Embryological studies on Trichloris (Poaceae, Chloridoideae)

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Abstract: The present study provides information on the development of the female and male gametophytes and seed ontogeny characteristics for *Trichloris*, and it discusses their taxonomic significance to the family Poaceae. *Trichloris* species are characterized by having 2–3 stamens per floret, tetrasporangiate anthers, a monocotyledonous development of the anther wall, a single middle layer of common origin with the tapetum, fibrous thickening in the cell walls of the endothecium, and a secretory tapetum. These characteristics are constant within Poaceae. The possession of bitegmic and tenuinucellate ovules, laterally positioned antipodals prior to their degeneration, a free exocarp, and an epiblast in the mature embryos gives *Trichloris* the typical characteristics described for other members of the subfamily Chloridoideae. The degradation of tapetal cells before the maturation of the pollen grain and the absence of aborted ovules indicate a high fertility rate, which is an important feature for perennial halophytes such *Trichloris* that do not possess elaborate seed dormancy systems.

Key words: Poaceae, gametophyte, pericarp, utricle, seed development, halophyte

1. Introduction

Trichloris E.Fourn. ex Benth. is a genus of perennial and warm-season grasses represented by only two species: *T. crinita* (Lag.) Parodi and *T. pluriflora* E.Fourn. (Parodi, 1919; Rúgolo and Molina, 2012). Both species are distributed throughout the Americas, from the southern USA to the center of Argentina (Cavagnaro et al., 2006), constituting important components of the arid and semiarid rangelands (Ragonese, 1955; Cabrera, 1994; Zabala et al., 2011).

These species are notable for their forage quality and resistance to extreme environmental conditions, such as drought and salinity (Quiroga et al., 2013; Báez et al., 2015; Marinoni et al., 2015; Olea et al., 2015). Studies aimed at obtaining useful information for the development of breeding programs and germplasm collections have reported sexual reproduction for both species (Gutiérrez et al., 2016; Kozub et al., 2017). However, detailed descriptions of the development of their gametophytes and fruits have not yet been provided. Although these aspects are important complements to reproductive

biology studies, it still remains one of the least investigated traits of the wild grasses (Lovisolo, 2011).

Embryological studies have been carried out to assess the phylogenetic relationships and to solve taxonomic problems in many groups of plants (Johri et al., 1992; Arias and Williams, 2008; Madrid and Friedman, 2010), even at the genus level (Berg, 2009). These types of studies are particularly important for *Trichloris* because its taxonomic position has undergone some modifications. This genus, which was often placed closely related to *Chloris,* was embedded in *Leptochloa* sensu stricto with another three species (*Leptochloa digitata* (R.Br.) Domin, *L. virgata* (L.) P.Beauv., and *L. chloridiformis* (Hack.) Parodi) (Peterson et al., 2012), and the recently proposed *Enteropogon mollis* (Nees) Clayton and *Chloris exilis* Renvoize (Peterson et al., 2015), as a result of molecular studies. Embryological studies may help to clarify some phylogenetic relationships; therefore, in this study, we provide a detailed description of the development of the female and male gametophytes, as well as seed ontogeny, for both species of *Trichloris*.

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2. Materials and methods

2.1. Studied species

Trichloris species are perennial, caespitose, rhizomatous or stoloniferous plants, with simple or branched culms. Their inflorescences are formed by spiciform racemes arranged into several whorls on an abbreviated axis. The spikelets are dorsally compressed and have 1–4 inferior fertile and perfect florets, 1–3 superior sterile and rudimentary florets, which are deciduous, with disarticulations above the glumes so that the florets fall as a unit. The glumes are two, shorter than the spikelet, persistent and hyaline. Lemmas are three-awned, with the central nerve prolonged in a well-developed edge and higher than the body of the lemma, and the other two of equal length, or smaller. Lemmas of the reduced sterile floret are generally smaller than those of the fertile ones. Paleas are of equal length to the lemmas, shortly bidentate or biaristulate. The lodicules are two and are welded to the palea, and a glabrous ovary has 2 styles and 2 feathery stigmas. The fruit is compressed dorsoventrally, with a wide ventral groove. It is characterized by an embryonic macula, up to the middle of fruit length or less, a basal punctiform, a very marked hilum, and dry endosperm (Nicora and Rúgolo, 1987).

T. crinita has inflorescences with 5–25 spiciform racemes, 5–18 cm in length, and spikelets with 1 inferior and fertile floret and 1–2 superior sterile and rudimentary florets. Conversely, the inflorescences of *T. pluriflora* have 7–20 racemes, 7–20 cm in length, 2–4 inferior and fertile florets and 1–3 superior sterile and rudimentary florets (Nicora and Rúgolo, 1987).

2.2. Plant material

The inflorescences employed in this study were collected from 8 populations of plants (4 of *T. crinita* and 4 of *T. pluriflora*) grown in the experimental field at the Faculty of Agricultural Sciences, National University of the Littoral (FCA-UNL), Esperanza, Argentina (31°27′S, 60°56′W). Each experimental population consisted of 30 plants arranged in a completely random design. These populations originated from seeds collected at different locations in Argentina (Table).

Specimens of both species preserved in the herbarium (SF) of the FCA-UNL were also employed for morphological observations. *T. crinita*: P10372 (Formosa), E354 (La Rioja), P9420 (Córdoba), E265 (Catamarca), P9319 (Córdoba). *T. pluriflora*: P10058 (Chaco), P10095 (Santiago del Estero), P8053 (Córdoba), P8501 (Formosa).

2.3. Embryological analysis

For this test, five inflorescences (racemes) from three plants of each population in different grown stages (n = 15 inflorescences per population) were utilized. The different growth development stages of inflorescences were delimited according to the criteria employed by Gutierrez et al. (2016): 1) start of inflorescence emergence from the leaf flag (<10% of inflorescence emerged); 2) half of inflorescence emerged (50% of inflorescence emerged); 3) end of emergence (inflorescence completely emerged from the flag leaf). These were fixed in formalin, acetic acid, and alcohol (Ruzin, 1999) and stored in 70% ethanol. The dissected material was treated with 20% hydrofluoric acid for 48 h to dissolve silica. The silica-free material was then dehydrated in an alcohol–xylol series and embedded in paraffin wax (Zarlavsky, 2014). The sections were cut with a rotary microtome at 7–9 µm thickness and stained with safranin and fast green (Zarlavsky, 2014). Photographs were taken using Motic and Leica microscopes with built-in digital cameras. Some morphological aspects of the spikelets, florets, and fruits were observed using a stereomicroscope.

Table. Origin of populations of *Trichloris* employed in this work.

Species	Population ID[1]	Province	GPS	
			Latitude	Longitude
T. crinita	INTA San Luis	INTA San Luis	-	-
	7078	Santiago del Estero	25°08′S	64°47′W
	6917	Formosa	25°41′S	59°03′W
	7602	Córdoba	29°51′S	64°40′W
T. pluriflora	6962	Córdoba	30°47′S	64°31′W
	7000	Salta	25°13′S	64°55′W
	7063	Salta	24°03′S	64°00′W
	7011	Jujuy	24°32′S	65°04′W

[1] Identification number of the specimen collected, present in the herbarium (SF) of the FCA-UNL.

3. Results

The embryological processes observed in fertile florets of *Trichloris crinita* and *T. pluriflora* presented the same development pattern. Because of this, a general description of the embryology for both species is provided.

3.1. Microsporangium

T. crinita had 3 stamens per fertile floret (Figure 1A) and *T. pluriflora* showed only 2 stamens in all fertile florets (Figure 1B). Despite having different numbers of stamens, the anthers in both species are dorsifixed, bithecial, tetrasporangiate, and of longitudinal dehiscence.

3.2. Development of anther wall

During the first stages of development, three initial layers are distinguished in the anther wall: an outer layer that gives rise to the epidermis, and two secondary parietal layers. The outermost parietal layer divides and produces the endothecium, and the inner secondary parietal layer forms the middle layer and tapetum. The middle layer is ephemeral and disintegrates at the beginning of microsporogenesis. Below this is the tapetum, composed of binucleate cells with thin walls and dense cytoplasm (Figure 2A). This layer remains intact until the release of the microspores and then progressively disintegrates. At the same time, a tapetal membrane with numerous orbicules differentiates (Figure 2B). The epidermal and endothecial cells become larger, and these are the only two

tissues that persist until anther maturity. The endothecial cells develop thickenings in their radial and inner tangential walls (Figure 2B).

