Protoplasma (2022) 259:141–153
https://doi.org/10.1007/s00709-021-01649-7

ORIGINAL ARTICLE

A comparative approach to understanding the ovule, seed, and fruit development in *Bulbostylis* (Cyperaceae: Cyperoideae: Abildgaardieae)

Andrea Guadalupe Reutemann[1,2] · Sebastián Elias Muchut[1] · Nora Graciela Uberti Manassero[1,2] · André Luís Laforga Vanzela[3] · María Gabriela López[4] · Abelardo Carlos Vegetti[1] · Ana Maria Gonzalez[4,5]

Received: 26 October 2020 / Accepted: 12 April 2021 / Published online: 27 April 2021

Abstract

In the present work, we study the ovule, seed, and fruit development in six *Bulbostylis* species in order to characterize the genus in a comparative approach and to identify the characteristics that can be used in taxonomy and phylogeny. Flowers and fruits at different developmental stages were analyzed using LM and SEM after processing according to standard techniques. The species studied have the following: anatropous and bitegmic ovules, weak crassinucellar ovules, obturator of integumentary origin, monosporic embryo sac of the *Polygonum* type, nuclear endosperm, hypostase formation, seed coat formed by tanniferous endotegmen and exotesta, and *Bulbostylis*-type embryo. On the other hand, the pericarp development constitutes the main variation within *Bulbostylis* since the cells of the exocarp may or may not present starch grains, and their inner periclinal walls may be slightly or deeply concave depending on the degree of development of the mesocarp sclereids. In a taxonomic context, the results herein obtained are in conflict with studies which suggest infrageneric groupings based on fruit micromorphology, and also with the relationship among the *Bulbostylis* species based on molecular analysis. This work contributes to a better understanding of the reproductive anatomy and embryology in *Bulbostylis*, and reveals the first insights about the origin of multiple embryos in Cyperaceae. Given the frequent presence of polyembryony in *Bulbostylis*, and the poor mention of this condition in the family, this work highlights an aspect in the anatomy of Cyperaceae that must be re-explored.

Keywords Achene · Anatomy · Development · Embryology · Polyembryony · Reproductive structures

Introduction

Bulbostylis Kunth (tribe Abildgaardieae) has *c.* 220 pantropical to warm temperate species, mainly concentrated in tropical Africa and South America. These species commonly grow in open, sunny habitats on dry or temporarily wet soils (Goetghebeur 1998; Govaerts et al. 2020). The high degree of similarity of the vegetative and reproductive organs among the *Bulbostylis* species is a great complication for establishing infrageneric groupings. The *Bulbostylis* species present leaves with long white hairs at the sheath; inflorescences with few-many spikelets (arranged in anthelodium or cephalodium) or reduced to a single spikelet; and perfect perianthless flowers, with 1–3 stamens, 3(-2)-fid styles with their base thickened and persistent (rarely deciduous) on the ripe fruit. The fruit in *Bulbostylis* is a trigonous (rarely biconvex) achene, about 0.6–1.8 mm long by 0.5–1.4 mm wide, with exocarp cells vertically elongated, and the mature embryo is classified as of the *Bulbostylis*-type embryo (Van

Handling Editor: Dorota Kwiatkowska.

✉ Andrea Guadalupe Reutemann
areutemann@fca.unl.edu.ar

[1] Instituto de Ciencias Agropecuarias del Litoral, Universidad Nacional del Litoral, Consejo Nacional de Investigaciones Científicas Y Técnicas, Kreder 2805, Esperanza, Santa Fe, Argentina

[2] Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina

[3] Universidade Estadual de Londrina, Londrina, Paraná, Brasil

[4] Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina

[5] Instituto de Botánica del Nordeste, Universidad Nacional del Nordeste, Consejo Nacional de Investigaciones Científicas Y Técnicas, Sargento Cabral 2131, Corrientes, Argentina

der Veken 1965; Kral 1971; Goetghebeur 1998; López 2012; Reutemann et al. 2012).

In general, only exomorphological characters of the mature achenes allow to distinguish among the species of this genus (Barros 1947; Svenson 1957; Pedersen 1969; Kern 1974; Kral 1971; Goetghebeur and Coudijzer 1985; Goetghebeur 1998; López 2012). In a taxonomic study of 20 *Bulbostylis* species from the Southern region of South America, López and González (2017) suggest informal groupings within the genus based on the micromorphology of the fruit surface. The proposed groups include species whose fruits have cells of the exocarp without silicophytoliths (group 1, eight species), with rounded silicophytoliths, occupying most of the cell (group 2, four spp.), and with conical silicophytoliths, located at the center of the cell or eccentrically (group 3, eight spp.).

However, recent phylogenetic analyses of *Bulbostylis*, based on nuclear (ITS region) and plastid sequences (*trn*L intron), have pointed out that these groups are not monophyletic (Reutemann et al. 2018). According to Reutemann et al. (2018), the "micromorphological patterns of the achene surface" are homoplastic and therefore unsuitable to propose infrageneric groupings within the *Bulbostylis*. Based on these data, it is necessary to find new traits that contribute to clarify the relationships within the genus. The study of unexplored reproductive structures in *Bulbostylis* could provide new characters to support the infra-generic groupings suggested by the morphology of the fruits (López and Gonzalez 2017), to support the relationships indicated by the molecular phylogeny of the genus (Reutemann et al. 2018), or to reveal unexpected relationships among the *Bulbostylis* species. The establishment of a phylogenetic framework in a group brings new opportunities for morphological studies and often helps to find problems in the definition of character states (Tobe 1989; Endress et al. 2000).

In many plant groups, anatomical and developmental studies of reproductive structures are a reliable source of traits to overcome taxonomic problems (Ornduff 1978; Endress 2011). For example, embryological findings such as pollen formation, the fate of the antipodes after fertilization, and the type of embryogeny have questioned the Cyperaceae-Poaceae closeness sustained by early research and have supported the closeness between the Cyperaceae and Juncaceae families (Shah 1967; Singh 1981), which is currently supported by the recent plastome phylogeny proposed by Givnish et al. (2018), where Cyperaceae is sister to Juncaceae.

Several taxa of Cyperaceae have been characterized embryologically and share some characters, such as follows: anatropous, bitegmic and crassinucellated ovule, hypostase and funicular obturator, *Polygonum* embryo sac with ephemeral antipodes, nuclear endosperm, embryogeny of the *Onagrad*-type, *Juncus*-variation, and albuminous seed with seed coat formed by exotesta and endotegmen (Khanna 1965; Shah 1965; Murty and Kumar 1967; Padhye 1968, 1971; Nijalingappa 1976, 1977, 1986; Padhye and Makde 1982; Makde and Bhuskute 1987; Johri et al. 1992; Nijalingappa and Palakshaiah 1998; Rudall 1997; Coan et al. 2008; Kellogg 2015; Rocha et al. 2015).

The available knowledge on the embryological aspects of Cyperaceae is too fragmentary for some genera (Coan et al. 2008). The anatomy and development of the seed and fruit, the organs from which they derive (i.e., the ovule and ovary, respectively), and the processes that take place within them have not been compared among the *Bulbostylis* species. Gonzalez and López (2010) studied the anatomy and development of the gynoecium and fruit in only two *Bulbostylis* species: *B. communis* M.G. López & D. Simpson, and *B. sphaerolepis* (Boeck) Beetle; however, these authors focused on characterizing the components of the pericarp that determine the differences in the achenes surface between these two species, without analyzing their embryology.

This article aims to provide a detailed description of the ovule morphology, megasporogenesis, megagametogenesis, and seed and fruit development in six *Bulbostylis* species belonging to groups 1 and 3 as proposed by López and Gonzalez (2017), and one species not characterized by these authors in order to characterize the genus in a comparative approach and to identify new traits which could support such groupings and thus contribute to the taxonomy of the genus.

Materials and methods

Plant material

Six *Bulbostylis* species were included in this study: *Bulbostylis brevifolia* Palla (A.G.Reutemann 186 [SF]); *Bulbostylis communis* M.G.López & D.A.Simpson (A.G.Reutemann 2, 189 [SF]); *Bulbostylis conifera* (Kunth) C.B.Clarke (AAI 26 [HRCB]); *Bulbostylis hirtella* (Schrad. Ex Schult.) Nees ex Urb. (M.G.López 363 [CTES]); *Bulbostylis juncoides* (Vahl) Kük. Ex Herter (A.G.Reutemann 7 [SF]); and *Bulbostylis wanderleyana* Prata & M.G.López (A.G.Reutemann 159 [SF]). For the scanning electron microscopy studies, only *B. communis*, *B. conifera*, and *B. juncoides* were analyzed. *Bulbostylis brevifolia* and *B. communis* belong to group 1 sensu López and Gonzalez (2017), while *B. hirtella*, *B. juncoides*, and *B. wanderleyana* belong to group 3; *B. conifera* was not studied by these authors.

