



# Megasporogenesis and megagametogenesis in *Hydrocleys nymphoides*, *Alisma plantago-aquatica*, and *Sagittaria montevidensis* (Alismataceae)

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## Abstract

Ovule morphology, megasporogenesis, and megagametogenesis processes were examined in *Hydrocleys nymphoides*, *Alisma plantago-aquatica*, and *Sagittaria montevidensis*. Each of these species belongs to a different clade within the Alismataceae family. It is worth mentioning that the genus *Hydrocleys* previously belonged to the Limnocharitaceae family but is now classified within the Alismataceae. Flowers in different developmental stages were processed following classical histological methods for their observation with bright-field microscope. The three species present an anatropous and bitegmic mature ovule. This is tenuinucellate in *A. plantago-aquatica* and *S. montevidensis* and pseudo-crassinucellate in *H. nymphoides*. Although all three species have the same type of megasporogenesis, they differ in the megagametogenesis and in the total number of nuclei and cells that form the mature gametophyte. *H. nymphoides* has a female gametophyte composed of four cells and four nuclei, while *A. plantago-aquatica* and *S. montevidensis* have a female gametophyte of five cells and six nuclei. The results are discussed according to the phylogenetic position of each of the species. Moreover, new types of megagametophyte development are described: *Hydrocleys* and *Sagittaria* types. The reduction of the female gametophyte with respect to the *Polygonum* type is found in families belonging to the ANA grade and in other aquatic families within the order Alismatales. We infer that the reduction in the number of cells and nuclei in the female gametophyte is characteristic of species that inhabit aquatic environments. Future studies in aquatic species belonging to other families would be necessary to confirm this hypothesis.

**Keywords** Ovule development · Megasporogenesis · Megagametogenesis · Female gametophyte · *Sagittaria* · *Hydrocleys* · *Alisma*

## Introduction

The order Alismatales is one of the most primitive divisions within the phylogeny of monocots (Davis et al. 2004; Givnish et al. 2006; Qiu et al. 2006; Ruhfel et al. 2014; Ross et al. 2016). It includes the largest clade of aquatic angiosperms, a

group of 12 exclusively hydrophytic families (Les and Tippery 2013). Between them, the Alismataceae family, is considered one of the oldest lineages, and plays an important role both in the systematics and in the knowledge of the evolutionary processes of flowering plants (Soltis et al. 2005).

According to Haynes et al. (1998), the Alismataceae family comprises 12 genera with approximately 80 species (Les et al. 1997). However, APG III also includes genera previously considered in Limnocharitaceae within this family, to maintain the family as a monophyletic group. Therefore, at present, Alismataceae comprises 17 genera, with approximately 113 species (Les and Tippery 2013). Chen et al. (2012) carried out a phylogenetic analysis based on multiple DNA sequences. The results support the fusion of Limnocharitaceae with Alismataceae as a single family. These authors described two well-supported clades based on the combined ITS data set, psbA, rbcL, and matK. Clade B

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consists of *Luronium*, *Damasonium*, *Baldellia*, and *Alisma*, and clade A is formed by the remaining genera of Alismataceae and Limncharitaceae. Recently, Li et al. (2022) identified three clades based on the analysis of 78 protein-coding genes (PVGs) from each of the chloroplast genomes. The results showed that clade B is composed of the genera *Butomopsis*, *Hydrocleys*, and *Limncharis*. On the other hand, clade C is formed by the genera *Alisma*, *Baldellia*, *Damasonium*, *Burnatia*, and *Luronium*. Finally, clade A is composed by the remaining genera of the Alismataceae family. In Argentina, there are six genera: *Hydrocleys* and *Limncharis* (clade B), *Alisma* (clade C), and *Sagittaria*, *Helianthium*, and *Echinodorus* (clade A).

The classification of Alismataceae based on morphological characters remains controversial today (Rogers 1983). This is probably because of morphological reduction and phenotypic plasticity of aquatic plants in various environments (Les et al. 2005, 2006). However, the embryological characters are stable and are less affected by the environment. Therefore, embryological characters are of considerable importance for taxonomy (Pandey 1997). The contributions of embryology to systematic can be considered as significant as those offered by other disciplines. It is considered a relevant tool to identify taxon relationships at all levels (Herr 1984).

The characteristics of the ovule and the female gametophyte development of *Hydrocleys nymphoides* (Willd.) Buchenau were described by Suessenguth (1920) and later by Johri (1938). Ward (1880) described for the first time the development of the embryo sac of *Alisma plantago-aquatica* L. and Bessey (1898) studied the development of the ovule. Other authors also investigated the development of the female gametophyte of this species: Fischer (1880), Schaffner (1896), Nitzschke (1914), Dahlgren (1928), and Johri (1936); however, the results obtained are controversial. Schaffner (1897) described the development of the embryo sac and the female gametophyte of *Sagittaria latifolia*. Cook (1907) described it for *S. lancifolia* and Dahlgren (1934) for *S. sagittifolia*. Johri (1935) compiled the information described by the previous authors and incorporated the results obtained by him in *S. guayanensis* and *S. graminea* in 1936. The most recent study of embryology within the genus belongs to *S. guayanensis* subsp. *lappula* (Wang et al. 1997).