3.3. Microsporogenesis and microgametogenesis

In longitudinal sections of young anthers, the microspore mother cells are usually arranged in one or two rows, which are distinguishable by their dense cytoplasm and conspicuous nuclei (Figure 3A). In successive stages, these cells become spherical and a callose wall is deposited between the plasmalemma and the primary wall. Once this callose wall is completely formed, the microspore mother cells enter into meiotic division and successive cytokinesis (Figures 3B and 3C), leading to the formation of isobilateral microspore tetrads (Figure 3D).

Subsequently, the callose wall disintegrates and the microspores are released from the tetrads. These cells increase in size and become spherical due to their large vacuolation, which causes a displacement of the nucleus to a peripheral position near the cell wall (Figure 3E). The microspores then undergo a mitotic division, giving rise to two cells: a small generative cell that occupies a parietal position, and a larger vegetative cell that inherits the large vacuole of the microspore (Figure 3F). Subsequently, the generative cell migrates to the central region and divides mitotically before the dehiscence of the anther (Figure 3G) into two sperm cells. Thus, at the time of anther dehiscence,
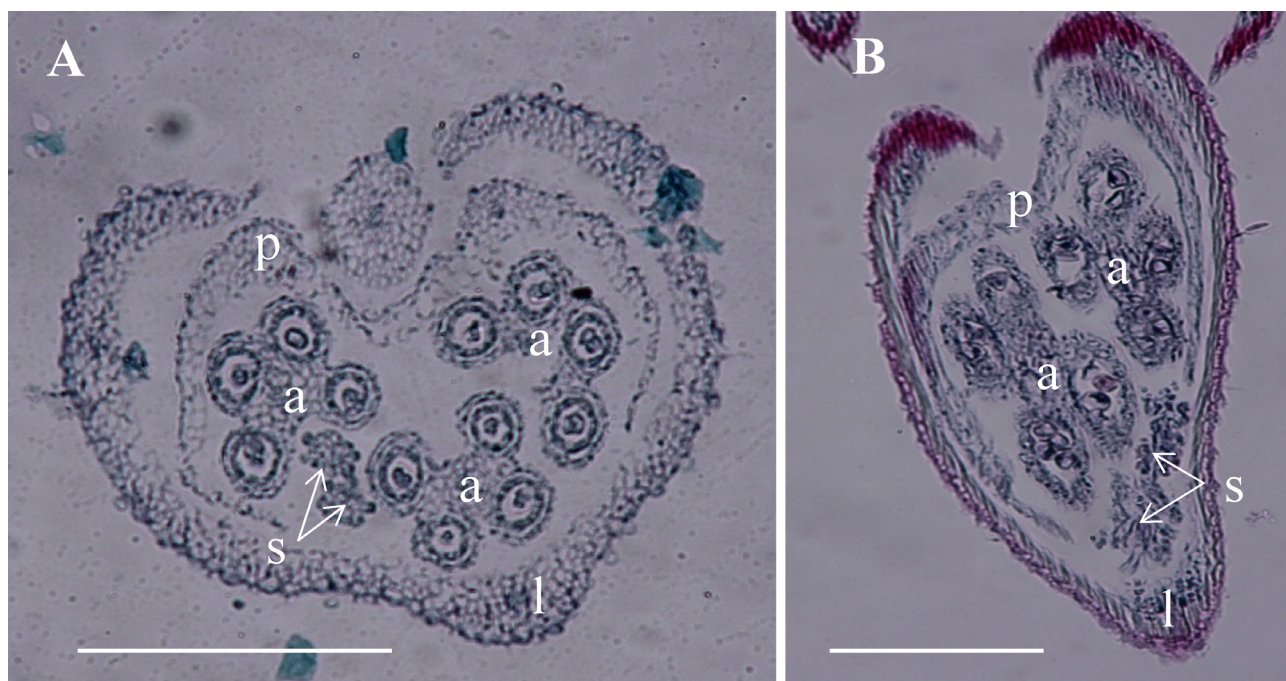

Figure 1. Transections of fertile florets of *Trichloris*: A) *T. crinita* floret showing three tetralocular anthers and a bifid stigma inside the palea and lemma; B) *T. pluriflora* floret with two tetralocular anthers, a bifid stigma, palea and lemma. a: Anther, s: stigma, p: palea, l: lemma. Bar: 500 μm.
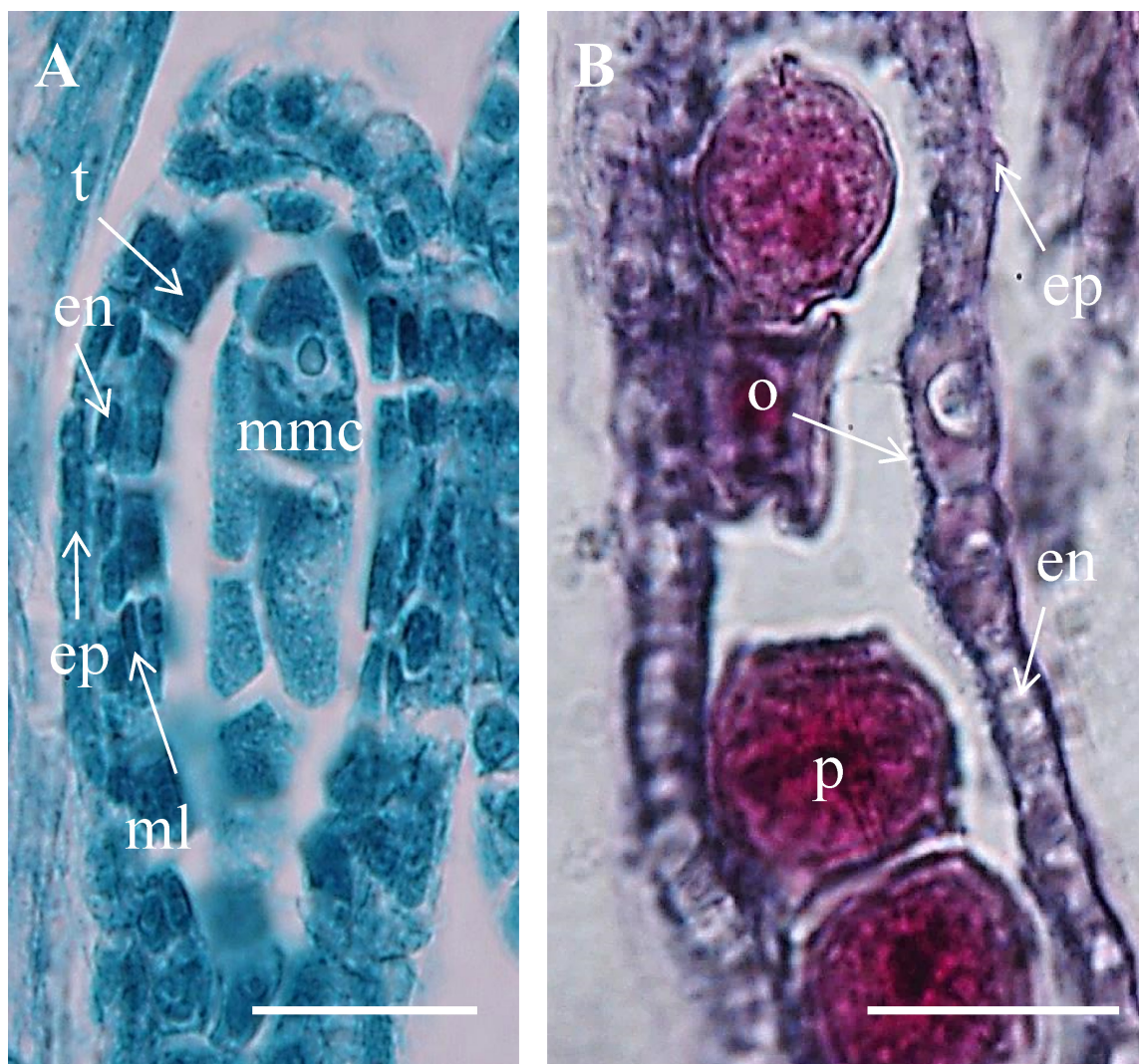
Figure 2. Anther section of Trichloris crinita in different development stages: A) immature anther composed by a tapetum, middle layer, endothecium, and epidermis, containing the microspore mother cells; B) mature anther composed only by a thickened endothecium and a barely perceptible epidermis, internally covered by numerous and diminutive orbicules, containing developed pollen grains. mmc: Microspore mother cell, ml: middle layer, en: endothecium, ep: epidermis, o: orbicules, p: pollen grains, t: tapetum. Bar: 50 μm.

the mature pollen grains are three-celled, spheroidal, and monoporate, with the vegetative cell cytoplasm full of starch grains (Figure 3G).