Samples were collected from wild populations in Argentina and Brazil. Voucher specimens were deposited in the Herbarium of Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Santa Fe (SF), and Instituto de Botánica del Nordeste, Corrientes (CTES) in Argentina, and

also in the Rioclarense Herbarium (HRCB) of the Instituto de Biociências, Universidade Estadual Paulista in Brazil.

Scanning electron microscopy

Spikelets from at least four individuals of each species fixed in FAA were dissected in 70% ethanol under a Nikon SMZ-10 stereomicroscope, and dehydrated in an ethanol series up to absolute ethanol. The material was then transferred for 2 h to pure acetone. The dehydrated samples were critical-point dried (EMITECH K850 Critical Point Dryer) with CO2 as intermediate fluid. The dried material was coated with gold–palladium and observed and photographed using a PHILIPS XL 30 scanning electron microscope.

Light microscopy

Spikelets containing flowers at different development stages from at least four individuals of each species were fixed in FAA (formalin:acetic acid:70% ethanol, 10:5:85, v/v; Johansen (1940)) for 48 h and preserved in 70% ethanol (Johansen 1940). Spikelets were soaked in 5% hydrofluoric acid for 24 h to dissolve the silica (Metcalfe 1971). The material was dehydrated in an ethanol series, embedded in paraffin (Ruzin 1999), and sectioned at 10–12 µm on a rotary microtome (Reichert, Austria). The anatomical sections were stained with either safranin, fast green, Mayer's hematoxylin, or safranin-astra blue combinations (Johansen 1940; Luque et al. 1996), and mounted with synthetic Canada balsam (Biopack). The results were documented by photomicrographs obtained with a Leica DM 1000 microscope fitted with a Canon EOS REBEL T2i capturing device and digital imaging software (EOS Utility).

Developmental sequences of each studied species are shown in Electronic Supplementary Materials (ESM1–ESM10).

Results

Aspect of flower development

In all the species studied, the androecium is the earliest whorl that initiates from an undifferentiated flower primordium (Fig. 1a). Subsequently, an annular ovary wall primordium and a central ovule primordium emerge (Fig. 1b, c). As development progresses, three stigma primordia appear on the top of the ovary wall, being the abaxial one somewhat delayed (Fig. 1d). The annular/cylindrical ovary wall grows up from its base until it completely encloses the ovule (Fig. 1e, f). Later, the lengthening of the stigmatic branches occurs, and the style originates (Fig. 1g); a superior ovary with a single ovule in basal position is thus formed (Figs. 1a–g and 2a–c).

Ovule development

The ovule primordium presents a dermal layer and the archesporial cell, which is larger than the other cells and has a subdermal origin (Fig. 2a–d). The initiation of the inner integument occurs by periclinal divisions of dermal cells located in a lateral position (Fig. 2c–d, white arrows). The subdermal archesporial cell divides asymmetrically, originating a large megasporocyte and a small parietal cell that undergoes anticlinal divisions (Fig. 2e–f); simultaneously, the outer integument initiation occurs (Fig. 2e, black arrow).

Afterwards, the ovule primordium grows and becomes curved (Fig. 2e–i). The curvature of the ovule progresses until it reaches 180° by growth of the nucellus and integuments (Fig. 2j). The inner integument grows more rapidly than the outer integument, completely surrounding the nucellus and delimiting the micropyle (Fig. 2j).

The anti-raphe region of the outer integument is left behind in its development, and its edges do not close to form the exostome (Fig. 2j). The inner portion of the outer integument remains in contact with the endostome; the further development of this portion results in a mass of thin-walled cells with dense cytoplasm that covers the endostome, constituting an integumentary obturator (Fig. 2j–l).

In the mature ovule, both the inner and outer integuments are two-layered (Fig. 2l shows these layers at the anti-raphe region), although the inner integument at the endostome presents 4–5 layers (Fig. 2k–l). The cells of the obturator acquire glandular characteristics with dense cytoplasm (Fig. 2l).

The vascular bundle runs through the funiculus and reaches the chalaza without branching (Fig. 2k). When the embryo sac is fully formed, the nucellus remains composed of several layers of cells and with a discernible uni-layered epidermis at the micropylar region (Fig. 2k–l).

Megasporogenesis and megagametogenesis

At the beginning of the inner integument formation, the archesporial cell increases in size and divides to form the megasporocyte and a parietal cell; the megasporocyte presents a dense and granular content and the nucleus occupies most of the cell volume, showing clear nucleoplasm and a large nucleolus (Fig. 2f–i; Fig. 3a).

Before the ovule becomes completely anatropous, and before the closing of the inner integument delimiting the endostome, the megasporocyte undergoes meiosis. Meiosis I is transverse and leads to the formation of dyad cells, of which the chalazal cell is slightly elongated (Fig. 3b). Meiosis II is also transverse and originates a linear tetrad of

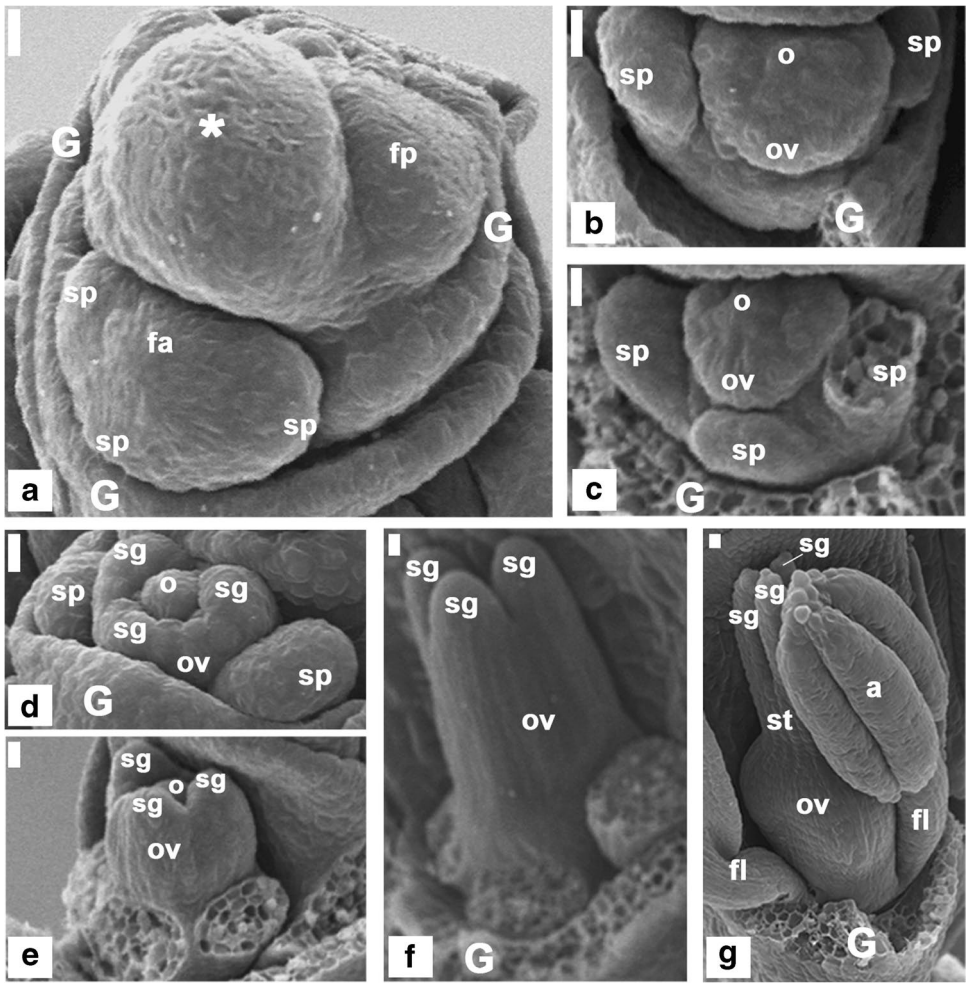

Fig. 1 SEM images of flower development in *Bulbostylis*. **a**–**c**, **e** *B. juncoides*. **d**, **g** *B. communis*. **f** *B. conifera*. **a** Apical view of a spikelet showing two early stages of flower development: an undifferentiated flower primordium, and another more developed flower primordium with stamen primordia and yet undifferentiated floral apex. **b** and **c** Flower primordium with an ovary wall primordium and an ovule primordium at the floral apex. **d** Next step in the differentiation of the flower primordium with a cylindrical ovary wall primordium and three stigma primordium, where the abaxial one is somewhat delayed; notice the single ovule primordium positioned in the center-basal region of the ovary. **e** and **f** Developing gynoecium with the ovary wall primordium completely enclosing the ovule, and with three distal stigma branches. **g** Gynoecium with differentiated stigma and style are differentiated. *a* anther, *fa* flower apex, *fp* flower primordium, *G* glume, *o* ovule (primordium), *ov* ovary wall (primordium), *sg* stigma (primordium), *sp* stamen (primordium), *st* style, * rachilla apex. Scale bars = 10 µm

megaspores (Fig. 3c). The chalazal megaspore of the tetrad enlarges and becomes functional, while the remaining three megaspores degenerate (Fig. 3c–d).

