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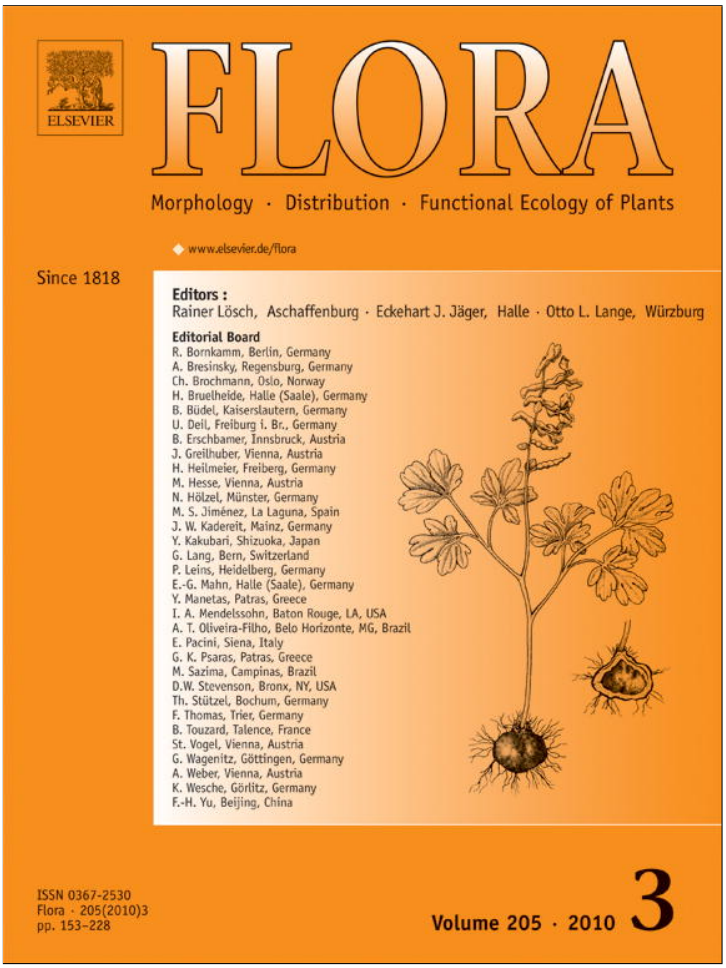

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Development and morphology of the gynoecium and nutlet in two South-American *Bulbostylis* (Cyperaceae) species

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ABSTRACT

The anatomy and ontogeny of the gynoecium and nutlets of two *Bulbostylis* species with different micromorphology, *Bulbostylis capillaris sensu* Barros and *B. major*, were analyzed. The specific aim of this work was to identify which part of the pericarp determines the differences in the nutlet surface between these two species. We found that pre-fertilization development is the same in both species, with differences between species appearing only after fertilization. In the nutlet of *B. major*, the exocarp forms a tuberculate primary sculpture that has nipple-like protuberances consisting of one conoidal silica body per cell, whereas the secondary sculpture is micro-granulose and is constituted by the cuticle. In the nutlet of *B. capillaris*, the primary sculpture is granulose and is formed by the presence of starch granules in the exocarp that do not degrade, whereas the secondary sculpture is smooth.

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Introduction

The family Cyperaceae comprises numerous systematically difficult taxa, thus presenting a special challenge to the plant taxonomist. One of the difficulties of the classification of sedges can be attributed to their substantially reduced floral and vegetative morphology (Guarise and Vegetti, 2008; Menapace, 1991).

The genus *Bulbostylis* Kunth (Cyperaceae-Cyperoideae-Abildgaardieae) includes approximately 150 species from tropical and subtropical habitats throughout the world (Bruhl, 1990; Goetghebeur, 1998; Muasya et al., 2000). Some species can be found in temperate areas, with the southern limit of the genus in South America at 40°S. The genus has two important centres of diversity: Africa and South America (especially central Brazil) and most species are found in open habitats such as savannas, in soils that are often sandy and damp at least during part of the year (López et al., 2007).

The nutlet (achene) surface is one of the most frequent characters studied for the delimitation of diverse genera of Cyperaceae: e.g., Schuyler (1971) on *Scirpus* L. and *Eriophorum* L.; Walter (1975), Toivonen and Timonen (1976), Raymond and Wujek (1983), Wujek and Menapace (1986), Menapace and Wujek (1987) and Standley (1987, 1990) on *Carex* L.; Ragonese et al. (1984) and Strong (2006) on *Rhynchospora* Vahl; Denton (1983), Lye (1983), López and Matthei (1995), Araújo and Longhi-Wagner (1997) on *Cyperus* L.; Menapace (1991, 1993) on *Eleocharis*; Browning et al. (1997) on *Bolboschoenus* (Ascherson) Palla; Wujek et al. (2001) on *Scleria* Bergius; Wujek et al. (1994) and Menapace (2003) on *Fimbristylis* Vahl.

In *Bulbostylis*, Goetghebeur and Coudijzer (1984) examined the nutlet surface of central African species using scanning electron microscope (SEM). On the other hand, in a revision for Austral America, where 26 species can be found, López (2007, 2008) found that the pattern of the nutlet surface was different in each species. These studies were restricted to morphological characters like the shape and the outline of the nutlet in a transverse section and to characters like the pattern of the surface, the size and shape of the exocarp, the thickness of the mesocarp and endocarp and the presence or absence of silica bodies. However, all these micromorphological characters were mostly analyzed only by SEM.

Goetghebeur (1998) also studied the organization of the fruit wall of Cyperaceae, and found that it generally presented three layers: the outer epidermis with conoidal silica bodies, two to three layers of longitudinally arranged fibres and transversal fibres in the most inner layer. In other studies, genera such as *Cyperus*, *Kyllinga* and *Scirpus* presented middle layers consisting of longitudinally arranged fibres (Khanna, 1965). In species of *Rhynchospora,* Ragonese et al. (1984) noted sclereids in the mesocarp.

The embryo of the Cyperaceae family has been studied and classified by Shah (1965), Van der Veken (1965) and Goetghebeur (1998). All these authors have described the embryo of *Bulbostylis* like *Bulbostylis* type, characterized by both the radicle and plumule point toward the micropyle.

The aims of the present study were (1) to provide a detailed documentation of the gynoecium and fruit structure, (2) to describe the ontogeny in two species of *Bulbostylis* with different

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nutlet micro-morphologies and (3) to resolve which structures determine the diverse patterns of the nutlet surface.