Most current embryological studies within the family date from the beginning of the twentieth century and lack photomicrographs that back their descriptions. Therefore, the objective of this work is to update and expand the embryological information of species representing each of the clades. Consequently, the study of the development of the female gametophyte of *Hydrocleys nymphoides* (Humb. & Bonpl. ex Willd.) Buchenau (Clade B), *Alisma plantago-aquatica* L (Clade C), and *Sagittaria montevidensis* Cham. & Schltld (Clade A) was carried out. Our hypothesis is that

embryological characters will be relevant to support the current molecular phylogeny and will help to clarify the evolutionary processes.

## Materials and methods

Flowers from populations of *Hydrocleys nymphoides*, *Alisma plantago-aquatica*, and *Sagittaria montevidensis* cultivated under natural conditions in the Lucien Haumann Botanical Garden, located in the Faculty of Agronomy of the University of Buenos Aires, Buenos Aires City, Argentina, were selected and collected. All species flowered during late spring and summer: *H. nymphoides* from December to February, *A. plantago-aquatica* from November to February, and *S. montevidensis* from November to March. Three successive samplings were carried out between the years 2020 and 2022. In each sampling, approximately 300 flowers were collected for each species in different stages of development, for their subsequent fixation in FAA (formol-acetic acid-alcohol). Vouchers of *H. nymphoides* (29758 BAA), *A. plantago-aquatica* (29755 BAA), and *S. montevidensis* (29757 BAA) were deposited in the Herbarium Gaspar Xuarez.

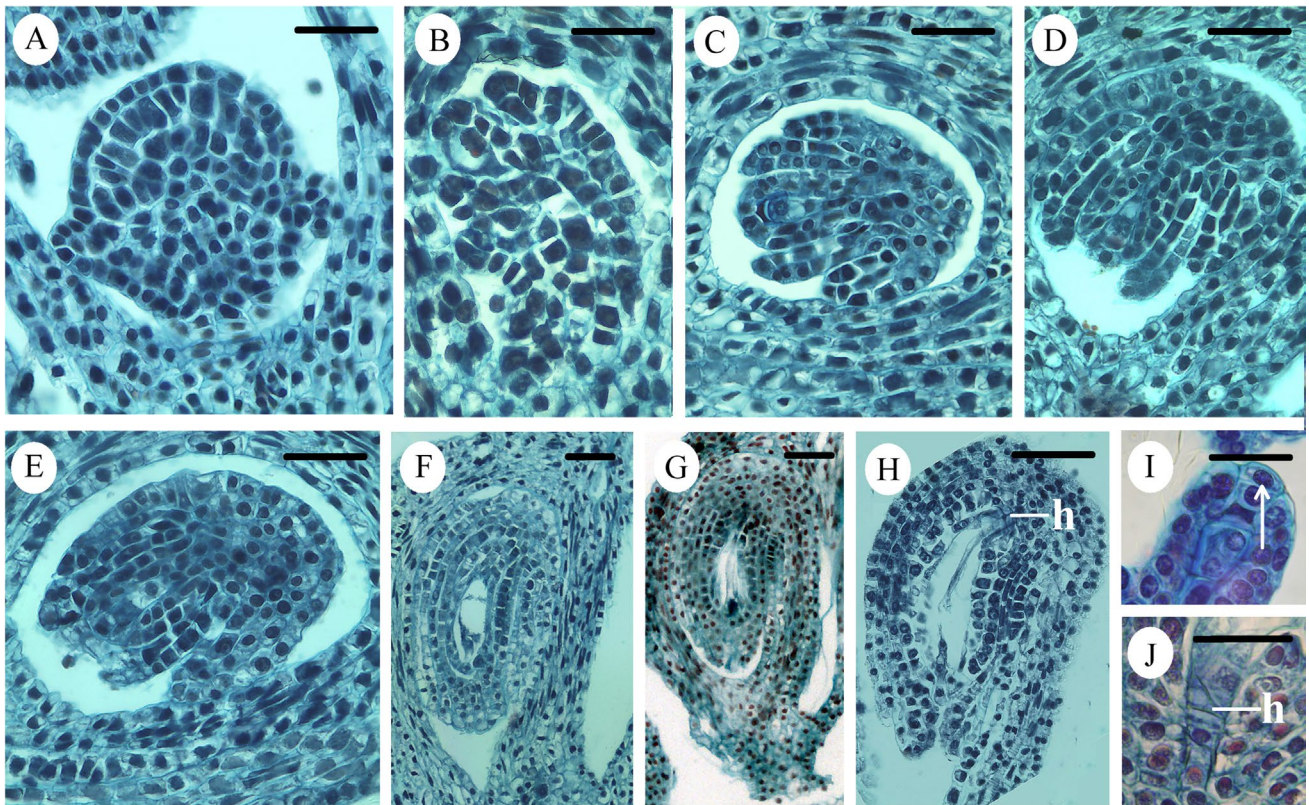
The pistils were subjected to classical histological techniques: ascending serial dehydration of alcohols (ethyl alcohol-xylol), paraffin embedding, 10- $\mu$ m-thick longitudinal sections with a rotary microtome, safranin-fast-green double staining, and finally mounting on synthetic resin (D'Ambrogio de Argüeso 1986). The photomicrographs were taken with a Motic microscope with a built-in digital camera DMWB1-223ASC.

## Results

### Ovule

The mature ovules of *H. nymphoides*, *A. plantago-aquatica*, and *S. montevidensis* are of the anatropous type. The ovules are initiated by periclinal divisions of the subdermal layer of the placental meristem, giving a small ovule primordium covered only by the anticlinally dividing dermal layer (Fig. 1A). The body of the ovule is curved 180°, so that the funicle is elongated, it is soldered on one side of the nucellus constituting the raphe, and the chalaza is opposite the hilum and the micropyle (Fig. 1B–E).