The functional megaspore undergoes three successive mitoses, forming an eight-nucleate *Polygonum*-type embryo sac (Fig. 3e–h). The egg apparatus at the micropylar pole is formed by a central egg cell and two lateral synergids. The filiform apparatus is well developed and visible in the histological sections due to its pecto-cellulosic composition (Fig. 3f, j–l). Three antipodal cells lie at the chalazal end (Fig. 3f–h). The two polar nuclei fuse to form the secondary nucleus before fertilization of the egg cell (Fig. 3i–j). At that time, the antipodes are already degenerating. Subsequently, double fertilization occurs, resulting in the formation of the zygote and endosperm mother cell (Fig. 3k–l). The filiform apparatus is still visible as a remnant of the degenerated synergid cells.

Seed development

Endosperm

After fertilization takes place, the endosperm mother cell divides before the division of the zygote, thus initiating the formation of the endosperm (Fig. 4a). In all the species studied, the endosperm is of the nuclear type. Successive

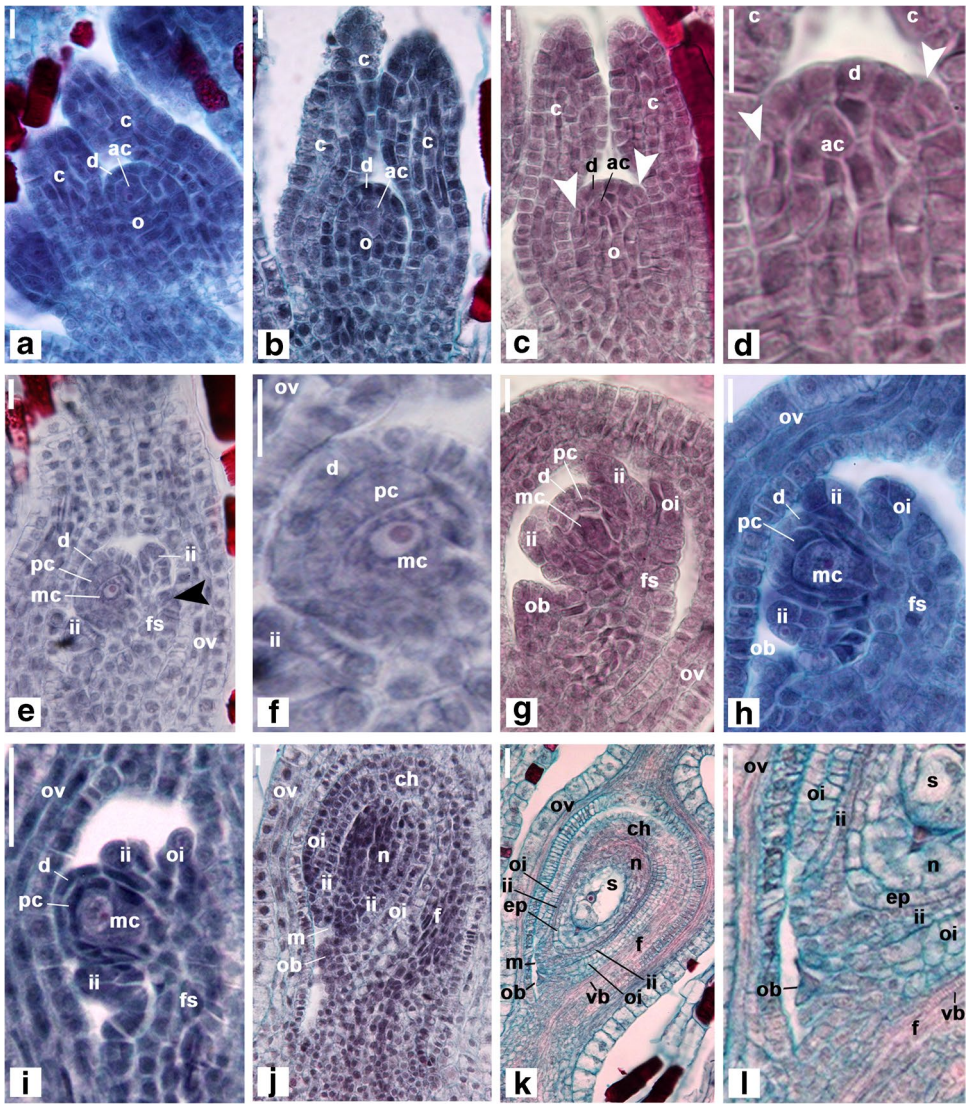

Fig. 2 Longitudinal sections of the ovule in *Bulbostylis* at different developmental stages (LM). **a**, **b**, **h**, **i**, **k**, **l** *B. communis*. **c**, **d**, **g** *B. hirtella*. **e**, **f** *B. brevifolia*. **j** *B. conifera*. **a**–**d** Ovule primordium with a dermal layer and a large subdermal archesporial cell; note in **c** and **d** the origin of the inner integument by periclinal division of dermal cells (white arrows). **e** Subsequent stage of ovule development, in which a parietal layer and a megasporocyte can be recognized under the dermal layer; note the outer integument initiation on the funicular side (black arrow). **f** Detail of **e** showing cells resulting from anticlinal divisions of the parietal cell. **g**–**i** Early stages of ovule curvature, where the initiation of the obturator is observed, and the internal and external integuments continue their differentiation. **j** Advanced stage of ovule curvature, in which the endostome and the obturator are recognizable in the micropylar region. **k** Mature ovule with embryo sac, the integuments covering the nucellus, the vascular bundle running through the funiculus, reaching the chalaza. **l** Detail of **k** corresponding to micropylar region and obturator. *ac* archesporial cell, *c* carpel, *ch* chalaza, *d* dermal layer, *ep* nucellar epidermis, *f* funiculus, *fs* funicular side, *ii* inner integument, *m* micropyle, *mc* megasporocyte, *n* nucellus, *o* ovule (primordium), *ob* obturator, *oi* outer integument, *ov* ovary wall, *pc* parietal cell, *s* embryo sac, *vb* vascular bundle. Scale bars: **a**–**h**, **j** = 10 µm; **i**, **k**, **l** = 50 µm

karyokinesis originate a set of nuclei, which are located towards the periphery of the cell due to the presence of a central vacuole (Fig. 4a). It is common to note numerous collapsed pollen tubes in the obturator (Fig. 4b).

Tannic substances are deposited in the cells of the endotegmen, and the same occurs in the cells of the nucellus near the chalaza, constituting a hypostasis (Fig. 4b–d). Subsequently, cells of the exotesta also become tanniniferous (Fig. 4e).

Each endosperm nucleus is surrounded by a mass of cytoplasm, delimited by a dense cortical array of microtubules and forming a nucleocytoplasmic domain (Fig. 4e–g). At this stage, the cells in the nucellus are progressively consumed and crushed except for the cells of