3.4. Ovary and ovule
The ovary in both species contains a basal, bitegmic, and tenuinucellate ovule. The micropyle is formed only by the inner integument (Figure 4A). As the integuments develop, the ovule begins to curve and increase its size until becoming hemitropous (Figures 4B and 4C). The ovule remains orthotropic until the stage of the tetrad of megaspores (Figures 5A and 5B).

3.5. Megasporogenesis and megagametogenesis
A single archaesporic cell per ovule differentiates under the nucellar epidermis and acts directly as the mother cell of the megaspores (Figure 5A). This mother cell, through meiotic divisions, gives rise to a tetrad of T-shaped megaspores (Figures 5B and 5C). The different stages of megasporogenesis and microsporogenesis can be observed simultaneously on stage-1 inflorescences (<10% of inflorescence emerged from the leaf flag).
The chalazal-most megaspore is the only one that remains functional; the others degenerate. The functional megaspore undergoes three successive mitotic
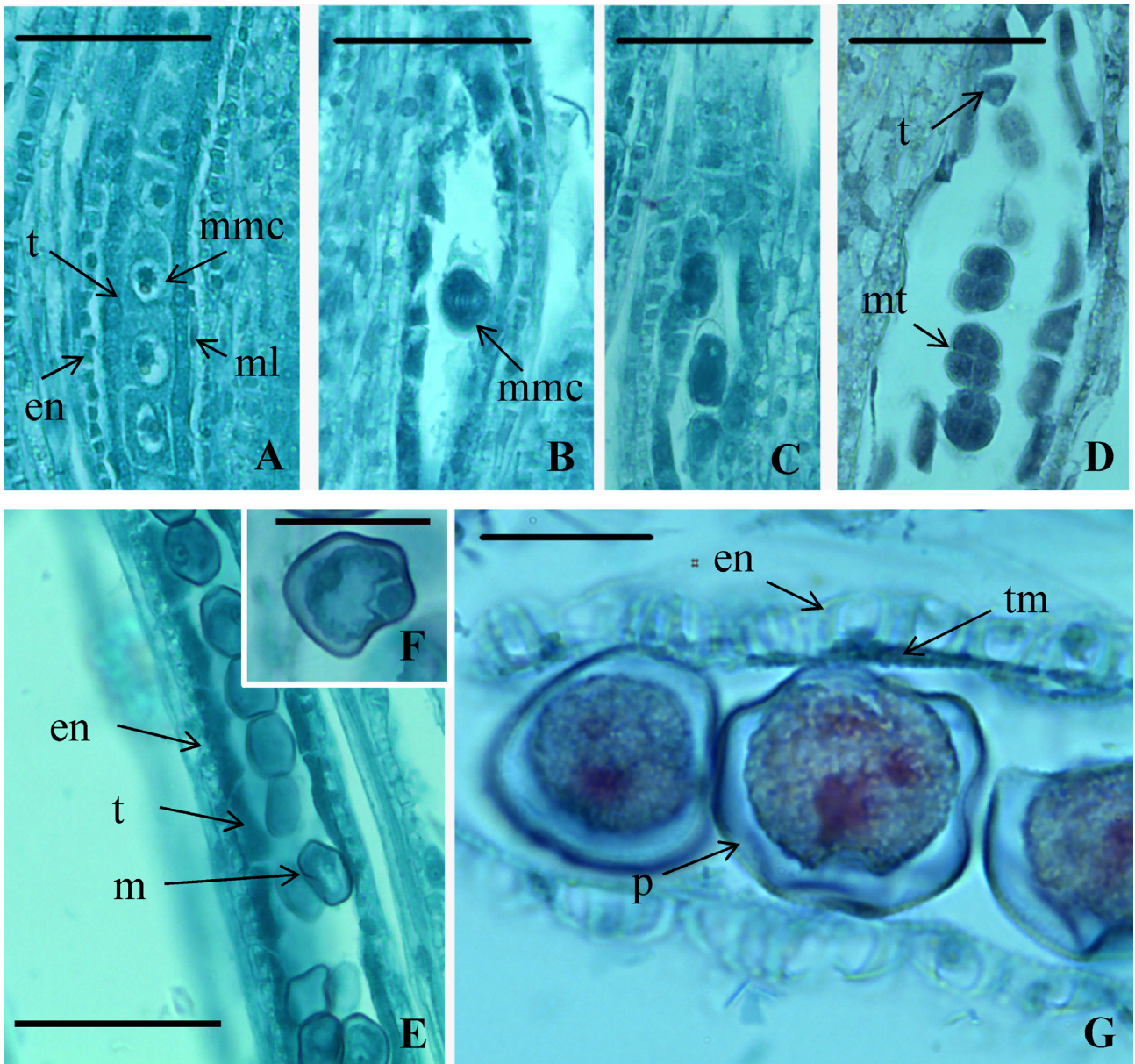
Figure 3. Anther sections of *Trichloris pluriflora* at different development stages: A) young anther, prophase into meiotic division I of the microspore mother cell, bar 100 µm; B) microspore mother cell in anaphase I, bar 100 µm; C) microspore mother cell in telophase I, bar 100 µm; D) isobilateral microspores tetrads, bar 100 µm; E) one-nucleated microspores with a large vacuole, bar 100 µm; F) bicellular pollen grains, bar 70 and 30 µm respectively; G) pollen grain starting its late maturation stage, where the cytoplasm of the vegetative cell fills the grain with starch grains, and mitosis of generative cell gives rise to two sperm cells, bar 30 µm. m: Microspore; mmc: microspore mother cell; en: endothecium; ml: middle layer; mt: microspore tetrads; p: pollen grain; tm: tapetal membrane.