Materials and methods

Herbarium specimens representing the broad geographical distribution of *Bulbostylis capillaris* (L.) C.B. Clarke (sensu Barros, 1945, 1947, 1960) and *B. major* Palla were used for general observations and SEM studies of the nutlet surface. In all cases, mature nutlets were selected. Species examined, location and voucher numbers are listed and fully documented in the Appendix.

For anatomical studies, the floral buds, flowers and fruits in various stages of development were collected and fixed in FAA (alcohol, formaldehyde, acetic acid, 90:5:5, v/v). The samples were dehydrated according to Gonzalez and Cristóbal (1997), and subsequently infiltrated in paraffin (Johansen, 1940). Transversal and longitudinal serial sections were cut at 10 μm with a rotary microtome. The sections were then stained with a safranin–astra blue combination (Luque et al., 1996). Next, the sections were examined and photographed using a Leica DM LB2 light microscope, equipped with polarized light and a drawing tube.

Scanning electron microscopy (SEM): entire and free-hand sections of mature nutlets were coated with gold–palladium using a Denton Vacuum Desk II sputter coater, and observed using a Jeol LV 5800 SEM.

In order to identify the cells of the mechanical layer of the pericarp, small pieces of mature nutlets were macerated with the technique of Boodle (1916). The dissociated elements were observed both through light and scanning electron microscopes, using the techniques previously described. To detect starch granules in the pericarp, small pieces were cleared with standard bleach for 1 min and stained with Lugol solution (I_2/IK) (Johansen, 1940).

Primary and secondary sculptures in the fruit of *Bulbostylis* were described according to Barthlott (1981).

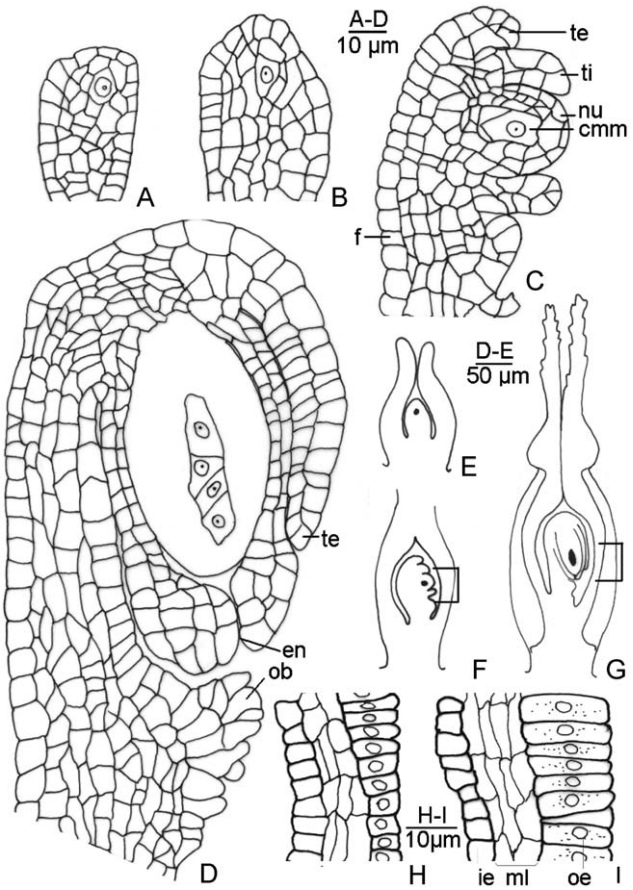

Fig. 1. Ovule and carpel wall in pre-fertilization stages. (A–D) Successive stages of ovule and megasporogenesis development in longitudinal sections. (D) Ovule with linear tetrad of megaspores. (E–G) Development of ovary, in longitudinal sections. (H–I) Ovary wall: (H) young and (I) mature. cmm, megaspore mother cell; en, endostome. (F) Funiculus. ie, inner epidermis; ml, middle layers; nu, nucellus; oe, outer epidermis; ob, obturator; te, outer integument; ti, inner integument. *B. capillaris*: A–B; *B. major*: C–I.

Results

We found that both species analyzed in this study have a tricarpellar, syncarpous, unilocular ovary, with a single anatropous ovule and basal placentation. The single style has a swollen base, known as stylopodium, and the stigma consists of three papillose stigmatic branches. The nutlet epidermis consists of longitudinally arranged elongated cells. The outer wall of these cells is plane to convex and may either have a small central protuberance like a rounded papilla or be smooth or with slender transverse wrinkles.

Ovary

In the gynoecium primordium, before the style and stigma differentiate, the ovary wall is formed by one layer of epidermis and two to four layers of parenchymatous cells (Fig. 1E and H).

At the young ovary stage (Fig. 1F and G), the cells of the carpel wall increase in size and acquire a different orientation: the cells of the outer epidermis expand in a radial direction perpendicular to the longitudinal axis, whereas the cells of the inner epidermis are disposed transversely to the vertical axis of the ovary (Fig. 1I).

In the mature ovary, the outer epidermis consists of a single palisade layer, whose cells are larger at the base of the ovary (Fig. 2A and G). These cells have cellulosic walls, and the external ones are thicker and covered by a smooth cuticle. These cells store large amounts of small starch granules. This layer lacks stomata.

The cells of the middle layers are thin and have a translucent cytoplasm (Fig. 2A and G). All along the ovary surface, the cells of the outer epidermis and middle layers adapt to each other forming a smoothly wavy boundary (Fig. 2A).

The inner epidermis is formed by only one layer of tabular and thin-walled cells, with partly covered ends (Fig. 2A and G). In the region adjacent to the carpellary bundles, the inner epidermis displays several layers of small cells (Fig. 2G).

Style

The stylopodium is formed by small, irregular, thin-walled parenchymatous cells intercalated with tanniniferous cells (Figs. 2A and 4A), which are also intermixed with tracheoids: short or variously elongated tracheid-like cells, with annular to helicoid secondary wall thickenings. The tracheoids are mostly located next to the three vascular bundles (Figs. 2A, F and 5E). Through the stylopodium and style runs a thin canal, which is continuous with the ovary locule. This canal is lined by transmitting tissue, which is formed by very small glandular cells of dense cytoplasm (Figs. 2A, D–F and 4A). The style is composed of parenchymatous tissue, covered with a smooth cuticle-bearing epidermis of large