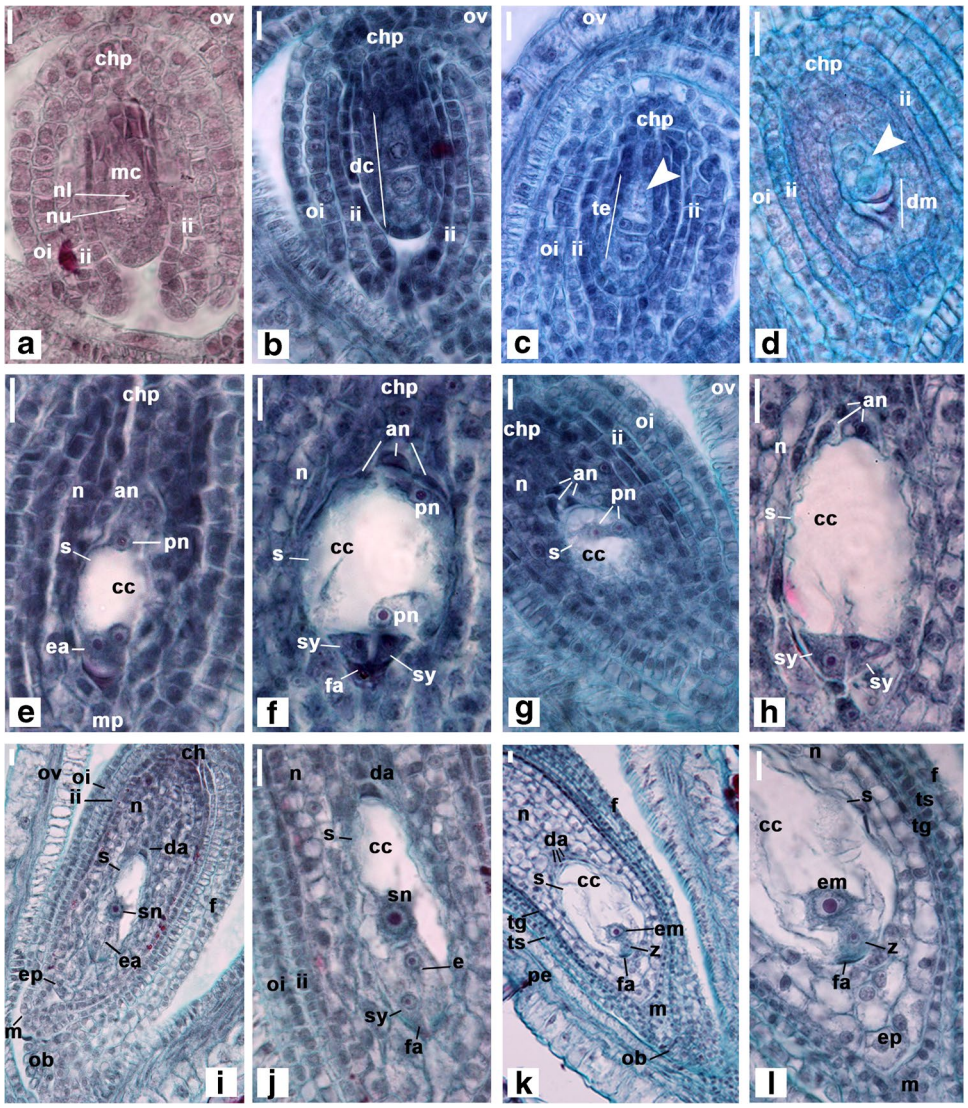

Fig. 3 LM images of embryo sac development in *Bulbostylis*, in longitudinal sections. **a** *B. hirtella*. **b**–**e**, **g**, **i**, **j** *B. communis*. **f**, **h**, **k**, **l** *B. juncoides*. **a**–**c** Megasporogenesis. **d**–**j** Megagametogenesis. **k** and **l** Newly fertilized embryo sac. *an* antipodal cell, *cc* central cell, *ch* chalaza, *chp* chalazal pole, *da* degenerate antipodal cell, *dc* dyad cells, *dm* degenerate megaspores, *e* egg cell, *ea* egg apparatus, *em* endosperm mother cell, *ep* nucellar epidermis, *f* funiculus, *fa* filiform apparatus, *ii* inner integument, *m* micropyle, *mc* megasporocyte, *mp* micropylar pole, *n* nucellus, *nl* nucleolus, *nu* nucleus, *ob* obturator, *oi* outer integument, *ov* ovary wall, *pe* pericarp, *pn* polar nucleus, *s* embryo sac, *sn* secondary nucleus, *sy* synergid cell, *te* megaspores tetrad, *tg* tegmen, *ts* testa, *z* zygote. The arrows in **c** and **d** indicate the functional megaspore before and after the first mitotic division, respectively. Scale bars = 10 µm

the nucellar epidermis in the micropylar zone surrounding the embryo (Fig. 4f–h).

After numerous nuclear divisions, cytokinesis occurs in the endosperm by progressing from the chalazal to the micropylar region until the endosperm becomes completely cellular (Fig. 4i).

At seed maturity, the cells of the endosperm are polygonal, arranged very compactly and storing food materials, mainly starch grains. Surrounding the endosperm, a layer of nucellar epidermis is preserved (Fig. 4j–l); this layer, on the sides of the embryo, has large, elongated cells in a radial direction with thin walls, and it also accumulates small starch grains (Fig. 4l).

Embryo

Embryogenesis begins in all species with the transverse division of the zygote, which results in a two-celled embryo consisting of an apical cell (towards the chalaza) and a basal cell (towards the micropyle) (Fig. 4f, g).

The mature embryo is widely turbinate and has the single cotyledon in terminal position. The embryonic axis is totally recurved, so both the radicle and plumule are in basal position. The plumule is surrounded by the cotyledonary sheath (Fig. 4l).

Polyembryony was recorded in some specimens of *B. brevifolia*, *B. communis*, *B. juncoides*, and *B. wanderleyana* (Fig. 5), where several (2–7) additional embryos are formed in the micropillar region. These apomictic embryos are started at the same time as the sexual embryo in *B. brevifolia*, *B. juncoides*, and *B. wanderleyana*; and their origin could be observed from cells in the nucellar region close to the sexual embryo (Fig. 5a–e, arrows). In *B. communis*, although we also observed two embryos formed inside the embryo sac, their origin could not be determined (image not shown). In all species, multiple embryos develop until

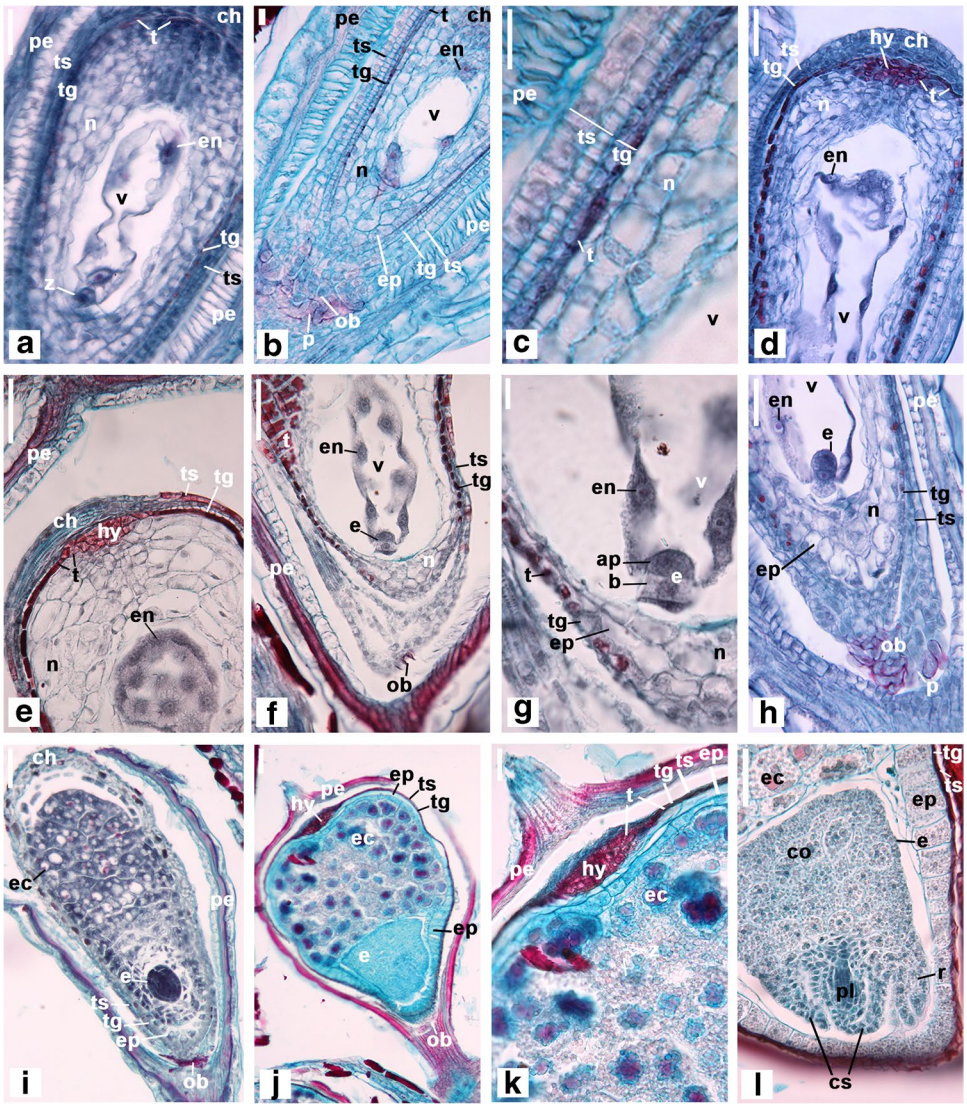

Fig. 4 LM images of endospermogenesis and embryogenesis in *Bulbostylis*, in longitudinal sections. **a–d**, **h–k** *B. communis*. **e–g**, **l** *B. hirtella*. **a** and **b** Early states of endosperm division. **c** Detail of **b**, showing the young seed coat derived from outer and inner ovule integuments. **d–h** Initial stages of embryo and endosperm development. **i** Later stage of embryo development, with endosperm in the process of cellularization. **j** Mature seed, surrounded by the fruit pericarp. **k** Detail of mature endosperm and the persistent hypostasis at the chalazal pole. **l** Detail of mature *Bulbostylis* embryo. *ap* apical cell, *b* basal cell, *co* cotyledon, *ch* chalaza, *cs* cotyledonary sheath, *e* embryo, *ec* endosperm cell, *en* endosperm nucleus, *ep* nucellar epidermis, *hy* hypostasis, *n* nucellus, *ob* obturator, *p* collapsed pollen tubes, *pe* pericarp, *pl* plumule, *r* radicle, *t* tanniferous cells, *tg* tegmen, *ts* testa, *v* vacuole, *z* zygote. Scale bars: **a**, **d–f**, **h–l** = 50 µm; **b**, **c**, **g** = 10 µm

the globular state (Fig. 5f–k); however, only one embryo reaches the adult stage, while the remaining embryos degenerate (Fig. 5l).