The three species are bitegmic, the internal integument develops first, and the development of the external integument begins later, in both cases through the periclinal division of the dermal layer. The two integuments present two layers of cells thick. The external integument does not participate in the formation of the micropyle, so it is constituted only by an endostome (Fig. 1F–H).



**Fig. 1.** Light microscopy (LM) photomicrographs of ovules at successive stages of development in *A. plantago-aquatica* (A–F), *S. montevidensis* (F), and *H. nymphoides* (H–J). **A** Ovule primordium.

**B–E** Ovule curvature process. **F–H** Mature ovule. **I** Periclinal division of the nucellar epidermis (arrow). **J** Detail of the hypostasis (h). Scale bar = 60  $\mu\text{m}$  (A–E, H–J); scale bar = 100  $\mu\text{m}$  (F–G)

In *S. montevidensis* and *A. plantago-aquatica*, the megaspore mother cell develops just below the nucellar epidermis defining a tenuinucellate type of ovule, while in *H. nymphoides*, the nucellar epidermis divides once periclinally giving a type of pseudo-crassinucellate ovule (Fig. 1I).

In the mature ovule of *H. nymphoides*, a group of cells of the nucellus, close to the chalaza and in direct contact with the female gametophyte, is distinguished by its thicker walls that stain intensely with the fast-green dye (Fig. 1J), thus constituting a hypostasis.

## Megasporogenesis

In *H. nymphoides*, *S. montevidensis*, and *A. plantago-aquatica*, the archesporic cell at the apex of the nucellus acts directly as the megaspore mother cell (MMC). This is distinguished by its larger volume and prominent nucleus compared to the surrounding nucellus cells (Fig. 2A).

Meiotic division I of the MMC results in a dyad, with a chalazal cell larger than the micropylar (Fig. 2B–D). The latter progressively aborts until the start of meiotic division II (Fig. 2E–F), which only occurs in the chalazal cell, resulting

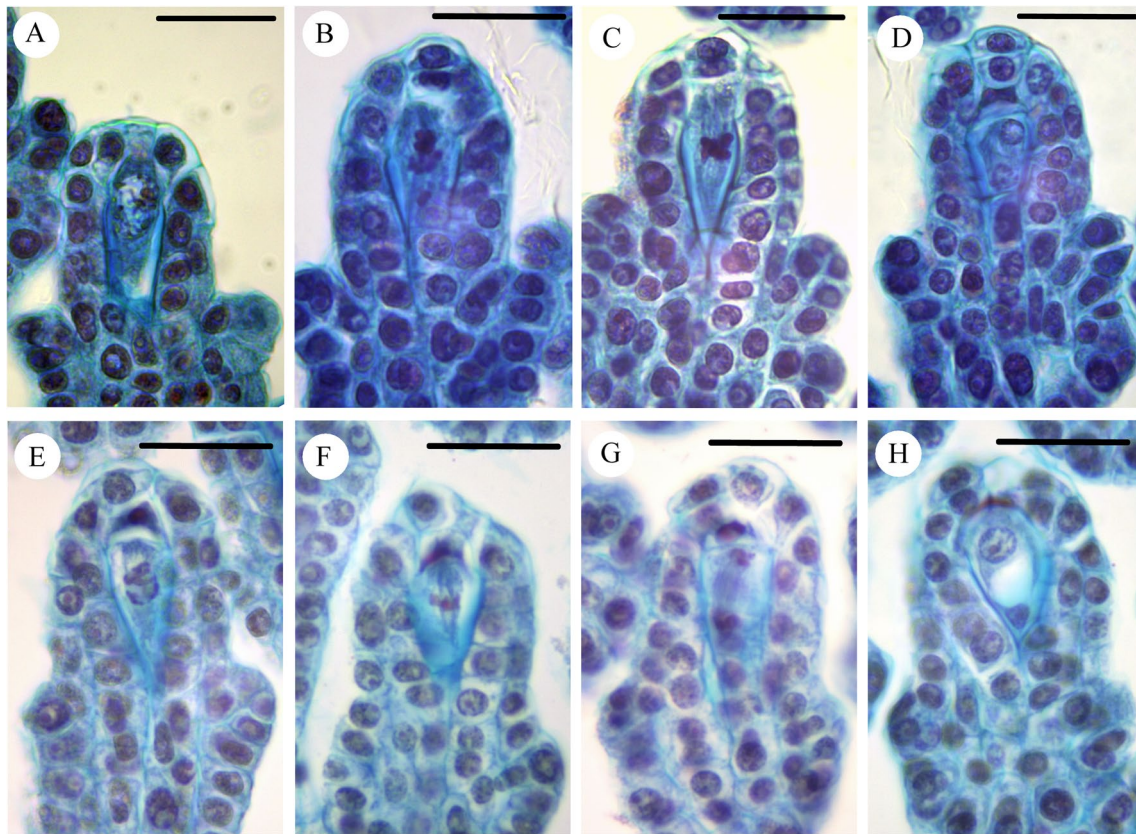
in two nuclei without cytokinesis (Fig. 2G), thus forming a binucleate coenomegaspore (Fig. 2H).

