Mariath JEA. 2015.** Ovary and ovule anatomy in the nidularioid complex and its taxonomic utility (Bromelioideae: Bromeliaceae). *Botanical Journal of the Linnean Society* **177:** 66–77.

O'Brien TP, **Feder N**, **McCully ME. 1964.** Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* **59:** 368–373.

Okimoto MC. 1948. Anatomy and histology of the pineapple inflorescence and fruit. *Botanical Gazette* **110:** 217–231.

Palací CA, **Brown GK**, **Tuthill DE. 2004.** The seeds of *Catopsis* (Bromeliaceae: Tillandsioideae). *Systematic Botany* **29:** 518–527.

Prado JPC, **Schimidt EC**, **Steinmacher DA**, **Guerra MP**, **Bouzon ZL**, **Vesco LLD**, **Pescador R. 2014.** Seed morphology of *Vriesea friburguensis* var. *paludosa* L. B. Sm. (Bromeliaceae). *Hoehnea* **41:** 553–562.

Rauh W, **Barthlott W. 1982.** Bromelienstudien I. Neue und wenig bekannte Arten aus Peru und anderen Lindern. *Tropische und Subtropische Pflanzenwelt* **39:** 1–35.

Rodrigues MT. 1987. Distribution of lizards of the genus *Tropidurus* in Brazil (Sauria, Iguanidae). In: Vanzolini PE, Ronald Heyer W, eds. *Proceedings of a workshop on Neotropical distribution patterns*. Rio de Janeiro: Academia Brasileira de Ciências, 305–315.

Sajo MG, **Prychid CJ**, **Rudall PJ. 2004a.** Structure and development of the ovule in Bromeliaceae. *Kew Bulletin* **59:** 261–267.

Sajo MG, **Rudall PJ**, **Prychid CJ. 2004b.** Floral anatomy of Bromeliaceae, with particular reference to the evolution of epigyny and septal nectaries in commelinid monocots. *Plant Systematics and Evolution* **247:** 215–231.

Santos-Silva F, **Mastroberti AA**, **Mariath JEA. 2015.** Capsule structure in three species of *Dyckia* (Bromeliaceae): ontogenetic and taxonomic issues. *Journal of the Torrey Botanical Society* **142:** 249–257.

Schulte K, **Barfuss MH**, **Zizka G. 2009.** Phylogeny of Bromelioideae (Bromeliaceae) inferred from nuclear and plastid DNA loci reveals the evolution of the tank habit within the subfamily. *Molecular Phylogenetics and Evolution* **51:** 327–339.

Silva KR, **Versieux LM**, **Oriani A. 2018.** Anatomy of vegetative organs, inflorescence axis and pedicel in the *Neoregelia bahiana* complex (Bromeliaceae): taxonomic and ecological importance. *Nordic Journal of Botany* **2018:** e01800.

Smith LB. 1934. Geographical evidence on the lines of evolution in the Bromeliaceae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **66:** 446–468.

Smith LB, **Downs RJ. 1974.** Pitcairnioideae (Bromeliaceae). *Flora Neotropica Monograph* **14:** 1–658.

Smith LB, **Downs RJ. 1979.** Bromelioideae (Bromeliaceae). *Flora Neotropica Monograph* **14:** 1493–2142.

Smith BG, **Harris PJ. 1995.** Polysaccharide composition of unlignified cell walls of pineapple [*Ananas comosus* (L.) Merr.] fruit. *Plant Physiology* **107:** 1399–1409.

Smith LB, **Till W. 1998.** Bromeliaceae. In: Kubitzki K, ed. *The families and genera of vascular plants*, *vol.* **4**. Berlin: Springer, 74–99.

Szidat L. 1922. Die Samen der Bromeliaceen in ihrer Anpassung an den Epiphytismus. *Botanisches Archiv* **1:** 29–46.

Taylor NP. 1991. The genus *Melocactus* (Cactaceae) in Central and South America. *Bradleya* **9:** 1–80.

Taylor N, **Zappi D. 2004.** *Cacti of eastern Brazil*. Kew: Royal Botanic Gardens.

Thadeo M, **Hampilos KE**, **Stevenson DW. 2015.** Anatomy of fleshy fruits in the monocots. *American Journal of Botany* **102:** 1–23.

Valido A, **Olesen JM. 2007.** The importance of lizards as frugivores and seed dispersers. In: Dennis AJ, Schupp EW, Green RJ, Westcott DA, eds. *Seed dispersal: theory and its application in a changing world*. Wallingford: CAB International, 124–147.

Varadarajan GS, **Gilmartin AJ. 1988.** Seed morphology of the subfamily Pitcairnioideae (Bromeliaceae) and its systematic implications. *American Journal of Botany* **75:** 808–818.

Venturelli M, **Bouman F. 1988.** Development of ovule and seed in Rapateaceae. *Botanical Journal of the Linnean Society* **97:** 267–294.

Versieux LM, **Wendt T**, **Louzada RB**, **Wanderley MGL. 2008.** Bromeliaceae da Cadeia do Espinhaço. *Megadiversidade* **4:** 99–110.