Seed coat

The seed coat in the mature seed is constituted of both the endotegmen and the exotesta, both layers showing tannic content. The remaining layers derived from the ovule integuments are flattened during the development of the seed. The hypostasis remains recognizable with tannic substances in the mature seed, without increasing in size (Figs. 4j–l, 5l).

Fruit development

In the early stages of the gynoecium development, the ovary wall consists of an outer epidermis, a 2–4 cell-layered mesophyll, and an inner epidermis (Fig. 6a–b). At the anthesis and during the development of the fruit, the number of these wall layers does not increase, but their cells undergo differentiation. The outer epidermis originates the exocarp, the mesophyll originates the mesocarp, and the inner epidermis originates the endocarp. The cells of the outer epidermis increase in size, and their outer periclinal walls become thicker but cellulosic; the cells of the inner epidermis elongate transversely and become spindle-shaped (Fig. 6c–d). Meanwhile, the cells of the middle layers become sclerenchymatous (Fig. 6d–n), and consequently, the mechanical layer is represented by the mesocarp.

The main differences among the species studied occur in the development of pericarp after fertilization and during the differentiation of the achene. In *B. brevifolia*, *B. communis*, and *B. hirtella*, the exocarp cells accumulate a discrete amount of starch grains, and their inner periclinal walls are slightly concave (Fig. 6f–j). On the other hand, in *Bulbostylis conifera*, *B. juncoides*, and *B. wanderleyana*, the exocarp

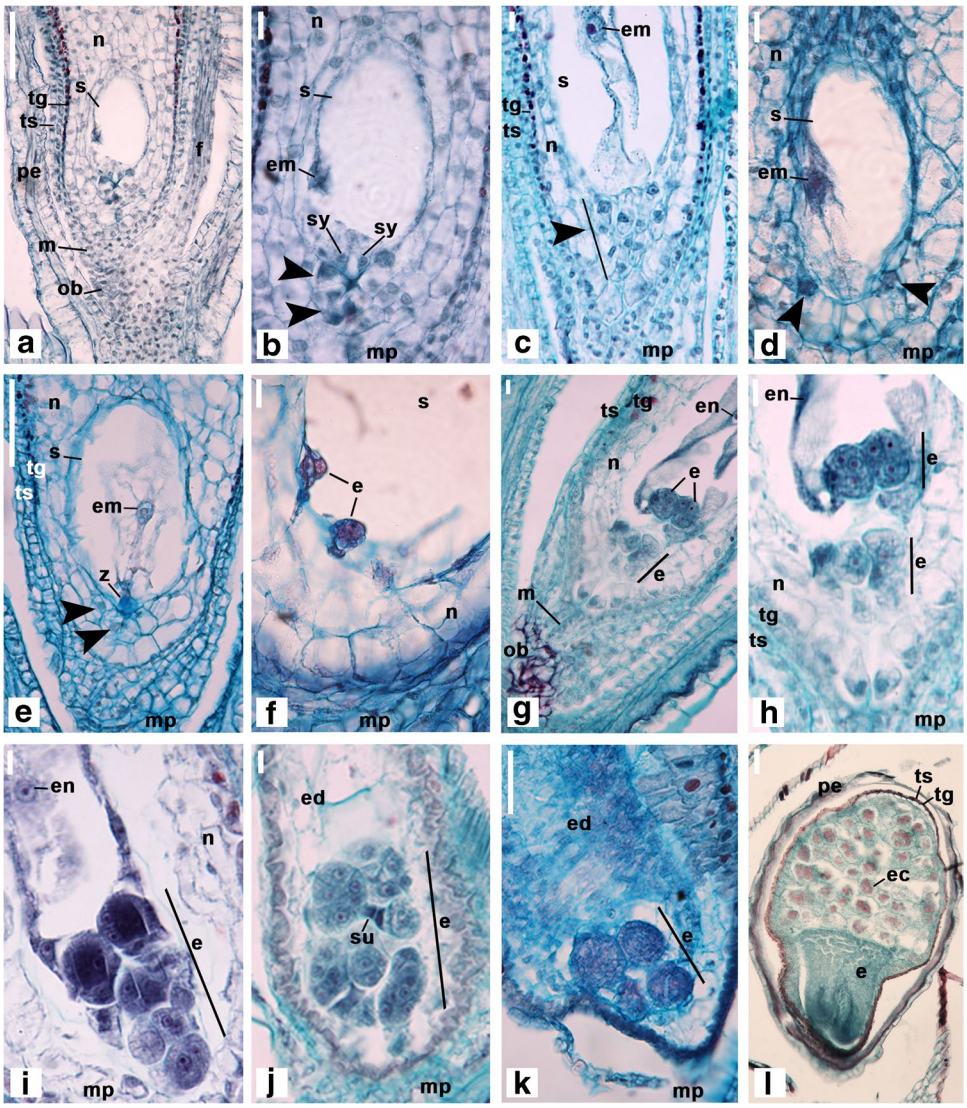

Fig. 5 LM images of polyembryony development in *Bulbostylis*, in longitudinal sections. **a**, **b**, **l** *B. brevifolia*. **c**, **g**–**j** *B. juncoides*. **d**–**f**, **k** *B. wanderleyana*. **a**–**e** Apomictic embryos initiation from nucellar cells at the micropylar pole. **f**–**k** Early stages of multiple embryos development. **l** Mature achene containing a seed with only one embryo. *e* embryo, *ec* endosperm cell, *ed* endosperm, *em* endosperm mother cell, *en* endosperm nucleus, *f* funiculus, *m* micropyle, *mp* micropylar pole, *n* nucellus, *ob* obturator, *pe* pericarp, *s* embryo sac, *su* suspensor, *sy* synergid cell, *tg* tegmen, *ts* testa, *z* zygote; arrows indicate the emergence of apomictic embryos from nucellar cells. Scale bars: **a**, **e**, **k**, **l** = 50 µm; **b**–**d**, **f**–**j** = 10 µm

cells do not develop starch grains, and their inner periclinal walls are deeply concave to adjust their shape to the protruding sclereids of the mesocarp (Fig. 6k–n).

Discussion

In *Bulbostylis*, floral development takes place according to the general pattern observed in Cyperoideae (Vrijdaghs et al. 2009; Reynders et al. 2012).

The ovule and seed development are shared among the *Bulbostylis* species studied here, such as follows: (1) anatropous and bitegmic ovules, as it has been pointed out by Gonzalez and López (2010) for *B. communis* and *B. sphaerolepis*, and as it has also been reported in many other taxa within Cyperaceae (Khanna 1965; Murty and Kumar 1967; Padhye 1968; 1971; Nijalingappa 1976, 1986; Makde and Bhuskute 1987; Johri et al. 1992; Goetghebeur 1998; Nijalingappa and Palakshaiah 1998; Coan et al. 2008); (2) weakly crassinucellar ovules, following the classification of Endress (2011); (3) obturator of integumentary origin; (4) monosporic embryo sac of the *Polygonum* type; (5) nuclear endosperm; (6) hypostase formation; (7) seed coat consisting of tanniferous endotegmen and exotesta; and (8) *Bulbostylis*-type embryo. Therefore, according to the development of the ovules and seeds, no characters have been observed to support the infrageneric groupings proposed by López and Gonzalez (2017) based on pericarp morphology, and no characters have been found to support interspecific relationships observed in molecular phylogenetic analysis presented by Reutemann et al. (2018).

Pericarp development constitutes the main variation among the *Bulbostylis* species found in this study. According to Gonzalez and López (2010), the role of mechanical protection is transferred from the seed coat to the pericarp and, in particular, to the mesocarp, which is in agreement