## Megagametogenesis

### *Hydrocleys nymphoides*

The chalazal nucleus of the coenomegaspore is smaller and progressively aborts. Only the micropylar nucleus of the coenomegaspore undergoes two mitotic karyokinesis giving rise to a pentanucleate embryo sac (Fig. 3A). After cytokinesis, the embryo sac differentiates into a five-nucleate female gametophyte composed of the egg cell, two synergids, a cell with a single polar nucleus, and the chalazal nucleus with signs of abortion. This latter is no longer observed when the gametophyte is fully mature, so the female gametophyte is tetranucleate and tetracellular (Fig. 3B–F).

The synergids have a large vacuole located in the chalazal region and the nucleus at the micropylar end; meanwhile, in the egg cell, the vacuole is in the micropylar region with the nucleus at the chalazal pole. The central cell has a single polar nucleus.



**Fig. 2.** LM photomicrographs of megasporogenesis in *Hydrocleys nymphoides*. **A** Detail of the megaspore mother cell (MMC). **B** Prophase I of the MMC. **C** Metaphase I of the MMC. **D** Telophase I of

the MMC. **E** The micropylar cell of the diade progressively aborts. **F** The chalazal megaspore in metaphase II. **G** The chalazal megaspore in telophase II. **H** Binucleate coenomegaspore. Scale bar = 30  $\mu$ m

### *Alisma plantago-aquatica* and *Sagittaria montevidensis*

The coenomegaspore presents the chalazal nucleus smaller than the micropylar (Fig. 4B–C). A first mitotic karyokinesis occurs in both nuclei, giving rise to a tetranucleate embryo sac with two micropylar nuclei of greater volume than the chalazal ones (Fig. 4D). The two micropylar nuclei undergo further mitotic karyokinesis resulting in a six-nucleated embryo sac (Fig. 4E–F). After cytokinesis, the mature female gametophyte consists of the egg apparatus formed by two synergids and an egg cell: a central cell with two polar nuclei and a single antipodal cell. One of the polar nuclei is located near the egg apparatus, while the other is close to the only antipodal cell, both separated by a large vacuole (Fig. 4G).

### Fertilization

After discharge of both male gametes through the pollen tube, one of them fertilizes the oosphere and the other to the polar nucleus in *H. nymphoides*. In *S. montevidensis* and *A. plantago-aquatica*, the two polar nuclei are not fused, and the

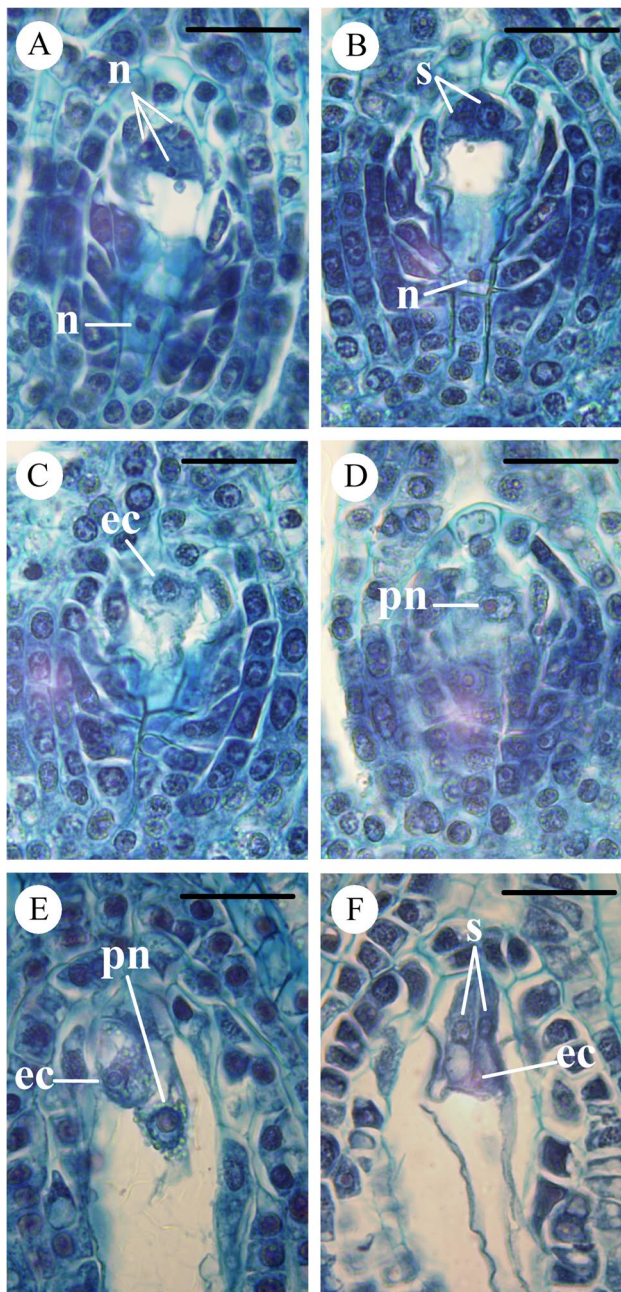
male gamete fertilizes only the one closest to the micropyle. In the chalazal zone, the single antipodal cell divides mitotically, giving rise to two cells. The remaining polar nucleus becomes polyploid by endomitosis. This nucleus is not fertilized and therefore is not part of the endosperm nucleus (Fig. 4H).

## Discussion

### Ovule

The ovule of the three species studied is anatropous and bitegmic. According to the position of the megaspore mother cell, the ovule is tenuinucellate in *S. montevidensis* and *A. plantago-aquatica* and pseudocrassinucellate in *Hydrocleys nymphoides*. This is consistent with previous studies by Johri (1938) on *Hydrocleys nymphoides*, Bessey (1898) on *Alisma plantago-aquatica*, and Schaffner (1897) and Cook (1907) on selected species of the genus *Sagittaria*.