karyokineses, to first form a binucleate embryonic sac (Figures 5D and 5E), then a tetranucleate and finally an octonucleate sac. Cytokinesis then gives rise to the formation of a megagametophyte of the *Polygonum* type, consisting of two synergids, an egg cell, a middle cell with two polar nuclei, and three antipodals. As the gametophyte continues its maturation, the antipodals proliferate by mitosis, reaching up to 12 antipodal cells, and the filar apparatus in the synergids is well differentiated (Figures 6 and 7). The development of the female gametophyte and the pollen grains are simultaneous events. These are already noticeable on stage-2 inflorescences (half of inflorescence emerged), when the florets start opening.
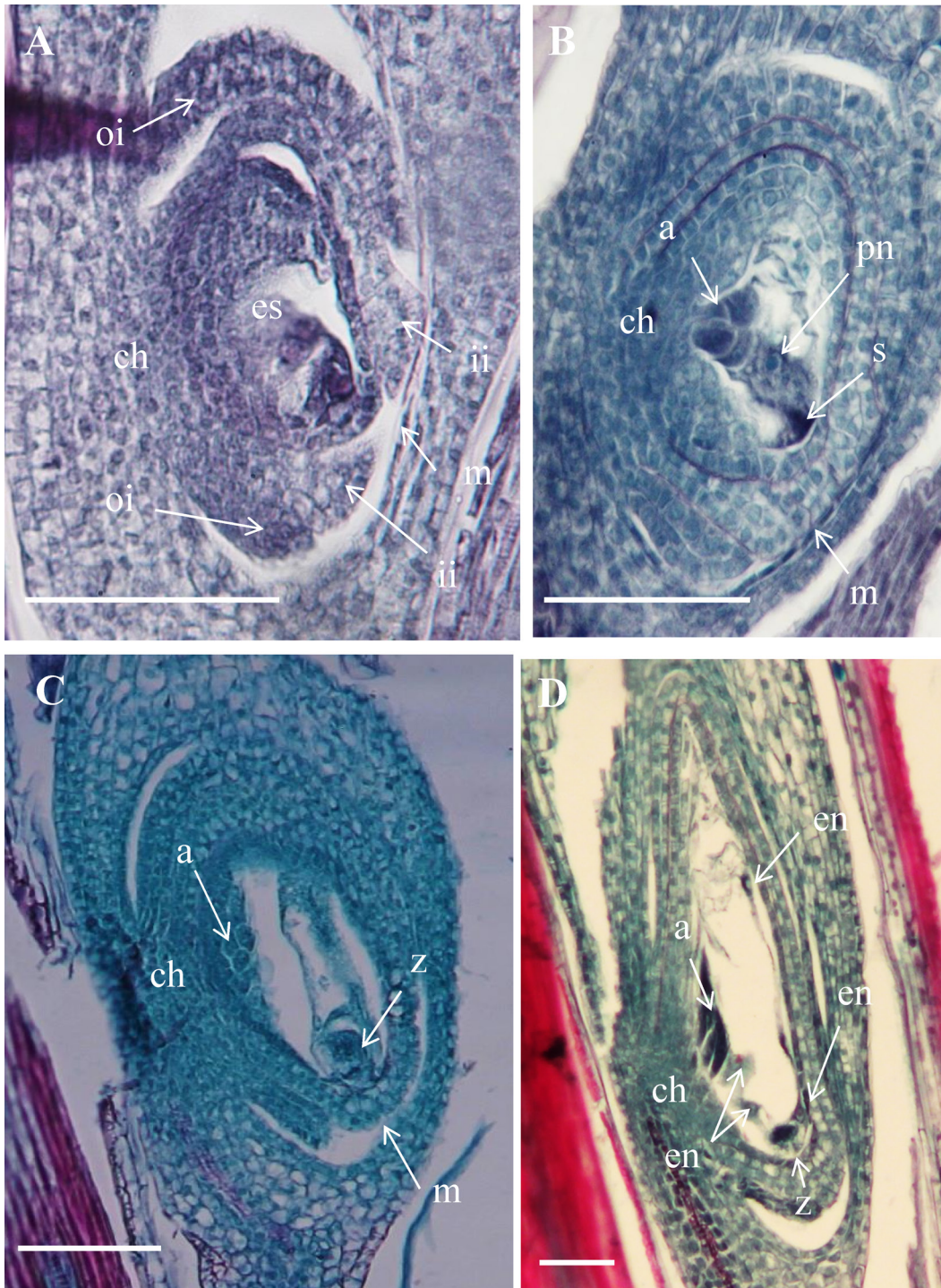
Figure 4. Longitudinal section of *Trichloris crinita* ovules in different developmental stages: A) tetranucleate embryo sac, where the formation of the micropyle by the inner integuments is observed; B) lateral view of a portion of the embryo sac shows the antipodal cells occupying the chalazal pole; C) postfertilization stage of a hemitropous ovule (note the right angle between the micropyle and chalaza), with a recently formatted zygote, a nuclear endosperm (not visible in this histological section), and the antipodes in a lateral position, next to the chalaza; D) advanced postfertilization ovule stage, with a nuclear endosperm and antipodes in a lateral position starting its degradation. a: Antipodes; es: embryonic sac; ch: chalaza; ii: inner integuments; m: micropyle; oi: outer integument; z: zygote. Bar: 50 μm.
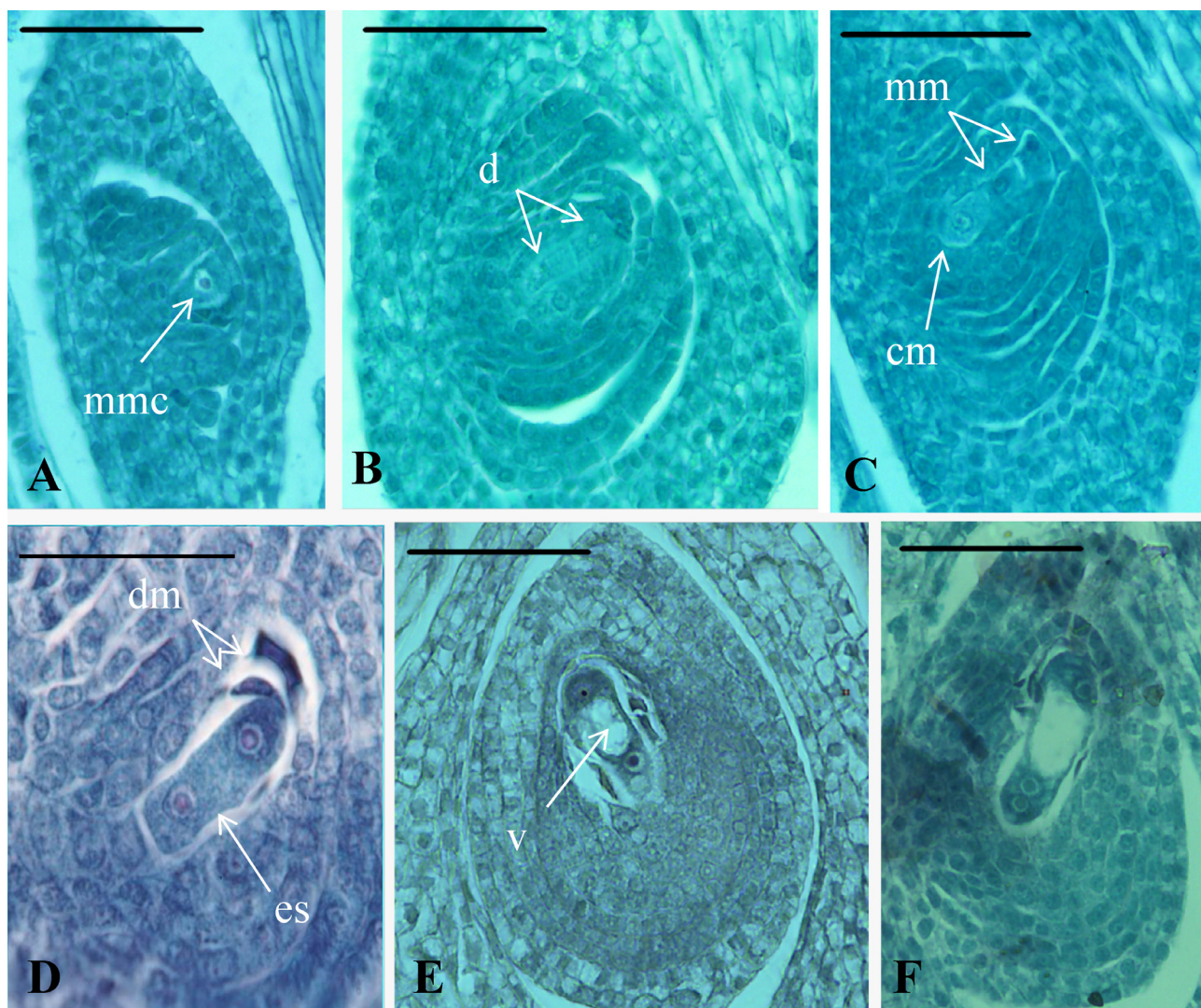
Figure 5. Development of the megagametophyte in *T. pluriflora*: a) mother cell of the megaspore; B) dyad as a result of the first division of the megaspores' mother cell; C) advanced T-shaped megaspore tetrad, with a hyaline chalazal megaspore (that will become the functional one) and the rest of the micropylar megaspores showing a dense cytoplasm indicating that they are closest to aborting (one of them keeps behind the megaspore located at the micropylar-most region and therefore is not observed in the figure); D) binucleate embryo sac as a result of the first mitotic division of the functional megaspore, and rest of the degenerated megaspores at the micropylar pole; E) binucleate embryo sac with a big central vacuole; F) tetranucleate embryo sac. cm: Chalazal megaspore; dm: degenerated megaspores; es: embryo sac; mmc: megaspore mother cell; mm: micropylar megaspores; v: central vacuole. Bar: 100 μm.