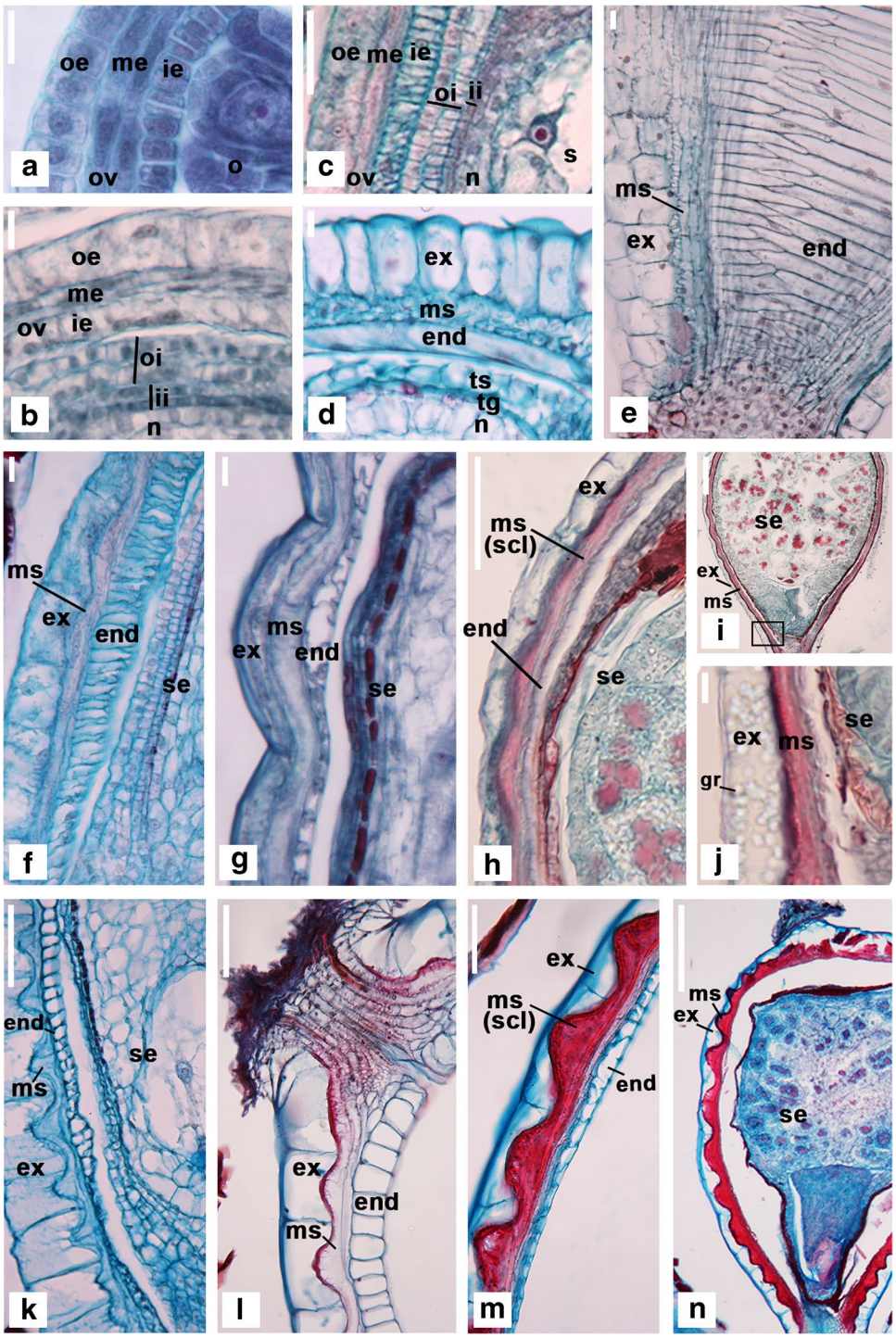

Fig. 6 LM images of pericarp development in *Bulbostylis*, in longitudinal sections (**a**, **c**, **e**–**n**) and in cross-sections (**b**, **d**). **a**, **c**, **f**, **g** *B. communis*. **b**, **h**–**j** *B. brevifolia*. **d** *B. juncoides*. **e** *B. hirtella*. **k**–**n** *B. wanderleyana*. Ovary wall during megasporogenesis (**a**) and megagametogenesis (**b**), consisting of two epidermis and 2–4 layers of parenchyma. **c** Ovary wall at the anthesis, when epidermis and mesophyll have progressed in their differentiation; observe in **b** and **c** both integuments of the ovule formed by two cell layers. **d** and **e** Pericarp at early stages of fruit development; note that in **d**, the seed coat is constituted only by two cell layers. **f**–**j** Pericarp differentiation in *B. brevifolia*, *B. communis*, and *B. hirtella*. **k**–**n** Pericarp differentiation in *B. conifera*, *B. juncoides*, and *B. wanderleyana*. *end* endocarp, *ex* exocarp, *gr* starch grain, *ii* inner epidermis, *ii* inner integument, *me* mesophyll, *ms* mesocarp, *n* nucellus, *o* ovule primordium, *oe* outer epidermis, *oi* outer integument, *ov* ovary wall, *s* embryo sac, *scl* sclereids, *se* seed, *tg* tegmen, *ts* testa. Scale bars: **a**, **b**, **d**–**g**, **j** = 10 µm; **c**, **h**, **k**–**m** = 50 µm; **i**, **n** = 100 µm

with the results presented in this work. Two conditions are observed in the species studied in relation to the anatomy of the pericarp: (1) exocarp cells with starch grains and slightly concave inner periclinal walls; or (2) exocarp cells without starch grains and deeply concave inner periclinal walls. These conditions could be taxonomically useful at level of species. However, species groupings based on these characteristics agree neither with those observed based on the micromorphology of fruits (López and Gonzalez 2017), nor with those suggested by molecular phylogenetic analysis (Reutemann et al. 2018).

Even though this work does not provide new characters to contribute to the infrageneric classification of the genus, it constitutes the most complete embryological research on *Bulbostylis*. In addition to providing basic data about the reproductive anatomy of the genus, it contributes to a better

understanding of the embryological aspects in Cyperaceae, revealing in addition the first insights about the origin of multiple embryos in this family.

At the beginning of the ovule formation, a single archesporial cell is recognized: these results had been reported in Cyperaceae even though some authors have occasionally detected more than one archesporial cell in *Eleocharis geniculata* (L.) Roem. & Schult. (Padhye 1968), and *E. atropurpurea* (Retz.) J.Presl & C.Presl (Nijalingappa 1976). Before the meiosis of the megasporocyte, the young ovule in *Bulbostylis* shows a single subdermal parietal layer; according to Endress (2011), the ovules with just one hypodermal cell layer between the megasporocyte and nucellus apex are called "weakly crassinucellar ovules." The formation of two layers of parietal cells, i.e., two subdermic parietal layers, has been frequently mentioned in Cyperaceae (Padhye 1968), but variations have been observed in different taxa: 3 layers of parietal cells in *Eleocharis geniculata*; more than 3 layers in *Fimbristylis quinquangularis* (Vahl) Kunth and *F. dichotoma* (L.) Vahl (Padhye 1968, and citations therein); 5–6 layers in *Scleria foliosa* Hochst. ex A.Rich. (Nijalingappa 1986); 2–3 layers in *Rhynchospora wightiana* (Ness) Steud. (Nijalingappa and Palakshaiah 1998); 3–4 layers in *R. consanguinea* (Kunth) Boeckeler and *R. rugosa* (Vahl) Gale; and 5–8 layers in *Hypolytrum bullatum* C.B.Clarke and *H. schraderianum* Nees (Coan et al. 2008).

The meiotic division of the megasporocyte produces a linear tetrad of megaspores, with the functional megaspore located at the chalazal region. The *Polygonum* embryo sac in *Bulbostylis* is finally constituted by seven cells and eight nuclei, as is common in most angiosperms (Maheshwari 1950). The antipodes degenerate early, and the polar nuclei fuse before the fertilization of the egg cell. Both the moment of the degeneration of the antipodes and the moment of the polar nuclei fusion seem to show certain variations within the family. The degeneration of antipodes has been cited before (*Rhynchospora colorata* (L.) H.Pfeiff.; Makde and Bhuskute (1987)) and after (*Eleocharis atropurpurea*, *E. congesta* D.Don, *Fimbristylis tetragona* R.Br., *Fuirena trilobites* C.B.Clarke, *Schoenoplectiella lateriflora* (J.F.Gmel.) Lye, Nijalingappa (1976); *Scleria foliosa*, Nijalingappa (1986); *R. rugosa*, *R. consanguinea*, *Hypolytrum bullatum*, *H. schraderianum*; Coan et al. (2008)) fertilization in different species of Cyperaceae.

Differences in the starting moment of the obturator have been reported within the family. In *Cyperus brevifolius* (Rottb.) Hassk., the obturator is already completely developed at the megaspores tetrad stage (Padhye 1971), unlike the *C. dubius* Rottb. (Padhye 1960), *C. flavidus* Retz. (Padhye and Kasture 1970), *C. sanguinolentus* Vahl (Tiwari 1969), and *C. iria* L. (Padhye 1971), where the obturator is organized at the mature embryo sac stage. On the other hand, there are differences in the behavior of the obturator post-fertilization: the obturator can degenerate (Padhye 1971; Makde and Bhuskute 1987; Nijalingappa and Palakshaiah 1998), or lignificate and persist at seed maturity (Gonzalez and López 2010), as was reported in the species studied in this work. However, regardless of the moment of initiation or duration of the obturator, its funicular origin is constant in the family (Khanna 1965; Padhye 1968; 1971; Nijalingappa 1976, 1986; Makde and Bhuskute 1987; Johri et al. 1992; Goetghebeur 1998; Nijalingappa and Palakshaiah 1998; Coan et al. 2008; Reynders et al. 2012). Only in two *Bulbostylis* species studied by Gonzalez and López (2010) was an integumentary origin for this structure identified. Based on such background and given the integumentary origin of the obturator in the six *Bulbostylis* species studied in our work, it is considered here that the origin of the obturator differs in *Bulbostylis* from the rest of Cyperaceae. Within the tribe Abildgaardieae, Padhye et al. (1970) mentioned that the obturator is scarcely developed in *Fimbristylis*.