The presence of a hypostasis in the ovule of *H. nymphoides* was described in the present study. This constitutes the first report on the presence of this ovular structure for



**Fig. 3.** LM photomicrographs of the female gametophyte development in *H. nymphoides*. **A** Five-nucleate stage of the embryo sac (arrows). **B–D** Three serial longitudinal sections of the mature 4-celled female gametophyte. **E** Detail of the egg cell, one of the synergids, and polar nucleus. **F** Detail of the two synergids and egg cell. Scale bar = 30  $\mu$ m (ec, egg cell; n, nucleus; pn, polar nucleus; s, synergid)

the family. Hypostasis is defined as a set of cells with thickened cell walls located between the region of the embryo sac and the chalaza, forming a relatively impermeable barrier between both regions (Rudall 2021). This structure delimits the growth of the female gametophyte, which represents a critical factor in its development.

## Megasporogenesis

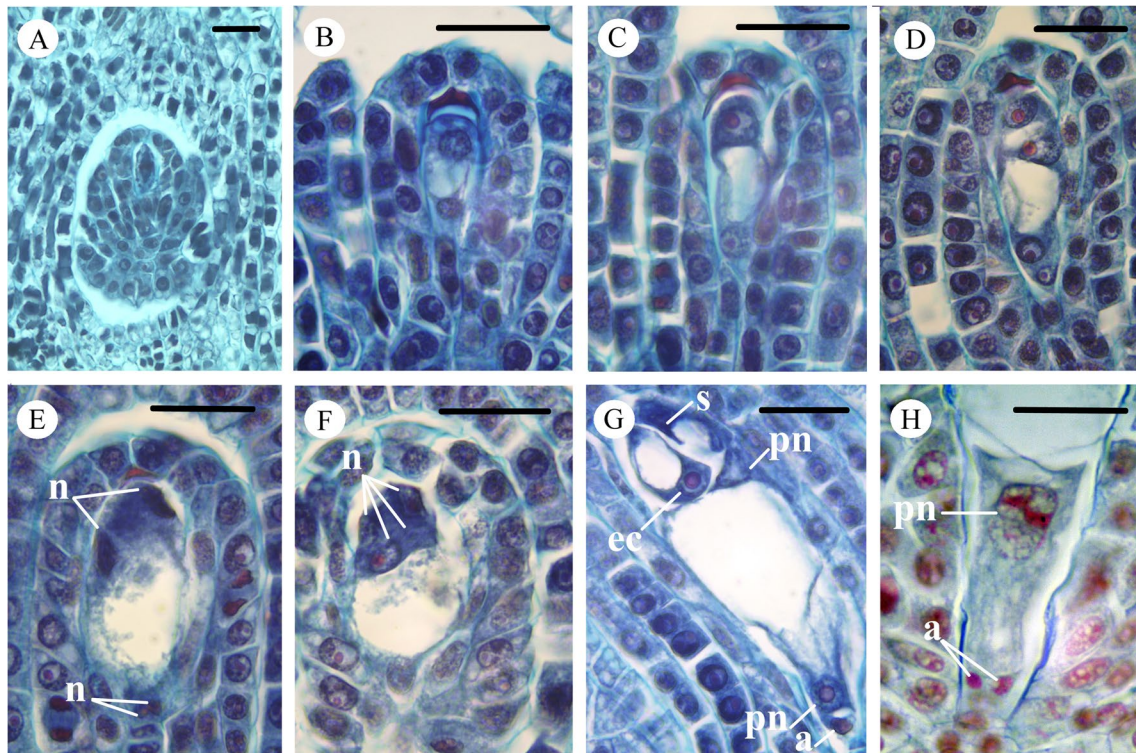
In the three described species, megasporogenesis is coincident, since after meiosis, two binucleated coenomegaspores are formed. The micropylar aborts, and the chalazal stands out for presenting a larger micropylar nucleus. Nevertheless, disparities were noted in comparison to earlier research. The first works in *Hydrocleys nymphoides* (Suessenguth 1920), in *Sagittaria latifolia* (Schaffner 1897), and *S. laurifolia* (Cook 1907) describe the formation of a linear megaspore tetrad that responds to the development of the Polygonum-type embryo sac. On the other hand, Johri (1935, 1936, 1938) described for *H. nymphoides*, *S. guayanensis*, and *S. graminea* the formation of two coenomegaspores with the abortive micropylar, coinciding with the development of the Allium-type embryo sac.

Many authors depict contradictory results in *A. plantago-aquatica* (Ward 1880; Fischer 1880; Schaffner 1896). According to Nitzschke (1914), the megaspore mother cell produces four free nuclei, of which three degenerate. Dahlgren (1928) describes the formation of a dyad of coenomegaspores. This observation was supported by Johri (1936) and our own findings.