3.6. Development of fruits and seeds

After fertilization, the ovule increases considerably in size, and the nuclear endosperm begins to form from mitotic divisions of the endospermogenetic nucleus. The zygote begins to divide when the endosperm already has several nuclei (Figure 7A). During these processes, the antipodes persist in a lateral position near the chalazal pole (Figures 4C and 4D). When the embryo exceeds its 4-cell stage, the endosperm cellularization begins and the antipodal cells finally disintegrate. The endosperm becomes completely cellular at the globular stage of the proembryo (Figure 7C). Thus, the endosperm development is of the nuclear type. At the same time, the outer integument disintegrates (Figures 7B, 7D, and 7F). The nucellar tissue is completely consumed, and the most peripheral stratum of the endosperm begins to differentiate into a single aleurone layer. The inner integument remains but undergoes a series of changes. Its inner cellular layer develops secondary thickenings in the walls and acquires a red coloration with safranin staining (Figures 7C and 7F). The layers
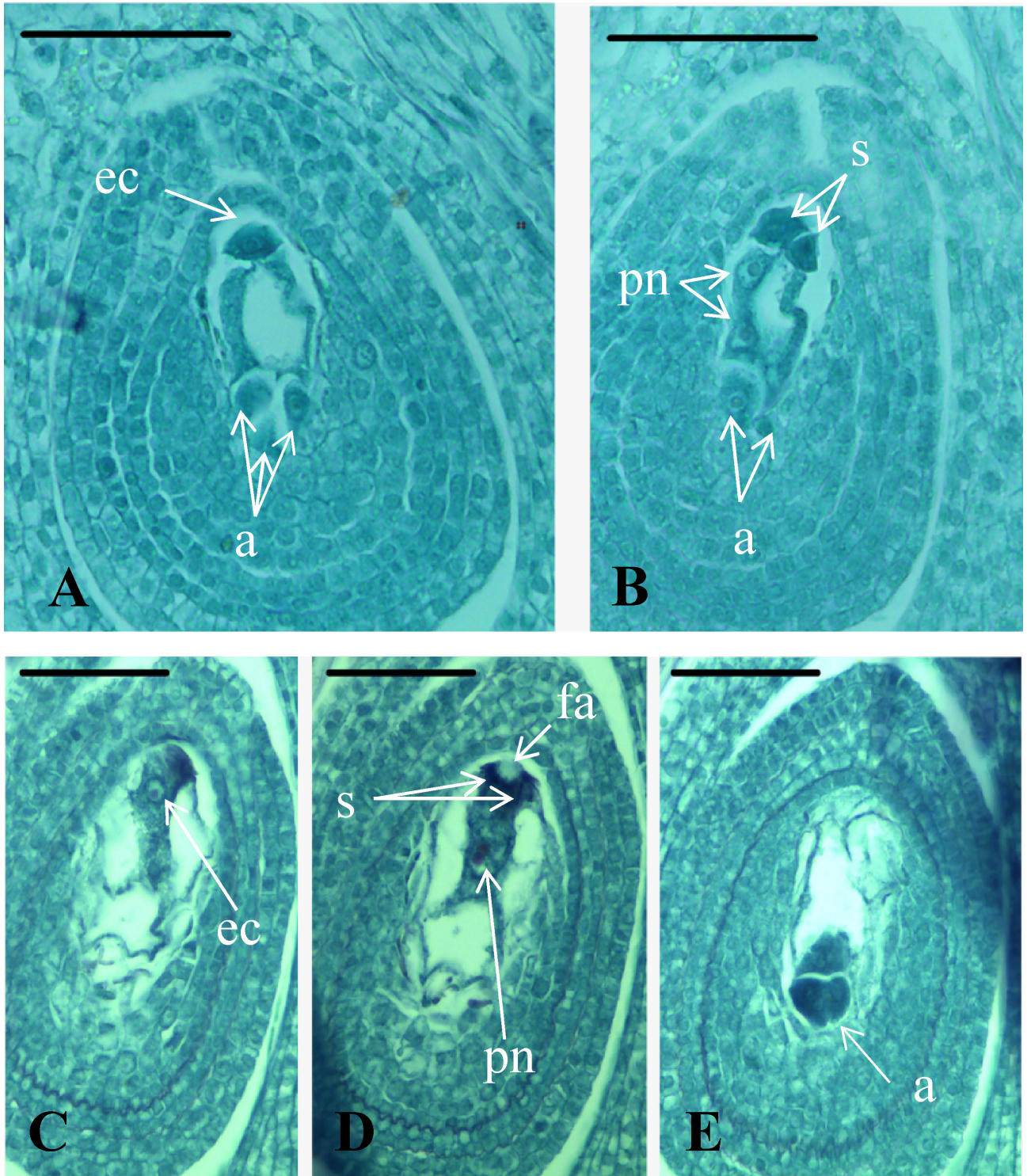
Figure 6. Megagametophyte of *Trichloris*: A, B) *T. pluriflora* megagametophyte shows two synergid cells, one egg cell, two polar nuclei, and a group of antipodes in proliferation; C–E) *T. crinita* megagametophyte with one egg cell, two synergid cells and its filar apparatus, two polar nuclei, and a group of antipodes in proliferation. a: Antipodes; fa: filar apparatus; pn: polar nucleus: ec: egg cell. S: synergid cells. Bar: 100 μm.
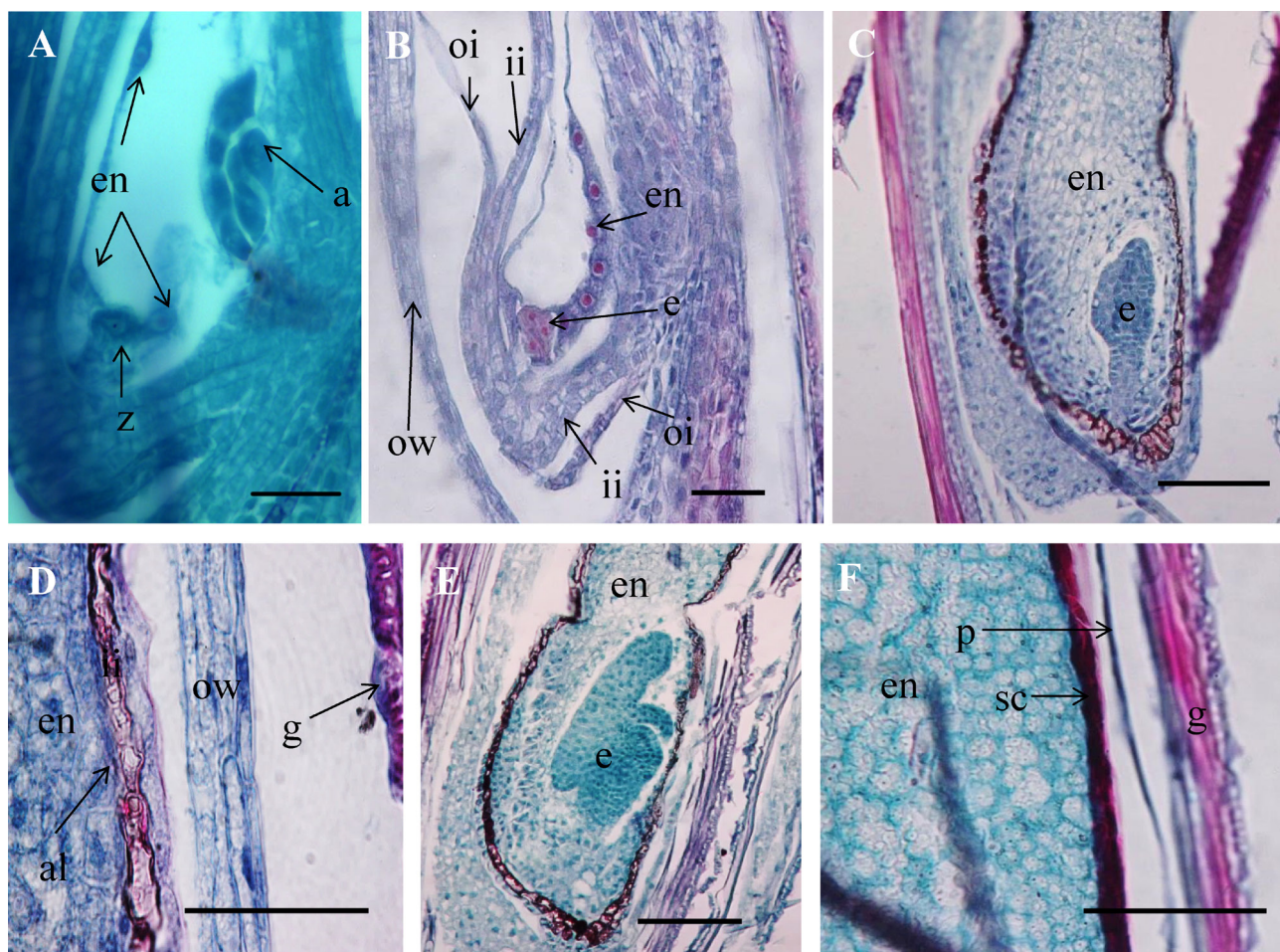
Figure 7. Longitudinal sections of postfertilized ovules of *Trichloris* at different stages of development: A) *T. crinita* ovule in a postfertilization stage with only 3 visible endosperm nuclei (of a total of 8 registered for this ovule in other histological sections not added in this figure), persistent antipodes, and the zygote showing its first cell division; B) 4-celled embryo stage of *T. pluriflora* with a nuclear endosperm; C, D) immature embryo of *T. pluriflora* with its suspensor and cellularized endosperm, and a detail of its seminal integument; E, F) immature embryo in *T. pluriflora* and a detail of its seminal integuments. a: Antipodals; al: aleurone layer; z: zygote; e: embryo; en: endosperm; g: glumella; ii: inner integument; oi: outer integument; ow: ovary wall; sc: seed coat. Bar: 100 μm.