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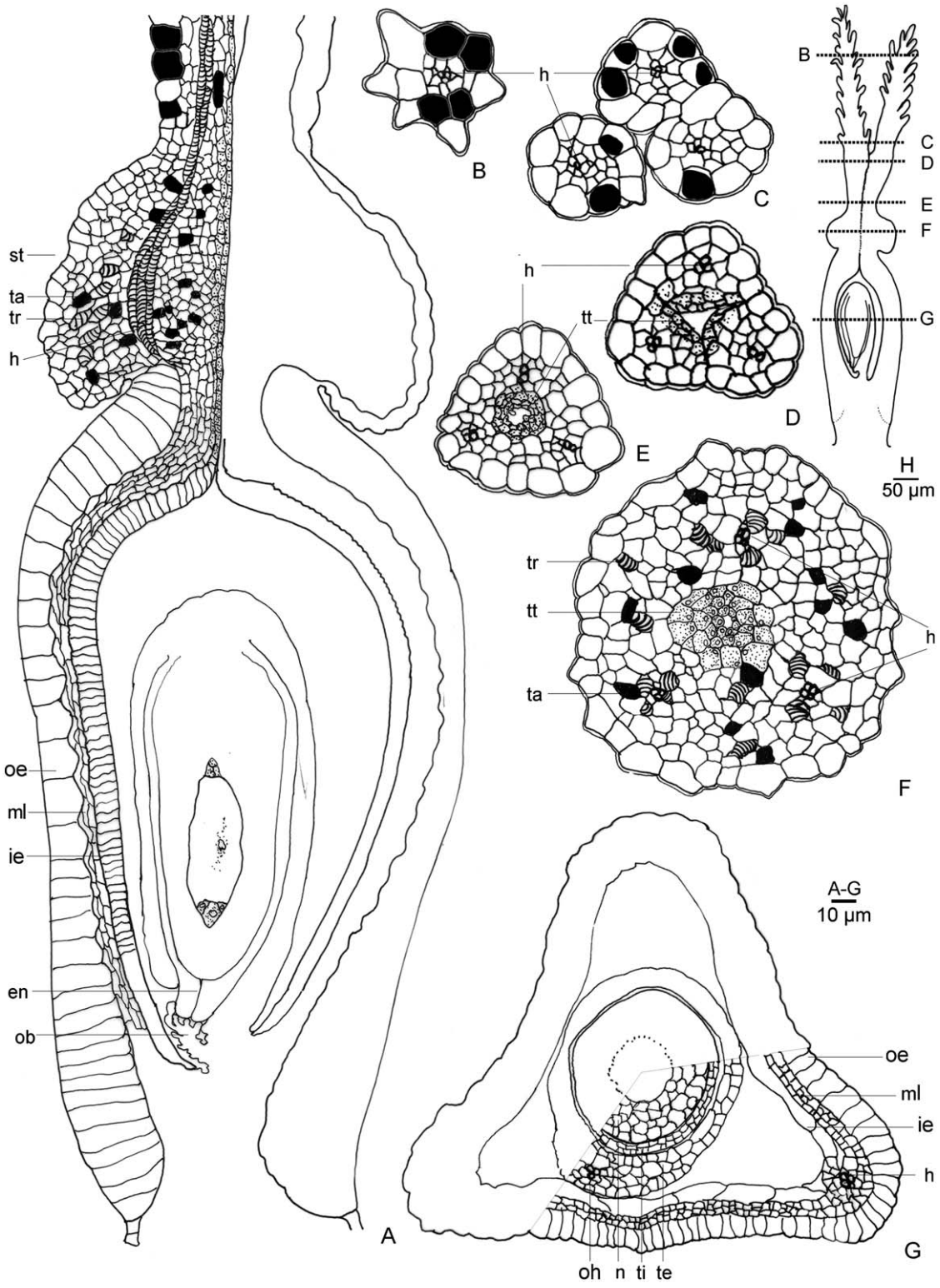

Fig. 2. Full-grown ovule and pistil of *B. major*. (A) Longitudinal section of the ovary, ovule and stylopodium. (B–G) Cross-sections of gynoecium as shown in Fig. H. (B, C) Stigmatic branches. (D, E) Style. (F) Stylopodium. (G) Ovary. (H) Longitudinal gynoecium scheme. en, endostome; h, bundle; ie, inner epidermis; ml, middle layers; nu, nucellus; oe, outer epidermis; ob, obturator; oh, ovular bundle; st, stylopodium; ta, tannin cell; te, outer integument; ti, inner integument; tr, tracheoids; tt, transmission tissue.

cells (Fig. 2E). In the mature nutlet, the stylopodium remains attached to the pericarp (Figs. 4H and 5A and H), its epidermal and parenchymatous cells collapse, showing a wrinkled surface, and its tanniniferous cells and tracheoids remain unaltered (Figs. 4H and 5E).

Stigma

The stigma is composed of three papillose branches (Fig. 2C and H) covered with a smooth and thin cuticle. The stigmatic surface is dry. There is one papilla per cell, formed by the distal

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region of the epidermal cell projecting upwards. Each papilla presents a special wall thickening formed by annular bands along its length and a lenticular thickening at the apex. These thickenings give it the appearance of a multicellular, articulated or moniliform trichome. *B. major* and *B. capillaris* show 5–8 and 3–5 annular bands, respectively (Fig. 4C and D). The epidermal cells between the papillae have large vacuoles in the cytoplasm that are completely full of tannic compounds (Figs. 2B and 4D).

Vascularization of the gynoecium

The vascular supply to the gynoecium derives from the last traces of the floral stele. Each carpel has one dorsal bundle and does not have marginal traces.

The three dorsal carpellary bundles are continuous through the stylopodium, supplying each stigmatic branch up to the last portions (Fig. 2C–F). The dorsal carpellary bundle runs through the middle layers of the carpel. These bundles are collateral and are mainly formed by xylem elements (Fig. 2G). The terminal cells of the bundles are xylem elements with annular thickenings (Figs. 2B and 4C). The ovular bundle derives from the final ramifications of the stele, runs through the funiculus and raphe, and ends blindly in the chalaza (Fig. 2G). In the mature fruit, the three carpellary bundles are not branched and run through the mesocarp.

Ovule anatomy and development

In both species the ovule primordium originates as a protuberance on the placenta at the base of the ovary (Fig. 1A and E). In the primordium, two zones are distinguishable: (1) a dermal layer where anticlinal divisions predominate and (2) a central mass of cells where divisions contribute to increase the volume of the primordium. In the latter, a megaspore mother cell covered by three to five layers of parietal cells, differentiates and results in a crassinucellate ovule. The megaspore is conspicuous because of its larger size and voluminous nucleus (Fig. 1A and B).