## Megagametogenesis

Suessenguth (1920) described the development of the embryo sac of *H. nymphoides* as Polygonum type, with 7 cells and 8 nuclei. Later, Johri (1938) defined the development of the embryo sac as a variant of the reduced Allium type, with only five nuclei instead of eight and five cells instead of seven. This author describes the formation of the embryo sac from a coenomegaspore with the micropylar nucleus of greater volume than the chalazal. The latter does not divide further and gives rise to a single ephemeral antipode. The micropylar nucleus after two mitotic divisions gives rise to the egg apparatus and the single polar nucleus. The results obtained in this work coincide partially with what was reported by Johri (1938). The embryo sac is bisporic, taking into account the type of megasporogenesis described, but the megagametogenesis studied presents some differences. The mature megagametophyte is composed of only 4 nuclei and 4 cells, because antipodal differentiation does not take place due to rapid abortion of the chalazal nucleus of the coenomegaspore. The formation of the embryo sac starts from a binucleated coenomegaspore like the “Allium” type, but the later events in its formation are completely different, so it is considered appropriate to define it as the “Hydrocleys” type.

The development of the mature female gametophyte of *Sagittaria montevidensis* was not previously described. Schaffner (1897) and Cook (1907), respectively, describe



**Fig. 4.** LM photomicrographs of the female gametophyte development in *A. plantago-aquatica* (A–G) and fertilization in *S. montevidensis* (H). **A** Megaspore mother cell in metaphase. **B–C** Coenomegaspores with detail of the aborted megaspore in the micropylar region. **D** Tetranucleate embryo sac. **E** The two micropylar nuclei in

metaphase. **F** Three of the four micropylar nuclei. **G** Mature female gametophyte. **H** Bicellular proembryo stage polyloid polar nucleus near the two antipodal cells. Scale bar = 60  $\mu\text{m}$  (A); scale bar = 30  $\mu\text{m}$  (B–G); scale bar = 15  $\mu\text{m}$  (H) (a, antipodal; ec, egg cell; n, nucleus; pn, polar nucleus; s, synergid)

the development of the mature gametophyte of *S. latifolia* and of *S. laurifolia* as Polygonum type with ephemeral antipodes. Johri studied the formation of the embryo sac of *S. guayanensis* (1935) and *S. graminea* (1936), which starts from a chalazal coenomegaspore giving rise to mature gametophytes with 6, 7, or 8 nuclei. This is the result of the last mitosis of development, which can occur only in the two micropylar nuclei, or in the two micropylar and one chalazal, or in the two micropylar and the two chalazal. Dahlgren (1934) observed the same embryo sac development with cases of 6, 7, and 8 nuclei for *S. sagittifolia*. More recently, Wang et al. (1997) found in *S. guayanensis* subsp. *lappula* a single type of mature gametophyte made up of eight nuclei and seven cells. Since the antipodes divide mitotically after fertilization, the variation in the number of nuclei could be expected. According to the observations made in this work, the mature gametophyte of *S. montevidensis* has six nuclei and five cells previous fertilization.

Nitzschke (1914) observed an eight-nucleated embryo sac for *Alisma plantago-aquatica*. Dahlgren's (1928) described the formation of the embryo sac from the chalazal coenomegaspore giving rise to a mature six-nucleated gametophyte. Johri (1936) confirmed these observations,

which coincide with what was described in the present study. The mature gametophyte of this species consists of six nuclei and five cells.

*Sagittaria montevidensis* and *Alisma plantago-aquatica* coincide in the characteristics of the megagametophyte. Until now, it was considered that it presented an Allium-type development because they start from a chalazal coenomegaspore. However, the Allium type has three antipodes, while *S. montevidensis* and *A. plantago-aquatica* have only one, with the two polar nuclei differing in size and position. Due to this, it is suggested to define the development of the megagametophyte of these species as "Sagittaria" type.

### Reduced gametophyte

The reduction of the mature gametophyte is observed in a couple of families belonging to the Alismatales order. Within the Butomaceae, Potamogetonaceae, and Scheuchzeriaceae families, a development pattern like that of the Polygonum type has been described. However, it should be noted that in these last two families, the existence of ephemeral antipodes is mentioned (Johri

et al. 2013). This presence of ephemeral antipodes could potentially indicate an incipient reduction process in the mature gametophyte or the fact that the antipodes have not been observed could be that they are not ephemeral, but rather that they do not develop. More studies are necessary to confirm it. In the case of Butomaceae, a family to which the genus *Hydrocleys* once belonged, gametophytes with 8 nuclei and 7 cells and 6 nuclei and 5 cells, as well as 4 nuclei and 4 cells, respectively, were identified (Fernando and Cass 1996).

Reduced gametophytes were also observed in some basal angiosperms such as genera belonging to the orders Nymphaeales and Austrobaileyales (Batygina et al. 1980; Galati 1985; Battaglia 1986; Van Miegroet and Dujardin 1992; Orban and Bouharmont 1998; Friedman 2001; Williams and Friedman 2004; Tobe et al. 2007; Zini et al. 2015). The female gametophytes of these orders exhibit a specific monosporic pattern in which two synergids, an egg cell and a cell, with a polar nucleus are found. This pattern of development of the female gametophyte is known as the *Schisandra* type, since it was first described by Batygina et al. (1980) in species of the genus *Schisandra* (Schisandraceae, Austrobaileyales). This four-nucleated, four-celled female gametophyte has been considered an ancestral character in flowering plants (Friedman 2001). Alismatales is considered the second oldest clade of monocots (Davis et al. 2004; Givnish et al. 2006; Graham et al. 2006; Qiu et al. 2006; Ruhfel et al. 2014). The results we obtained from the three species from different genus and clades within the Alismataceae and show a clear reduction in the number of cells that form the female gametophyte, ranging from five cells and six nuclei (*S. montevidensis* and *A. plantago-aquatica*) to four cells and four nuclei (*H. nymphoides*) as in basal angiosperms. It is interesting to note that in all these species, the endosperm would be diploid, as a result of the fertilization of a single polar nucleus. In *S. montevidensis* and *A. plantago-aquatica*,