of the ovary wall also persist, forming the pericarp that surrounds the seed, but forming it as a very light loose layer (Figure 7F).

The mature fruit contains a lateral embryo formed by a scutellum whose base is well differentiated and separated from the coleorhiza, a coleoptile protecting the caulinar apex, a coleorhiza covering the future radicle, and the epiblast that is located in the mesocotyl, opposite the scutellum (Figures 8A and 8B). Fruits of different development grades are in stage-3 inflorescences (inflorescence completely emerged from the flag leaf).

4. Discussion
This work constitutes the first record of the development of the female and male gametophytes and seed ontogeny

for *Trichloris crinita* and *T. pluriflora* in detail. The patterns of development observed are similar in both species. Therefore, these are described and discussed at genus level. The number of stamens per floret is a character not clearly recorded in the bibliography for *Trichloris*. Some authors point out this genus is characterized by the presence of 3 stamens per floret (Burkart, 1969); however, others argue that it presents only 2 (Molina and Rúgolo de Agrasar, 2006; Zuloaga et al., 2012). Nevertheless, the number of stamens observed per floret was 3 for *T. crinita*, but only 2 for *T. pluriflora*. This reduction in the number of stamens is common in grasses and is recognized as a characteristic of taxonomic, phylogenetic, evolutionary, and reproductive importance (Boechat et al., 2001; Rudall et al., 2005; Barnabás et al. 2008; Sajo et al., 2008; Nakamura et al.,
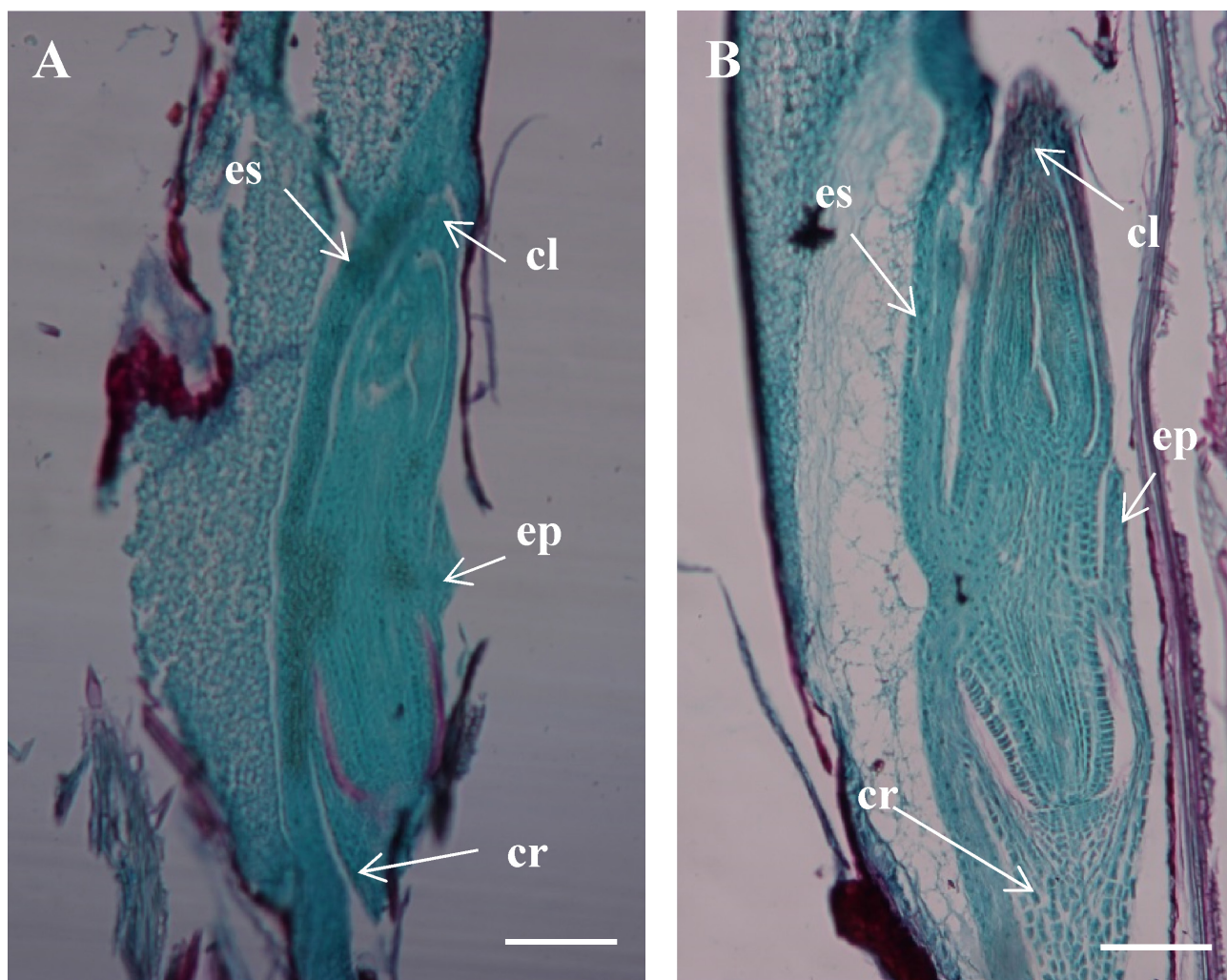
Figure 8. Mature embryo of: A) *Trichloris pluriflora*; B) T. *crinita*. cl: Coleoptile; cr: coleorhiza; ep: epiblast; es: scutellum. Bar: 100 μm.

2010). In this case, the lower proportion of fruits per floret obtained by *T. pluriflora* in comparison to *T. crinita* (Gutierrez et al., 2016) could be due to the reduction in the number of their stamens. However, further studies should be performed to test this hypothesis.