The nucellar apex is curved downwards by the increasing cellular divisions; the growth of both integuments begins simultaneously (Fig. 1C and F). The inner integument is formed by anticlinal and oblique divisions from dermal layers of the ovular primordium in an annular zone around the nucellus. The outer integument is also developed from a few cells of the dermal layer, appearing as a discontinued ring at the concave side of the ovule primordium. By the time that the nucellus reaches 90° of curvature, each integument is two cell layers thick (Fig. 1C). At this stage, the funiculus becomes visible, and is thicker on the abaxial side of the curvature (Fig. 1C). Finally, the nucellus is inverted and an anatropous ovule is formed (Fig. 1D and G). The inner integument envelops the nucellus, forming an endostome at the micropylar end, whereas the outer integument remains shorter (Fig. 1D). The megaspore mother cell completes meiosis, developing four megaspores, of which the chalazal one is the largest and the only one remaining, since the other three megaspores degenerate.

In a mature ovule, previous to fecundation, the number of integument layers does not increase. Each integument consists of two cell layers (Figs. 2G and 4B). These cells have translucent cytoplasm and evident nucleus. The inner epidermis of the inner integument has cells with a large tannin-containing vacuole (Fig. 4E). The outer integument is shorter, so that the micropyle is formed only by the endostome, where the tissue of inner integuments is up to five cell layers thick (Figs. 1D and 2A). The epidermis of the inner part of the outer integument, incompletely formed, consists of irregular cells that form a papillose surface, developing an obturator, which is in direct contact with the endostome. The obturator is completely developed at the megaspore tetrad stage (Fig. 1D).

The ovule has an embryo sac of the *Polygonum* type (Figs. 2A and 4B). The nucellus cells next to the chalaza have tannic compounds.

Ontogeny of the seed and the fruit

The lignification of the cells of the obturator is the first obvious change after fertilization. The obturator does not degenerate but its walls thicken and remain even in advanced stages of the embryonal development (Fig. 4G).

After fertilization, the only layer of the ovule integuments that continues differentiation is the inner epidermis of the inner integument. The epidermis cells increase in size, their cytoplasm is filled with a large tannic-containing vacuole (Fig. 4E), the internal tangential wall becomes hemispheric to wavy, incrusted between nucellar cells and they finally come into direct contact with the cells of the endosperm (Fig. 4F). A conspicuous, refringent cuticle covers these cells and the remaining layers of both integuments are crumpled (Fig. 4F and G). In young seeds, the tanniniferous cells of the nucellus, next to the chalaza, increase in size and develop a hypostasis.

The divisions of the primary endosperm nuclei and the daughter nuclei are not followed by cytokinesis and the resulting nuclei occupy the periphery of the embryo sac, around a large central vacuole. The endosperm wall formation is initiated at the chalazal side and finishes next to the embryo at globular stage (Fig. 4F and G). The endosperm has polygonal thin-walled cells and abundant reserve substances are stored in the form of globoid and prismatic protein bodies.

The embryo has a wide turbinate form with a terminal cotyledon and the embryonic axis is totally recurved; radicle and plumule in basal position are oriented toward the micropyle (Fig. 3K and L). The plumule is protected by one cotyledonary sheath, and is shorter than the radicle, which is covered by a calyptra (Fig. 3K and L). A mainly procambial trace links the radicle with the plumule and the cotyledon (Fig. 3K and L).

In *B. capillaris* the formation of one to four embryos was observed in the same embryo sac (Fig. 4G). Although these embryos are always located at the micropylar zone of the ovule, we were not able to obtain histological sections where their origin was observed. Only one embryo continues the development; the others degenerate at the globular stage.

Pericarp

The mature nutlets of *B. major* are obovoid, trigonous, basally attenuated, and 1.5 mm long × 1–1.25 mm wide; the stylopodium is cylindrical. The surface of the nutlet, as observed with SEM, consists of longitudinally elongated cells, and the outer wall is convex, with one central nipple-shaped protuberance per cell (Fig. 5A and B). In *B. capillaris* the fully grown nutlets are pear-shaped, trigonous and 0.75–1.2 mm long × 0.5–0.75 mm wide with a conical stylopodium. The surface is made up of longitudinally elongated cells, whereas the outer wall is granular, and plane to slightly convex (Fig. 5H and I).

During the ontogeny of both species, the number of wall layers of the carpel does not increase during the development of the fruit, only its differentiation takes place. It is in the post-fertilization stage of the ontogenetic development when the differences between the species studied in this work appear. Fig. 3A shows the spatial organization of the different layers of the pericarp.

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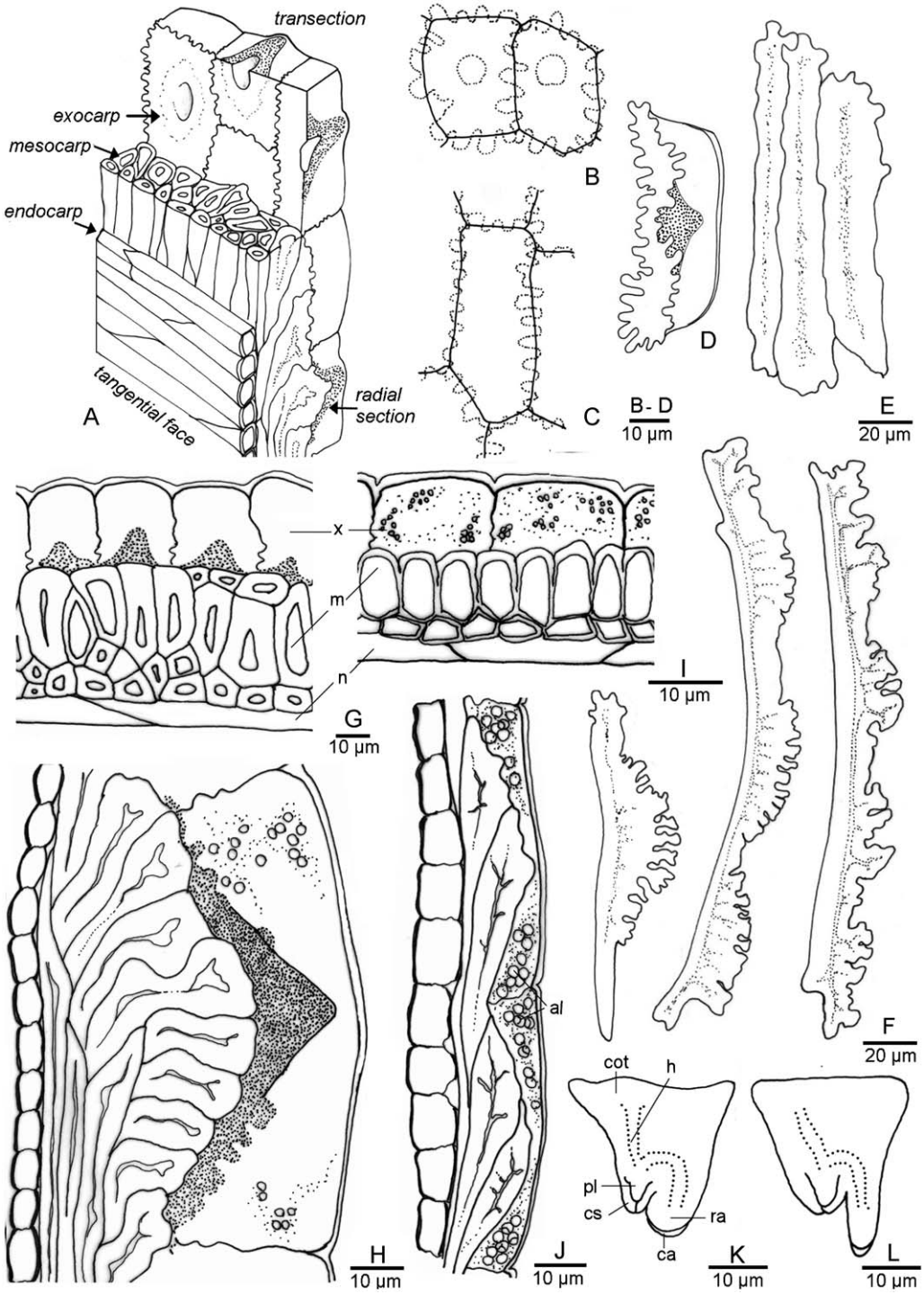