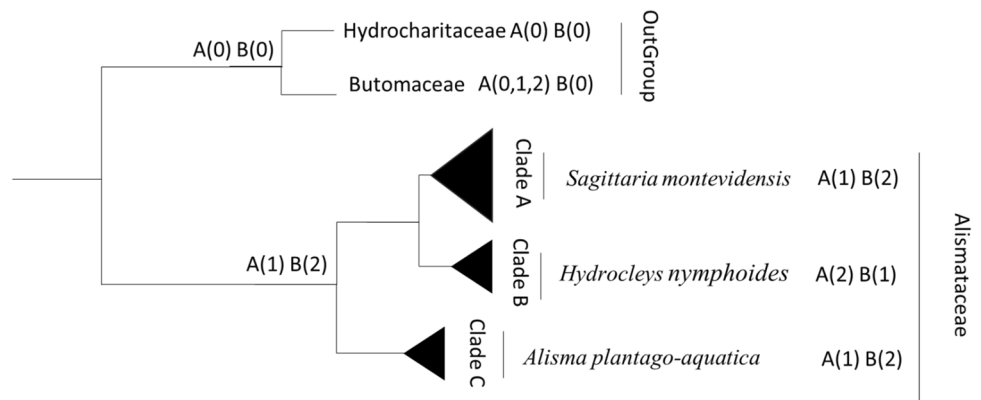
although there are 2 polar nuclei, only one is fertilized, so the endosperm is also 2n.

It is possible to infer that the reduction in the number of cells and nuclei that compose the female gametophyte could be related to acceleration in its maturation as a consequence of rapid floral development. This could result in an adaptive advantage in the environment in which the species develops, since the reduced gametophyte occurs in species that inhabit aquatic or marshy environments. To confirm this hypothesis, it is necessary to carry out studies related to biology and floral phenology.

### Conclusions

We can identify differences and similarities in the development of the embryo sac and the characteristics of the female gametophytes in a representative of each clade of the family Alismataceae. The mapping of the characters (Fig. 5) demonstrates a hypothetical common ancestor to the three clades of the family with a possible tenuinucelled ovule type and a number of cells of the female gametophyte of six nuclei and five cells, with a clear reduction of the gametophyte and change of ovule type to pseudo-crassinucellate in clade B. Analyzing the outgroup, a polymorphy of the character number of cells of the gametophyte is observed in the Butomaceae family. The study of other species belonging to the clades is necessary to sustain the results achieved. These findings are relevant to determine the inclusion of genera that previously belonged to Limnocharitaceae in this family. The results obtained reveal an identical megasporogenesis in the three species and only *A. plantago-aquatica* (clade C) and *S. montevidensis* (clade A) coincide in megagametogenesis (Fig. 6).

**Fig. 5.** Composition of the mature gametophyte and type of ovule indicated in a shorted phylogeny postulated by Li et al. (2022). Composition of the mature gametophyte (character A), (0) 8 nuclei 7 cells, (1) 6 nuclei 5 cells, (2) 4 nuclei 4 cells; ovule type (character B), (0) crassinucellate, (1) pseudo-crassinucellate, (2) tenuinucellate



	Megaspороgenesis			Megagametogenesis			
	Megaspore mother cell	Meiosis I	Meiosis II	Mitosis I	Mitosis II	Mitosis III	Mature embryo sac
<i>Hydrocleys nymphoides</i>						Absent	
<i>Alisma plantago-aquatica</i> and <i>Sagittaria montevidensis</i>						Absent	

**Fig. 6.** Schemes of megasporogenesis and megagametogenesis in *H. nymphoides*, *A. plantago-aquatica*, and *S. montevidensis*

**Author contribution** MN, BG, and MG conducted the research. MN wrote the manuscript. BG and MG reviewed and completed the information. GZ and SR prepared, processed, and observed the preparations. All authors read and approved the final manuscript.