Regarding the development of anthers and pollen grains, the species of *Trichloris* are characterized by having tetrasporangiate anthers, a monocotyledonous development of the anther wall, a single middle layer with a common origin with the tapetum, fibrous thickening in the cell walls of the endothecium, and a secretory tapetum. These characteristics are constant within Poaceae (Nakamura et al., 2010); however, exceptions exist (Kellogg, 2015). In some early divergent grasses, such as *Anomochloa*, *Pharus*, and *Streptochaeta*, the anther wall formation is of the reduced type (with only three layers) (Sajo et al., 2007, 2008, 2012). Another variable character among grasses is the number of nuclei

in the tapetal cells (Bhanwra, 1988; Nakamura et al., 2010; Kellogg, 2015), even among species of the same genus (Astegiano, 1989; Lovisolo, 2011). Both species of *Trichloris* had binucleate tapetal cells, and they were degraded after the microspore release. The last is important considering that tapetal cell differentiation and subsequent disintegration should be coincident with the anther postmeiotic developmental program (Li et al., 2006). Premature or delayed degradation of tapetum is associated with male sterility (Li et al., 2006; Lovisolo, 2011), which was not observed in the studied species.

The development of orbicules observed in *Trichloris* was also described for numerous grass species and constitutes a characteristic trait for this family (Lovisolo, 2011). The morphology of these structures has been employed to assess systematics relationships at generic and tribal levels in many angiosperm families (Vinckier

and Smets, 2003; Gotelli et al., 2016) including Poaceae (Lovisolo and Galati, 2012). Due to this, the comparative study of orbicule morphology of *Trichloris* represents an interesting alternative to elucidate their systematic relationships.

The shape of the ovules is a variable feature within Poaceae, even among species of the same genus (Febulaus and Pullaiah, 1994; Lovisolo and Galati, 2007; Lovisolo, 2011). However, common characteristics have been described for the species belonging to the subfamily Chloridoideae: its ovules are typically tenuinucellate, bitegmic, and campylotropous, and the outer integument extends close to the micropyle without forming it (Bhanwra, 1988; Nakamura et al., 2009). *Trichloris* shows similar characteristics, but its ovules are hemitropous. However, contradictions exist in the literature in this respect. Some authors argue that campylotropous ovules are rare among angiosperms and that ovules in Poaceae are typically hemitropous (Endress, 2011), whereas others argue that ovules are hemicampylotropous in this family (Anton de Triquell, 1987). On the other hand, it should be mentioned that none of the observed ovules of the *Trichloris* species presented abortive characteristics.

The development of the megagametophyte in *Trichloris* corresponds to the *Polygonum* type, as mentioned in previous work (Gutierrez et al., 2015; Kozub et al., 2017). This is a common feature within Poaceae (Anton and Cocucci, 1984; Nakamura et al., 2009; Lovisolo, 2011). However, the arrangement of the megaspores is a character that can vary between species of the same genus, and even within the same species (Bhanwra, 1988; Febulaus and Pullaiah, 1994). This is not the case for *Trichloris*, since both species showed tetrads of megaspores in a "T" arrangement.

The location of the antipodals within the mature megagametophyte also varies within the family, but apart from a few exceptions, they are typically located laterally in Arundinoid, Pooideas, and Chloroideas (Bhanwra, 1988; Lovisolo, 2011). In accordance with this, the antipodals in *Trichloris* are also located laterally. In addition, antipodal proliferation was observed. Although this proliferation is a general trend in grasses, some basal species, such as in the genus *Streptochaeta*, retain the plesiomorphic condition of only three antipodes (Sajo et al., 2008).

Among the most relevant aspects that differentiate the subfamilies is the morphology of the mature embryo (Lovisolo, 2011). The presence of a more or less developed epiblast seems to be a constant characteristic among the Chloridoideae (Reeder, 1957; Peterson et al., 2010;

Lovisolo, 2011). Epiblasts were observed in both species of *Trichloris*, so the results obtained in this work support this assertion.

The type of fruit described for *Trichloris* corresponds to a specialized form of caryopsis with a free pericarp called a utricle. This characteristic was observed in several genera belonging to the same subfamily: *Eragrostis*, *Dactiloctenium*, *Disakisperma*, *Dinebra*, *Eleusine*, *Leptochloa*, *Tripogon*, *Sporobolus*, *Acrachne*, *Chloris*, *Perotis*, and *Tragus* (Bhanwra, 1988; Astegiano, 1989; Nakamura et al., 2009; Lovisolo, 2011; Snow et al., 2013; Richard et al., 2015). This feature was also found in some bamboos and is considered a derived condition within Poaceae (Kellogg, 2015). However, the relative adnation of the pericarp is recognized as an important character defining natural groups in Chloridoideae (Liu et al., 2005; Peterson et al., 2010), so it is a useful character for phylogenetic inference in *Lepthochloa* sensu lato (Snow, 1998). According to Snow et al. (2013), in the newer and narrower circumscription of *Leptochloa* (Peterson et al., 2012), only *L. chloridiformis* (Snow, 1998) has pericarps that readily disassociate in water. However, this characteristic was also observed in the two *Trichloris* species that are also embedded in that group. Despite the importance of this character, the development and structure of these fruits among Poaceae have not been widely studied (Kellogg, 2015).

In conclusion, the development of pollen grains and the megagametophyte in *Trichloris* follows the general pattern observed in most Poaceae and Chloridoideae. Further studies involving orbicule morphology and/or the development of seminal integuments in related species could help to clarify the proposed phylogenetic relationships for this genus. This is especially significant for Chloridoideae, due to the particularities observed in their fruits, and because their phylogenetic relationships also remain an object of study. In addition, the fertility evidence found in these species could explain the high percentage of fruit establishment observed in previous studies (Gutiérrez et al., 2016). This is an important feature for these species, since, like other perennial halophytes from subtropical regions (Gul et al., 2013), they do not possess elaborate seed dormancy systems.

References

Anton AMRZ, Al-Shehbaz FO, Barboza IA, Belgrano GE, Freire MJ, O'Leary SE, Rotman N, Rúgolo AD, Salariato ZE, Múlgura DL (2012). Flora Argentina: Flora vascular de la República Argentina. Buenos Aires, Argentina: Instituto de Botánica Darwinion (in Spanish).

Anton AM, Cocucci AE (1984). The megagametophyte grass and its possible phylogenetic implications. Plant Syst Evol 146: 117-121.

Anton de Triquell A (1987). Grass gametophytes: their origin, structure and relation with the sporophyte. In: Soderstrom TR, editor. Grass Systematics and Evolution: International Symposium Held at the Smithsonian Institution. Washington, DC, USA: Smithsonian Institution Press, pp. 11-20.

Arias T, Williams JH (2008). Embryology of *Manekia naranjoana* (Piperaceae) and the origin of tetrasporic, 16-nucleate female gametophytes in Piperales. Am J Bot 95: 272-285.

Astegiano ME (1989). Biología reproductiva de *Sporobolus indicus* (Poaceae) en relación a la interacción gametofito-esporofito. Kurtziana 20: 65-94 (in Spanish).

Báez CG, Agüero RO, Ernst RD, Ruiz MA (2015). Caracterización morfológica, biomasa aérea y calidad en distintas poblaciones de *Trichloris crinita*. Archivos de Zootecnia 64: 49-56 (in Spanish).

Barnabás B, Jäger K, Fehér A (2008). The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ 31: 11-38.

Berg RY (2009). Embryo sac, endosperm, and seed of *Nemophila* (Boraginaceae) relative to taxonomy, with a remark on embryogeny in *Pholistoma*. Am J Bot 96: 565-579.

Bhanwra RK (1988). Embryology in relation to systematics of Gramineae. Ann Bot 62: 215-233.

Boechat SDC, Longhi-Wagner HM (2001). The genus *Eragrostis* (Poaceae) in Brazil. Iheringia Série Botânica 55: 23-169.

Burkart A (1969). Flora ilustrada de la provincia de Entre Ríos. Colección Científica del INTA 2: 551 (in Spanish).

Cabrera AL (1994). Enciclopedia Argentina de agricultura y jardinería: regiones fitogeográficas Argentinas. (Fascicle 1), Vol. II. Buenos Aires, Argentina: ACME (in Spanish).