Fig. 3. (A) Block diagram of portion of the pericarp. (B, C) Exocarp cells in front view. (D) Exocarp cell in three-dimensional lateral view. (E, F) Sclereids of macerated mesocarp. (G, I) Cross section of young pericarp. (H, J) Longitudinal section of the mature pericarp. (K, L) embryos. al, starch grains; ca, calyptra; cot, cotyledon; cs, cotyledonary sheath; h, bundle; m, mesocarp; n, endocarp; pl, plumule; ra, radicle; x, exocarp. *B. major*: A–D, G–H and K; *B. capillaris*: E–F, I–J and L.

Exocarp

The exocarp derives from the outer epidermal cells of the carpel wall (Fig. 3A, G and I). The following differences can be observed between the two species studied:

B. major: in front view the exocarp cells are approximately polygonal (Fig. 5B). The outline of the external wall is smooth (Fig. 5B), whereas the internal wall is much undulated (Fig. 3B). These characters are easily observed in dissociated material (Fig. 3D) or in SEM micrographs, with previous elimination of the outer wall (Fig. 5F). The cuticle that covers the cell is finely granulose (Fig. 5B).

The exocarp cells observed in cross-section have an outer wall that is straight to slightly convex, thick and lignified. The radial walls are straight towards the external face and undulated towards the internal face. The internal wall is concave and adjusts

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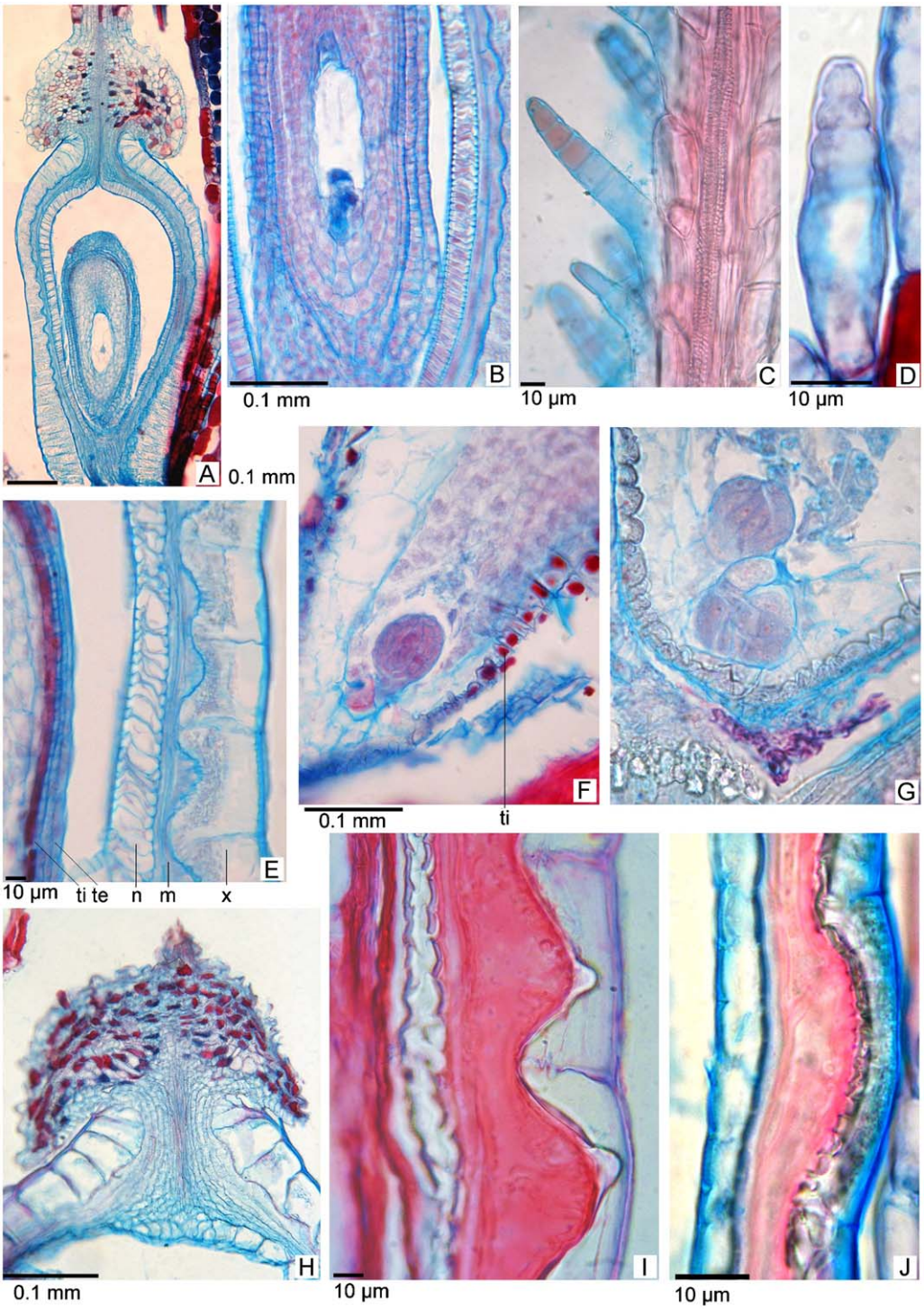