In other angiosperm groups, the obturator and the substances secreted by this tissue apparently guide the growth of the pollen tubes to the micropylar region of the ovules through chemotropism (Maheshwari 1950; Oriani and Scatena 2012). We found several collapsed pollen tubes in the obturator, probably indicating a common function of the obturator in *Bulbostylis*. According to Cortez et al. (2012) and Caetano et al. (2013), in Melastomataceae, the apomictic species show commonly high levels of pollen inviability, making sexual reproduction in these species less likely. However, sporophytic apomixis frequently occurs associated with sexual reproduction, where normal double fertilization stimulates the development of adventitious embryos (Caetano and Cortez 2014). This could be occurring in the species of *Bulbostylis* that develop apomictic embryos.

The nuclear endosperm of *Bulbostylis* has complete endosperm cellularization as in most Cyperaceae, progressing from chalazal to micropylar region in the species studied here. Such direction of cellularization differs from the centripetal cellularization reported for *B. communis* and *B. sphaerolepis* by Gonzalez and López (2010), and from the other Cyperaceae in which the endosperm cytogenesis starts at the micropylar region (Nijalingappa 1977), or is simultaneous along the sac periphery (Nagaraj and Nijalingappa 1973; Nijalingappa 1977), or starts around the embryo (Nijalingappa 1986; Nijalingappa and Palakshaiah 1998). No chalazal haustoria or micropillars were observed in *Bulbostylis*, as reported in some species of *Scleria* and *Rhynchospora* (Nijalingappa 1986; Nijalingappa and Palakshaiah 1998; and citations therein).

The endosperm as well as the embryo is surrounded by the nucellar epidermis, which is persistent to seed maturity. The nucellar epidermis is constituted by cells of different morphology, according to their location: (1) radially

elongated in the micropylar area, adjacent to the embryo; and (2) flat in the contact region with the endosperm. Coan et al. (2008) also refer to a persistent nucellar layer which stores oil globules in the ripe seed of the *Hypolytrum* and *Rhynchospora* species. Khanna (1965) identifies in *Cyperus rotundus* L. the persistence of nucellus until the advanced globular embryo stage, in 1–2 (3 in the micropylar region) layers, but the author mentions that it is later consumed and replaced by endosperm. Makde and Bhuskute (1987) in *R. colorata*, as well as Nijalingappa and Palakshaiah (1998) in *R. wightiana*, mention the presence of an endosperm epidermis whose cells contain oil globules and form an "oil sheath." It is possible that such an oil sheath corresponds to the persistent nucellar epidermis observed by Coan et al. (2008) in *Hypolytrum* and *Rhynchospora*, and to the nucellar epidermis detected here for *Bulbostylis*. However, no histochemical tests for oils in particular were performed in this study.

A hypostase of tannic cells starts forming after fecundation, remaining at seed maturity in all the studied species of *Bulbostylis*. This structure is widely represented in the family and constitutes a typical character of Cyperaceae (Johri et al. 1992). According to Boesewinkel and Bouman (1995), the hypostase is involved in closing the opening of the chalazal in mature seeds, but these, as most additional functions attributed to this structure (e.g., participating in hormone secretion during the growth of the embryo sac, taking part in the flow of nutrients from the chalazal bundle to the embryo sac, constituting a histological barrier to prevent the excessive growth of the embryo and endosperm), are speculations.

The morphology of the mature embryo of the *Bulbostylis* species studied here represents a *Bulbostylis*-type embryo (according to Van der Veken 1965), since the embryo has a totally recurved embryonic axis so that both the radicle and plumule are in basal position. Within the tribe Abildgaardieae, the *Bulbostylis*-type embryo is a synapomorphy of the *Bulbostylis-Nemum* clade (Semmouri et al. 2019). Ancestral state reconstruction studies suggest that the *Bulbostylis*-type embryo derives from the *Fimbristylis*-type embryo (Semmouri et al. 2019). The *Fimbristylis*-type embryo also has the plumule in a basal position but, unlike the *Bulbostylis*-type embryo, it shows the radicle in lateral position (Van der Veken 1965).

Polyembryony in the early stages of embryogeny seems to be a frequent phenomenon in *Bulbostylis*, since it has been detected in four out of the six species studied here. The occurrence of additional embryos was not constant in all the individuals from the same species, which is frequently observed in species with embryos originated by nucellar or integumentary cells (Maheshwari 1950). Recently, Gonzalez and López (2010) have observed more than one embryo at the globular stage in *B. communis*, but their origin has not been determined. We have found evidence of the nucellar origin of apomictic embryos in *Bulbostylis*. However, additional embryos that seemed to be located within the embryo sac were observed in some preparations. Further study will be needed to clarify whether the apomictic embryos are only derived from nucellar cells in *Bulbostylis*, or whether there is a formation of embryos by cells of the embryo sac other than the egg. Another likely situation regarding additional embryos originated outside the embryo sac is that these come to lie inside the embryo sac and are nourished by the endosperm (Maheshwari 1950).

Few studies mention the presence of multiple embryos in Cyperaceae. Only in two seeds of *Cyperus articulatus* L. has Shah (1965) observed an embryo sac with twin embryos. The author mentioned that the additional embryo seemed to have originated from a synergid cell (in the illustration, two embryos are seen at the globular stage). Shah (1965) also refers to the work by Braun (1860, work not seen), where twin embryos in *Carex pendula* Huds are reported. Juguet (1967) observed the formation of a double embryo sac in *Carex myosuroides* Vill. and *C. arenaria* L. There are no studies in the family that explore and document the origin and development of apomictic embryos, nor studies that explore their frequency and evolution in a phylogenetic context. Therefore, additional studies are needed to investigate the occurrence of polyembryony in other genera in order to explore how widespread it is in Cyperaceae, to characterize the origin and development of apomictic embryos in different clades within the family, and to study the evolutionary importance of polyembryony in Cyperaceae.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00709-021-01649-7.

Acknowledgements The authors are grateful to the staff of Bernardino Rivadavia Museum (Buenos Aires, Argentina) for their technical assistance during SEM sessions. We are also grateful to the anonymous reviewers whose comments and suggestions were helpful in improving the manuscript.

Funding This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 2013–0473 to A.R., and PICT 2019–01678 to A.R.), and Universidad Nacional del Litoral, Santa Fe, Argentina (CAI + D2020-50520190100078LI to A.R.).

Declarations

Ethics approval and consent to participate Ethics approval not applicable.

All authors have seen and agree with the content of this manuscript.

Consent for publication All authors agree with the publication of this manuscript.

Conflict of interest The authors declare no competing interests.

References

Barros M (1947) Cyperaceae. In: Descole H (ed) Genera et Species Plantarum Argentinarum. Guillermo Kraft, Buenos Aires, pp 273–297

Boesewinkel FD, Bouman F (1995) The seed: structure and function. In: Kigel J, Galili G (eds) Seed Development and Germination. Marcel Dekker, New York, pp 1–24

Caetano APS, Teixeira SP, Forni-Martins ER, Carmello-Guerreiro SM (2013) Pollen insights into apomictic and sexual *Miconia* (Miconieae, Melastomataceae). Int J Plant Sci 174:760–768. https://doi.org/10.1086/669927

Caetano APS, Cortez PA (2014) Reprodução assexuada. In: Rech AR, Agostini K, Oliveira PE, Machado IC (eds) Biologia da Polinização. Editora Projeto Cultural, Rio de Janeiro, pp 93–111

Coan AI, Alves MV, Scatena VL (2008) Comparative study of ovule and fruit development in species of *Hypolytrum* and *Rhynchospora* (Cyperaceae, Poales). Pl Syst Evol 272:181–195. https://doi.org/10.1007/s00606-007-0636-9

Cortez PA, Carmello-Guerreiro SM, Teixeira SP (2012) Understanding male sterility in *Miconia* species (Melastomataceae): a morphological approach. Aust J Bot 60:506–516. https://doi.org/10.1071/BT12076

Endress PK, Baas P, Gregory M (2000) Systematic plant morphology and anatomy-50 years of progress. Taxon 49:401–434. https://doi.org/10.2307/1224342

Endress PK (2011) Angiosperm ovules: diversity, development, evolution. Ann Bot 107:1465–1489. https://doi.org/10.1093/aob/mcr120

Givnish TJ, Zuluaga A, Spalink D, Soto Gomez M, Lam VKY, Saarela JM, Sass C et al (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. Am J Bot 105:1888–1910. https://doi.org/10.1002/ajb2.1178

Goetghebeur P (1998) Cyperaceae. In: Kubitzki K (ed) The families and genera of vascular plants, vol 4. Springer-Verlag, Berlin, pp 141–190

Goetghebeur P, Coudijzer J (1985) Studies in Cyperaceae 5. The Genus *Bulbostylis* in Central Africa. Bull Jard Bot Belg 55:207–259. https://doi.org/10.2307/3668016