Cavagnaro PF, Cavagnaro JB, Lemes JL, Masuelli RW, Passera CB (2006). Genetic diversity among varieties of the native forage grass *Trichloris crinita* based on AFLP markers, morphological characters, and quantitative agronomic traits. Genome 49: 906-918.

Endress PK (2011). Evolutionary diversification of the flowers in angiosperms. Am J Bot 98: 370-396.

Febulaus GNV, Pullaiah T (1994). Embryology of *Aristida* (Poaceae). Taiwania 38: 38-48.

Gotelli MM, Galati BG, Zarlavsky G (2016). Pollen development and anther morphology in 14 species of Rhamnaceae. Plant Syst Evol 302: 1433-1444.

Gul B, Ansari R, Flowers TJ, Khan MA (2013). Germination strategies of halophyte seeds under salinity. Environ Exp Bot 92: 4-18.

Gutiérrez HF, Richard GA, Cerino MC, Pensiero JF (2016). Sistema reproductivo de *Trichloris* (Poaceae, Chloridoideae, Chlorideae). Boletín de la Sociedad Argentina de Botánica 51: 111-122 (in Spanish).

Johri BM, Ambegaokar KB, Srivastava PS (1992). Comparative Embryology of Angiosperms. Berlin, Germany: Springer-Verlag.

Kellogg EA (2015). Pollen. In: Kellogg EA, editor. Flowering Plants: Monocots. Berlin, Germany: Springer International Publishing, pp. 63-67.

Kozub PC, Barboza K, Galdeano F, Quarin CL, Cavagnaro JB, Cavagnaro PF (2017). Reproductive biology of the native forage grass *Trichloris crinita* (Poaceae, Chloridoideae). Plant Biol 19: 444-453.

Li N, Zhang DS, Liu HS, Yin CS, Li XX, Liang WQ, Yuan Z, Xu B, Chu HW, Wang J et al. (2006). The rice tapetum degeneration retardation gene is required for tapetum degradation and anther development. Plant Cell 18: 2999-3014.

Liu Q, Zhao NX, Hao G, Hu XY, Liu YX (2005). Caryopsis morphology of the Chloridoideae (Gramineae) and its systematic implications. Bot J Linn Soc 148: 57-72.

Lovisolo MR (2011). Estudios embriológicos sobre algunas gramíneas nativas útiles (Poaceae) presentes en la Depresión del Salado, Buenos Aires, Argentina. PhD, Universidad Nacional de Buenos Aires, Buenos Aires, Argentina (in Spanish).

Lovisolo MR, Galati BG (2007). Ultrastructure and development of the megagametophyte in *Eleusine tristachya* (Lam.) Lam. (Poaceae). Flora 202: 293-301.

Lovisolo MR, Galati BG (2012). Diversidad de orbículas en Poaceae. Boletín de la Sociedad Argentina de Botánica 47: 87-96 (in Spanish).

Madrid EN, Friedman WE (2010). Female gametophyte and early seed development in *Peperomia* (Piperaceae). Am J Bot 97: 1-14.

Marinoni L, Bortoluzzi A, Parra-Quijano M, Zabala JM, Pensiero JF (2015). Evaluation and improvement of the ecogeographical representativeness of a collection of the genus *Trichloris* in Argentina. Genet Resour Crop Ev 62: 593-604.

Molina AM, Rúgolo de Agrasar ZE (2006). Flora Chaqueña Argentina. Formosa, Chaco y Santiago del Estero. Buenos Aires, Argentina: INTA (in Spanish).

Nakamura AT, Longhi-Wagner HM, Scatena VL (2010). Anther and pollen development in some species of Poaceae (Poales). Braz J Biol 70: 351-360.

Nakamura AT, Longhi-Wagner HM, Scatena VL (2009). Ovule, fruit and seed development in species of Poaceae (Poales). Braz J Biol 32: 165-176.

Nicora EG, Rúgolo de Agrasar ZE (1987). Los géneros de gramíneas de América Austral. Buenos Aires, Argentina: Hemisferio (in Spanish).

Olea LE, Sosa C, Fernández M, Toll Vera JR, Nicosia MG, Agüero SN, González Coletti A, Ojeda Férez E (2015). Valor proteico del rebrote de primavera de *Trichloris pluriflora* Fourn. en la Llanura Deprimida Salina Semiárida de Tucumán. Revista Agronómica del Noroeste Argentino 35: 53-58 (in Spanish).

Parodi LR (1919). Las Chlorideas de la República Argentina. Revista de la Facultad de Agronomía y Veterinaria 2: 233-335 (in Spanish).

Peterson PM, Romaschenko K, Arrieta YH (2015). A molecular phylogeny and classification of the *Eleusininae* with a new genus, *Micrachne* (Poaceae: Chloridoideae: Cynodonteae). Taxon 64: 445-467.

Peterson PM, Romaschenko K, Johnson G (2010). A classification of the Chloridoideae (Poaceae) based on multi-gene phylogenetic trees. Mol Phylogenet Evol 55: 580-598.

Peterson PM, Romaschenko K, Snow N, Johnson G (2012). A molecular phylogeny and classification of *Leptochloa* (Poaceae: Chloridoideae: Chlorideae) sensu lato and related genera. Ann Bot 109: 1317-1330.

Quiroga RE, Fernández RJ, Golluscio RA, Blanco LJ (2013). Differential water-use strategies and drought resistance in *Trichloris crinita* plants from contrasting aridity origins. Plant Ecol 214: 1027-1035.

Ragonese AE (1955) Plantas tóxicas para el ganado en la región central Argentina. Revista de la Facultad de Agronomía UNLP 31: 133-336 (in Spanish).

Reeder JR (1957). The embryo in grass systematics. Am J Bot 756-768.

Richard GA, Pensiero JF, Cerino MC, Galati BG, Gutiérrez HF (2015). Reproductive biology of *Sporobolus phleoides* Hack. (Poaceae), an endemic halophyte grass of Argentina. Plant Syst Evol 301: 1937-1945.

Rudall PJ, Stuppy W, Cunniff J, Kellogg EA, Briggs BG (2005). Evolution of reproductive structures in grasses (Poaceae) inferred by sister-group comparison with their putative closest living relatives, Ecdeiocoleaceae. Am J Bot 92: 1432-1443.

Rúgolo ZE, Molina AM (2012) Trichloris E. Fourn. ex Benth. In: Zuloaga FO, Rúgolo ZE, Anton AM, editors. Flora vascular de la República Argentina. Monocotyledoneae, Poaceae: Aristidoideae a Pharoideae (Suppl. 1), Vol. 3. Córdoba, Argentina: Graficamente, pp. 167-170 (in Spanish).

Ruzin SE (1999). Plant Microtechnique and Microscopy. New York, NY, USA: Oxford University Press.

Sajo MG, Longhi-Wagner H, Rudall PJ (2007). Floral development and embryology in the early-divergent grass *Pharus*. Int J Plant Sci 168: 181-191.

Sajo MG, Longhi-Wagner HM, Rudall PJ (2008). Reproductive morphology of the early-divergent grass *Streptochaeta* and its bearing on the homologies of the grass spikelet. Plant Syst Evol 275: 245-255.

Sajo MG, Pabón-Mora N, Jardim J, Stevenson DW, Rudall PJ (2012). Homologies of the flower and inflorescence in the early-divergent grass *Anomochloa* (Poaceae). Am J Bot 99: 614-628.

Snow N (1998). Caryopsis morphology of *Leptochloa sensu lato* (Poaceae, Chloridoideae). SIDA Contributions to Botany 18: 271-282.

Snow N, Peterson PM, Romaschenko K (2013). Systematics of *Disakisperma* (Poaceae, Chloridoideae, Chlorideae). PhytoKeys 26: 21-70.

Vinckier S, Smets E (2003). Morphological and ultrastructural diversity of orbicules in Gentianaceae. Ann Bot 92: 657-672.

Zabala JM, Widenhorn P, Pensiero JF (2011). Germination patterns of species of the genus *Trichloris* in arid and semiarid environments. Seed Sci Technol 39: 338-353.

Zarlavsky GE (2014). Histología Vegetal: técnicas simples y complejas. Buenos Aires, Argentina: Sociedad Argentina de Botánica (in Spanish).