Fig. 4. (A–D) Longitudinal section. (A) Full-grown ovary with stylopodium. (B) Detail of the mature ovule. (C–D) Stigmatic papillae. (E) Longitudinal sections of the young pericarp. (F) Globular embryo. (G) Polyembryony. (H) Longitudinal section of the stylopodium. (I–J) Longitudinal section of the mature pericarp. m, mesocarp; n, endocarp; te, outer integument; ti, inner integument; x, exocarp; *B. major*: A–B, D–F and H–I; *B. capillaris*: C, G and J.

its shape to the protruding cells of the mesocarp (Fig. 4E and I). In the young fruit the cells of the exocarp have many compound starch granules that degrade during the silica accumulation (Fig. 3H). A conoidal silica body, which protrudes to the exocarp cell lumen, is formed between the exo- and the mesocarp (Figs. 3A, G, H, 4I and 5C). In surface view of the mature fruit the top of the silica body appears like a nipple-shaped protuberance in each exocarp cell (Fig. 5A and B). When the development of the fruit is completed the silica impregnates all the internal walls of the exocarp cells (Fig. 5D).

B. capillaris: in front view the exocarp cells are tetragonal to hexagonal and have a granular surface and a smooth cuticle (Fig. 5H and I). These cells have a smooth outline in external face view and a much undulated one in the internal face (Figs. 3C and 5I, K, M). In cross-section the outer wall is straight, thicker and cellulosic (Fig. 5J). The inner wall is slightly concave with respect

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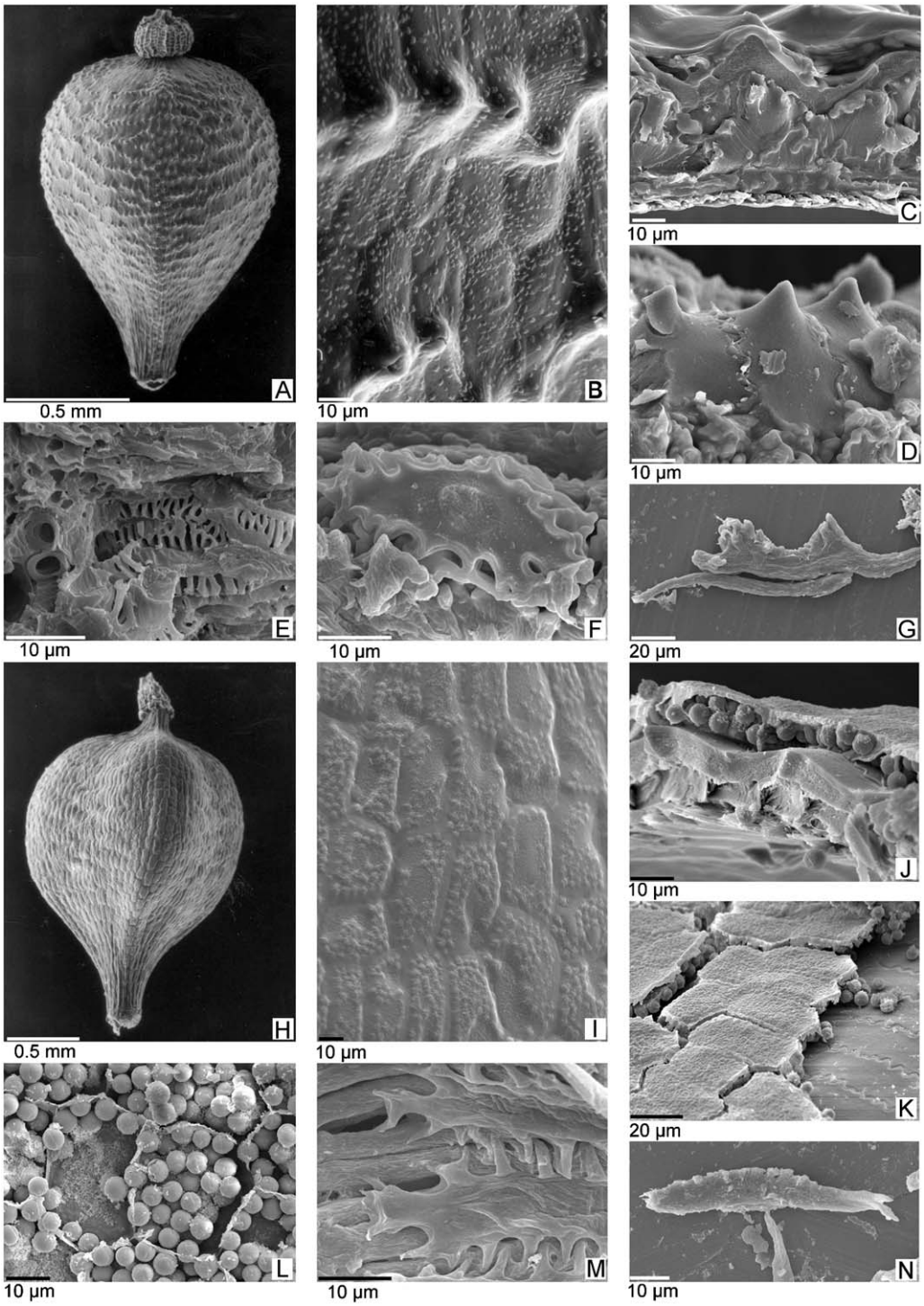

Fig. 5. SEM photographs. (A, H) Nutlet. (B, I) Detail of exocarp surface. (C, J) Cross-section of the pericarp. (D) Exocarp cell without tangential wall, showing silica body. (E) Tracheoids of stylopodium. (F, M) Inner wall of exocarp cell. (G, N) Sclereids of macerated exocarp. (K) Exocarp surface. (L) Exocarp without tangential wall, showing starch granules. *B. major*: A–G; *B. capillaris*: H–N.

to the surface (Figs. 3J and 4J). The cytoplasm is fully occupied by compound starch granules formed by 4–10 elements (Figs. 3J and 5K, L). This species lacks silica bodies.

Mesocarp

The mesocarp is composed of several layers of sclereids derived from the middle layers of the carpels (Fig. 3A). The mesocarp comprises two to three layers in *B. capillaris* and up to five layers in *B. major* (Fig. 3G–J). The dissociated material shows that the external wall has several excrescences (Figs. 3F and 5G, N). These excrescences fit together very tightly, which makes them difficult to see in the longitudinal cuts of the pericarp (Fig. 4I and J). The wall of the sclereid is lignified and extremely thick, with simple and occasionally branched pits; the lumen is almost fully obliterated at maturity (Figs. 3E, F and 4I, J).