Gonzalez AM, López MG (2010) Development and morphology of the gynoecium and nutlet in two South-American *Bulbostylis* (Cyperaceae) species. Flora 205:211–220. https://doi.org/10.1016/j.flora.2009.02.002

Govaerts R, Jimenez-Mejias P, Koopman J, Simpson D, Goetghebeur P, Wilson K, Egorova T, Bruhl J (2020) World checklist of Cyperaceae. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet http://wcsp.science.kew.org/. Accessed 30 Jan 2020

Johansen DA (1940) Plant microtechnique. McGraw-Hill Book Co, New York

Johri BM, Ambegaokar KB, Srivastava PS (1992) Comparative embryology of angiosperms, vol 2. Springer-Verlag, Berlin, pp 974–981

Juguet M (1967) Polyembryonie chez le *Kobresia bellardii* (All.) Degld. Bull Soc Bot Fr 114:7–8, 277–279. https://doi.org/10.1080/00378941.1967.10838360

Kellogg EA (2015) Embryology. In: Kubitzki K (ed) Flowering Plants. Monocots. The Families and Genera of Vascular Plants, vol 13. Springer, Cham, pp 45–53. https://doi.org/10.1007/978-3-319-15332-2_4

Kern JH (1974) Cyperaceae. In: van Steenis CGGJ (ed) Flora Malesiana I, Noordhoff International Publishing, Leiden, pp 7:107–187

Khanna P (1965) A contribution to the embryology of *Cyperus rotundus* L., *Scirpus mucrinatus* L., and *Kyllinga melanospora* Nees. Can J Bot 43:1539–1547. https://doi.org/10.1139/b65-163

Kral R (1971) A treatment of *Abildgaardia, Bulbostylis* and *Fimbristylis* (Cyperaceae) for North America. Sida 4:57–227

López MG (2012) Citología, morfología y taxonomía del género *Bulbostylis* (Cyperaceae) para América Austral. Ph Thesis, Universidad Nacional del Nordeste, Corrientes, Argentina

López MG, Gonzalez AM (2017) Micromorfología y estructura de los frutos en *Bulbostylis* (Cyperaceae) y su valor sistemático en las especies de América Austral. Bol Soc Argent Bot 52:69–87. https://doi.org/10.31055/1851.2372.v52.n1.16909

Luque R, Sousa HC, Kraus JE (1996) Métodos de coloração de Roeser (1972) -modificado- e Kropp (1972) visando a substituição do azul de astra por azul de alcião 8 GS ou 8GX. Acta Bot Bras 10:199–212. https://doi.org/10.1590/S0102-33061996000200001

Maheshwari P (1950) An introduction to the embryology of angiosperms. McGraw-Hill, New York

Makde KH, Bhuskute SM (1987) Embryology of *Kyllinga monocephala* (Cyperaceae) and its systematic position. Plant Syst Evol 156:143–150. https://doi.org/10.1007/BF00936069

Metcalfe CR (1971) Anatomy of the monocotyledons: V. Cyperaceae. Oxford University Press, Oxford

Murty YS, Kumar V (1967) Development of the female gametophyte and embryo in *Fimbristylis diphylla* Vahl. Proc Indian Acad Sci 65B:185–191. https://doi.org/10.1007/BF03052209

Nagaraj M, Nijalingappa BHM (1973) Embryological studies in *Cyperus alopecuroides* Rottb. Proc Indian Acad Sci 77B:252–263. https://doi.org/10.1007/BF03045598

Nijalingappa BHM (1976) Sporogenesis and gametogenesis in some Cyperaceae. Proc Indian Acad Sci 83:66–72

Nijalingappa BHM (1977) Post-pollination studies in some Cyperaceae. Proc Indian Acad Sci 85:391–398. https://doi.org/10.1007/BF03052247

Nijalingappa BHM (1986) Embryology of *Scleria foliosa* (Cyperaceae). Plant Syst Evol 152:219–230. https://doi.org/10.1007/BF00989429

Nijalingappa BHM, Palakshaiah CR (1998) Embryological studies in *Rhynchospora wightiana* (Cyperaceae). In: BhatiaShukla BAK, Sharma HL (eds) Plant Form and Function. Angkor Publishers, New Delhi, pp 225–236

Oriani A, Scatena VL (2012) Floral anatomy of *Xyrids* (Poales): contributions to their reproductive biology, taxonomy, and phylogeny. Int J Plant Sci 173:767–779. https://doi.org/10.1086/666664

Ornduff R (1978) Reproductive characters and taxonomy. Syst Bot 3:420–427. https://doi.org/10.2307/2418754

Padhye MD (1960) A contribution to the life history of *Kyllinga triceps* Rottb. Bot Soc Coll Sci, Nagpur, Bull 1:1–15

Padhye MD (1968) Male and female gametophytes of *Eleocharis geniculata* Roem. et Schult. Proc Indian Acad Sci 67B:54–60. https://www.ias.ac.in/article/fulltext/secb/067/02/0054-0060

Padhye MD (1971) Studies in Cyperaceae. III. Life history of *Kyllinga brevifolia* Rottb. with a brief discussion on systematic position of *Kyllinga*. Bot Gaz 132: 172–179. https://www.jstor.org/stable/2474090

Padhye MD, Kasture SW (1970) Studies in Cyperaceae. V. Gametophytes and fertilization in *Pycreus globossus* (All.) *var. niligiricus* (Stead) CBCl. J Biol Sci 13:37–42

Padhye MD, Makde KH (1982) Embryogeny in some Cypereae. Plant Syst Evol 139:279–287. https://doi.org/10.1007/BF00989330

Padhye MD, Chaube SN, Iyer AV (1970) Studies in Cyperaceae. VIII. Gametophytes and fertilization in two members of Cyperaceae. J Indian Bot Soc 49:86–91

Pedersen TM (1969) Cyperaceae. In: Cabrera AL (ed) Flora de la Provincia de Buenos Aires. Colección Científica del INTA, Buenos Aires, pp 315–421

Reutemann AG, Lucero LE, Guarise NJ, Vegetti AC (2012) Structure of the Cyperaceae inflorescence. Bot Rev 78:184–204. https://doi.org/10.1007/s12229-012-9098-z

Reutemann AG, Ardissone RE, López MG, Muchut S, Boldrini I, Vegetti AC, Giussani L (2018) Phylogenetic relationships in *Bulbostylis* (Cyperaceae: Abildgaardieae) inferred from nuclear and plastid DNA sequence data. System Biodivers 16:441–452. https://doi.org/10.1080/14772000.2018.1442885

Reynders M, Vrijdaghs A, Larridon I, Huygh W, Leroux O, Muasya M, Goetghebeur P (2012) Gynoecial anatomy and development in Cyperoideae (Cyperaceae, Poales): congenital fusion of carpels facilitates evolutionary modifications in pistil structure. Plant Ecol Evol 145:96–125. https://doi.org/10.5091/plecevo.2012.675

Rocha DM, Andrade CGTJ, Vanzela ALL (2015) Female gametophyte development in *Eleocharis sellowiana* (Cyperaceae): a species with atypical nuclear staining and polarity of the megaspore mother cell. Plant Syst Evol 301:1287–1292. https://doi.org/10.1007/s00606-014-1145-2

Rudall PJ (1997) The Nucellus and Chalaza in monocotyledons: structure and systematics. Bot Rev 63:140–181. https://doi.org/10.1007/BF02935930

Ruzin SE (1999) Plant microtechnique and microscopy. Oxford University Press, New York

Semmouri I, Bauters K, Léveillé-Bourret É, Starr JR, Goetghebeur P, Larridon I (2019) Phylogeny and systematics of Cyperaceae, the evolution and importance of embryo morphology. Bot Rev 85:1–39. https://doi.org/10.1007/s12229-018-9202-0

Shah CK (1965) Embryogeny in some Cyperaceae. Phytomorphology 15:1–9

Shah CK (1967) A taxonomical evaluation of the families Cyperaceae and Juncaceae. Symp Newer Trends in Taxonomy. Nat Inst Sci India, New Delhi 34:248–256

Singh V (1981) Taxonomy of angiosperms. Rastogi Publications, New Delhi

Svenson HK (1957) Poales-Cyperaceae. North American. Flora 18:540–556

Tiwari DK (1969) Embryological studies in the Cyperaceae. A contribution to the embryology of *Pycreus sanguinolentus* Nees. J Biol Sci 12:21–30

Tobe H (1989) The embryology of angiosperms: its broad application to the systematic and evolutionary study. Bot Mag Tokyo 102:351–367. https://doi.org/10.1007/BF02488572

Van der Veken P (1965) Contribution a l'embryographie systématique des Cyperaceae-Cyperoideae. Bulletin du Jardin botanique de l'État a Bruxelles 35:285–354. https://www.jstor.org/stable/3667185

Vrijdaghs A, Muasya AM, Goetghebeur P, Caris P, Nagels A, Smets E (2009) A floral ontogenetic approach to questions of homology within the Cyperoideae (Cyperaceae). Bot Rev 75:30–51. https://doi.org/10.1007/s12229-008-9021-9