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Endocarp

The endocarp develops from the inner epidermis of the carpel and is composed of elongated cells, with tapered ends that are transversely arranged in the fruit (Fig. 4A, G, I). These cells have slightly thicker but not lignified tangential walls (Fig. 3H and J).

Discussion

Ovule and seed development

In both *Bulbostylis* species studied, the development of the ovule concurs with the description given by Goetghebeur (1998). The megaspore mother cell divides meiotically and a monosporic embryo sac of the *Polygonum* type is formed. This pattern is also consistent with that of Cyperaceae in general (Goetghebeur, 1998), *Fimbristylis dichotoma* (L.) Vahl (Gupta, 1962) and species of *Kyllinga* Rottb. (Khanna, 1965; Padhye, 1971). The development of both *Bulbostylis* species studied in this work is also similar to that of *Fimbristylis*: the ovule reaches the anatropous curvature when the tetrad of megaspores is observed. At this stage the integuments achieve their maximum development.

The slower development of the outer integument at the raphal side is a common situation in an anatropous ovule (Bouman, 1984). In the ovular primordium of *Bulbostylis*, the small protuberance developed in the raphal side of the funiculus becomes a glandular obturator, thus suggesting it has an integumentary origin. Goetghebeur (1998) characterized the family Cyperaceae with a funicular obturator that appears in early development simultaneously with the meiosis of the megaspore mother cell. Padhye (1971) observed the same in *Kyllinga brevifolia* Rottb., and found that in other species (like *K. triceps* Rottb., *Pycreus globosus* (All.) Rchb. and *Cyperus iria* L.) the obturator is organized only when the embryonic sac is fully formed. According to Bouman (1984) this structure usually degenerates after fertilization. The lignification of the obturator and its conspicuous presence, even in the seeds, is remarkable.

The hypostasis appears as a cluster of tanniniferous cells in early development of the ovule and persists in mature seeds. A similar condition has been observed in a few species of Cyperaceae (Khanna, 1965; Padhye, 1960, 1971), and according to Werker (1997) and Bouman (1984) the presence of the hypostasis is a feature of the family.

The seed does not develop a seed coat with a mechanical layer, which is expected, because the fruit is indehiscent. In *Bulbostylis* nutlets, the role of mechanical protection is transferred from the seed coat to the pericarp, and in particular, to the mesocarp. The seed coat itself is usually undifferentiated. The inner epidermis of the inner integument develops the endotegmen, with tanniniferous cells.

The endosperm is nuclear and becomes cellular by centripetal cellularization. This type has also been found in *Kyllinga* Rottb., *Cyperus* L. and *Scirpus* L. (Khanna, 1965; Padhye, 1971) as well as in other Cyperaceae (Munro and Linder, 1997). This is one of the differences with Juncaceae (Munro and Linder, 1997).

The embryos of the two species studied correspond to the *Bulbostylis* type (Van der Veken, 1965). Polyembryony was observed only in *B. capillaris*: up to four embryos reach the globular stage of development, but only one of them completes its growth. Further examinations are required to determine the origin of multiple embryos. Shah (1965) described polyembryony from synergids in *Cyperus articulatus* L.; Juguet (1967) mentioned the presence of a double embryo sac in *Kobresia bellardii* (All.) Degld. and *Carex arenaria* L. Thus, although its origin is not always the same, it seems that polyembryony is a common condition in Cyperaceae.

Fruit development

At the pre-fertilization stage, the structure of the gynoecium is the same in both *B. capillaris* and *B. major*. A typical tricarpellar ovary normally has three dorsal and three marginal bundles (Esau, 1977). However, the two *Bulbostylis* species studied presented only three main bundles, and because these bundles were continuous through the style and stigmatic branches, we considered them as dorsal bundles. On the basis of the position of the dorsal bundles, together with the absence of other carpellary bundles, a reduction of carpellary supply can be deduced.

In the present work two types of specialized cells were described in the stylopodium: tanniniferous cells and tracheoids, the second is mentioned for the first time for Cyperaceae family. Tracheoids are typical elements of the vein endings of the leaves, where their functions are associated with water storage, air storage and mechanical support (Lersten and Bender 1976; Metcalfe and Chalk, 1979; Rao, 1991; Tucker, 1964). In reproductive organs, tracheoids have been described in seeds of Bignoniaceae (Lersten et al., 2002) and Papilionoideae where they form the "tracheid bar" (Lersten, 1982), and their functions are associated with seed dispersion and short distance water transport (Fahn, 1990; Fahn and Cutler, 1992; Lersten et al., 2002; Rao, 1991).

The stylopodium persists in the mature fruit, which is indehiscent. Consequently, the seed coat functions are transferred to the pericarp. Therefore, we consider that the presence of tracheoids in the stylopodium could be related to the water uptake for germination. The other idioblasts found in the stylopodium are tanniniferous cells. Their presence is usually associated with resistance to pathogens as well as with the delay in the decomposition of plant parts in the soil (Evert, 2007).

Based on morphological characters, the style of angiosperms is described as dry and corresponding to Group I (Heslop-Harrison and Shivanna, 1977). In both *Bulbostylis* species studied, the stylopodium and the style are hollow and the canal is lined with a glandular transmitting tissue. The stigmatic branches also have a papillose epidermis, which Goetghebeur and Coudijzer (1984) described as formed by moniliform trichomes. Since our observations show that each papilla is unicellular and seems to be articulated, we named them "pseudo-articulated". A similar characteristic was described by Raynal (1973) in the tribe Fimbristylideae (Abildgaardieae).

During the ovary ontogeny and fruit development, the outer epidermis of the carpel is the tissue that undergoes the greatest differentiation. In the *Bulbostylis* fruit wall, we described the middle layers as composed of sclereids with an elaborate shape, which can be observed only in dissociated material. The exocarp cells also have an undulated internal wall in close contact to the excrescences of the sclereids. Both species have starch granules in the exocarp of young nutlets. Only in *B. major* does the process of silicification take place where starch granules degraded during ontogeny; in addition, one silica body is formed in each cell. Starch granules, responsible for the smooth granulosity on the mature fruit surface, were found in all mature fruits of the *B. capillaris* analyzed. Ragonese et al. (1984) reported the presence of starch granules associated with silica bodies in *Rynchospora scutellata* Griseb. but did not use ontogenic analysis to make a correlation between both structures. A similar situation occurs in *Cyperus rotundus* L., which presents starch granules in the exocarp (Khanna, 1965). In the genus *Cyperus*, the presence of silica bodies is one of the features used in the differentiation of species (Denton, 1983).

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Taking into consideration the importance of the micro-morphological characters of the nutlets in Cyperaceae, and the fact that this fruit represents a unit of dissemination, we recommend the terminology proposed by Corner (1976) and Barthlott (1981) for the description of the seed surface. In *Bulbostylis* the three layers of the carpellary wall undergo modifications during the development of the fruit, without increasing the number of layers. Because of this, the wall of this fruit is described as non-multiplicative.

The differences between both species studied appear after fertilization, and are determined by a combination of several characters of the exocarp layer. In *B. major,* the primary sculpture is tuberculate due to the presence of one silica body per cell, whereas in *B. capillaris,* it is granular due to the presence of starch granules that are distinguishable when the outer cell wall is wrinkled. In *B. major,* the secondary sculpture is specified by the fine relief of the cell wall and the surface is micro-granular due to the presence of short cuticular granules, whereas in *B. capillaris* there is no secondary sculpture and the surface is smooth. These characters are independent of the habitat in which the plant grows.

Conclusions

The present work describes new characters in *B. major* and *B. capillaris*, with reference to the ovules and embryo development (presence and permanence of the obturator, hypostasis, polyembryony), pistil (anatomy and vascularization of the ovary wall, tracheoids in stylopodium, style and stigma anatomy) and fruit surface pattern.

The ontogenetic analysis of the nutlets provides a concrete basis for understanding the differences between species and the presence or absence of silica bodies is one of the most important characters that determine the differences between the pattern of the nutlet surface of each species studied.

We thus propose to use the specific and generally accepted terminology of seed coat (Barthlott, 1981; Corner, 1976) for the descriptions of the nutlet.

Micro-morphological characters such as shape, surface and structure of the exocarp are recognized as valuable in the diagnosis of both of *Bulbostylis* species studied.

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Appendix. Collection data of *Bulbostyls* studied, specimens are from CTES Herbarium, unless otherwise indicated.

Bulbostylis major. ARGENTINA. **Corrientes**, Empedrado: *López* et al. 101 (MO), *Schulz* 12250. Ituzaingó: *Carnevali* 5479, *Krapovickas* et al. 11978, *Quarín* et al. 2768, *Tressens* et al. 6615, *Vanni* et al. 3035. Santo Tomé: *Pedersen* 9151, *Schinini* et al. 23734. **Entre Ríos**, Concordia: *Burkart* 995. **Jujuy**, Capital: *Cabrera* et al. 30644. **Misiones**, Capital: *Arbo* et al. 1857, *Guillén* et al. 432. San Ignacio: *Krapovickas* et al. 28727, *Krapovickas* et al. 44623. San Javier: *Krapovickas* et al. 15261. San Pedro: *López* et al. 316. BOLIVIA. **Chuquisaca**: *Saravia Toledo* et al. 11716. BRAZIL, **Paraná**, Curitiba: *Dombrowski* 5939. Palmeira: *Hatschbach* et al. 35889, *Kummrow* 1468, *Pedersen* 10933. **Rio Grande do Sul,** Quarai: *Pedersen* 12562. Guaíba: *Pinto Silva* 3957. **São Paulo**, Angatuba: *Souza* et al. 10652. Itapeva: *Souza* et al. 8625. Suzano: *Hashimoto* 19966. PARAGUAY. **Alto Paraná:** *López* et al. 267. **Amambay**, Cerro Corá: *Krapovickas* et al. 14174, *Morrone* et al. 433, (SI). **Caaguazú:** *López* et al. 215. **Canindeyú:** *Schinini* et al. 33389. **Cordillera:** *Pedersen* 10084. **Guairá:** *Bordas* 1594. URUGUAY. **Rivera:** *Dematteis* et al. 1494-A, *Pedersen* 13875. **Tacuarembó:** *Dematteis* et al. 1816, *Pedersen* 11627.

Bulbostylis capillaries. ARGENTINA. **Chaco**, Alte. Brown: *Bordón* 654. Colonia Benitez: *Schulz* 671. San Fernando: *Schulz* 14304. **Corrientes**, Capital: *López* 54. Concepción: *Vanni* et al. 4086. Empedrado: *Schinini* et al. 12801. Esquina: *Ahumada* et al. 1348. General Paz: *Carnevali* et al. 2550. Goya: *Krapovickas* et al. 17815, *Schinini* et al. 18917. Itatí: *Carnevali*, 592. Ituzaingó: *Carnevali* 6205B. Mburucuyá: *Vanni* et al. 4073. Mercedes: *Carnevali* 3812, *Sosa* et al. 50. Monte Caseros: *Ahumada* 2789, *Schinini* et al. 17577, *Schinini* et al. 18343. San Cosme: *Schinini* et al. 27493. San Roque: *Carnevali* 2136 bis. **Entre Ríos**, Federación: *Bacigalupo* et al. 954, *Burkart* 22481, *Troncoso* et al. SI-27266 (SI). **Formosa**, Pilcomayo: *Guaglianone* et al. 543. **Misiones**, San Ignacio: *Rodriguez* et al. 399. **Santa Fé.** Gral. Obligado**:** *Castigliani* et al. 75124. **Santiago del Estero**, Choya: *Ulibarri* 974. BRAZIL **Paraná**, Pinhão: *Hatschbach* et al. 55251. **Rio Grande do Sul:** *Krapovickas* et al. 22812. **Porto Alegre:** *Lindeman* ICN 20803. PARAGUAY. **Amambay:** *Krapovickas* et al. 46122. **Boqueron**, col. Mennonita, Lolita: *Vanni* et al. 1807. **Caaguazú:** *Arsamendia* 32. **Central**, Lambaré: *Arenas* 368, *Krapovickas* et al. 45205, *Mereles* 310 (FCQ). **Concepción:** *Lurvey* 105, *Mereles* 1319 (FCQ). **Cordillera:** *Degen* 409 (FCQ). Areguá: *Mereles* 794. Cerro Tobatí: *Zardini* et al. 1987. **Guairá** *Schinini* et al. 25076. **Itapúa:** *Schinini* et al. 28372. **Olimpo**, Pto. María Auxiliadora: *Arenas* 352. **San Pedro**: *Soria* 6457. URUGUAY. **Paysandú:** *Marchesi* et al. MVFA 20856, **Flores:** *Solis Neffa* et al. 119.

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