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## Declarations

**Competing interests** The authors declare no competing interests.

## References

- APG (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 161:105–121
- Battaglia E (1986) Embryological questions: 7. Do new types of embryo sac occur in *Schisandra*. *Ann Bot* 44:69–82
- Batygina TB, Kravtsova T, Shamrov II (1980) Comparative embryology of some representatives of the orders Nymphaeales and Nelumbonales. *Bot Zh* 65:1071–1087
- Bessey EA (1898) The comparative morphology of the pistils of the Ranunculaceae, Alismaceae and Rosaceae. *Bot Gaz* 26:297–314
- Chen LY, Chen JM, Gituru RW, Wang QF (2012) Generic phylogeny, historical biogeography and character evolution of the cosmopolitan aquatic plant family Hydrocharitaceae. *BMC Evol Biol* 12(1):1–12
- Cook MT (1907) The embryology of *Sagittaria lancifolia* L. *Ohio Nat* 1907(7):97–101
- D'Ambrogio de Argüeso A (1986) Manual de técnicas en histología vegetal. Hemisferio sur, Buenos Aires
- Dahlgren KVO (1928) (1928) Die Embryologie einiger Alismataceen. *Svensk Bot Tidskr* 22:1–17
- Dahlgren KVO (1934) Die Embryosackentwicklung von *Echinodorus macrophyllus* und *Sagittaria sagittifolia*. *Planta* 21:602–612
- Davis JJ, Stevenson DW, Petersen G, Seberg O, Campbell LM, Freudenstein JV, Gandolfo M (2004) A phylogeny of the monocots, as inferred from *rbcl* and *atpA* sequence variation, and comparison of methods for calculating jackknife and bootstrap values. *Syst Bot* 29(3):467–510
- Fernando DD, Cass DD (1996) Development and structure of ovule, embryo sac, embryo, and endosperm in *Butomus umbellatus* (Butomaceae). *Int J Plant Sci* 157(3):269–279
- Fischer A (1880) Zur Kenntnis der Embryosackentwicklung einiger Angiospermen. *Jenaische Zeitschr. F. Naturaleza*, 14.
- Friedman WE (2001) Comparative embryology of basal angiosperms. *Curr Opin Plant Biol* 4(1):14–20
- Galati BG (1985) Estudios embriológicos en *Cabomba australis* (Nymphaeaceae) I. La esporogénesis y las generaciones sexuales. *Bol Soc Argent Bot* 24:29–47
- Givnish TJ, Pires JC, Graham SW, McPherson MA, Prince LM, Patterson TB, Sytsma KJ (2006) Phylogenetic relationships of monocots based on the highly informative plastid gene *ndhF*. *Aliso: J System Floris Bot* 22(1):28–51
- Graham SW, Zgurski JM, McPherson MA, Cherniawsky DM, Saarela JM, Horne ES, Smith SY, Wong WA, O'Brien HE, Biron VL, Pires JC, Olmstead RG, Chase MW, Rai HS (2006) Robust inference of monocot deep phylogeny using an expanded multigene plastid data set. *Aliso* 22:3–21
- Haynes RR, Les DH, Holm-Nielsen LB (1998) Alismataceae. In: *Flowering Plants: Monocotyledons*. Springer, pp 11–18
- Herr JM (1984) Embryology and taxonomy. In: Johri BM (ed) *Embryology of angiosperms*. Springer, Berlin, Heidelberg, pp 647–696
- Johri BM (1935) Studies in the family Alismaceae: II. *Sagittaria sagittifolia* L. In: *Proceedings/Indian Academy of Sciences*, vol 1. 7, Springer India, New Delhi, pp 340–348
- Johri BM (1936) Studies in the family Alismaceae: IV. *Alisma plantago* L.; *Alisma plantago-aquatica* L. and *Sagittaria graminea* Mich. In *Proceedings/Indian Academy of Sciences* (4, 2, 128–138). New Delhi: Springer India.
- Johri BM (1938) The embryo sac of *Hydrocleys nymphoides* Buch. *Beih Bot zbl* 48:165–172
- Johri BM, Ambegaokar KB, Srivastava PS (2013) *Comparative embryology of angiosperms*, vol 1/2 (Vol. 1). Springer Science & Business Media
- Les DH, Cleland MA, Waycott M (1997) Phylogenetic studies in Alismatidae, II: evolution of marine angiosperms (seagrasses) and hydrophyly. *Syst Bot*:443–463



- Les DH, Moody MA, Jacobs S (2005) Phylogeny and systematics of Aponogeton (Aponogetonaceae): the Australian species. *Syst Bot*:503–519
- Les DH, Moody MA, Soros C (2006) A reappraisal of phylogenetic relationships in the monocotyledon family Hydrocharitaceae (Alismatidae). *Aliso*:211–230
- Les DH, Tippery NP (2013) In time and with water... the systematics of alismatid monocotyledons. *Early Events Monocot Evol* 83:118–164
- Li ZZ, Lehtonen S, Martins K, Wang QF, Chen JM (2022) Complete genus-level plastid phylogenomics of Alismataceae with revisited historical biogeography. *Mol Phylogenet Evol* 166:107334
- Nitzschke J (1914) Beiträge zur Phylogenie der Monocotyledonen, gegründet auf der Embryosackentwicklung apokarper Nymphaeaceen und Helobien. *Beiträge z. Biolog, d. Pflanzen* 12:223–267
- Orban I, Bouharmont J (1998) Megagametophyte development of *Nymphaea nouchali* Burm. f. (Nymphaeaceae). *Bot J Linn Soc* 126:339–348
- Pandey AK (1997) Introduction to the embryology of Angiosperms. CBS, New Delhi, p 453
- Qiu YL, Li L, Hendry TA, Li R, Taylor DW, Issa MJ, White AM (2006) Reconstructing the basal angiosperm phylogeny: evaluating information content of mitochondrial genes. *Taxon* 55(4):837–856
- Rogers GK (1983) The genera of Alismataceae in the southeastern United States. *J Arnold Arbor* 64:383–420
- Ross TG, Barrett CF, Soto Gómez M, Lam VK, Henriquez CL, Les DH, Graham SW (2016) Plastid phylogenomics and molecular evolution of Alismatales. *Cladistics* 32(2):160–178
- Rudall PJ (2021) Evolution and patterning of the ovule in seed plants. *Biol Rev* 96(3):943–960
- Ruhfel BR, Gitzendanner MA, Soltis PS, Soltis DE, Burleigh JG (2014) From algae to angiosperms—inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. *BMC Evol Biol* 14(1):1–27
- Schaffner JH (1896) The embryo sac of *Alisma plantago*. *Bot Gaz* 21:123–132
- Schaffner JH (1897) (1897) The life history of *Sagittaria variabilis*. *Bot Gaz* 23:252–273
- Soltis DE, Soltis PS, Endress PK, Chase MW (2005) Angiosperm phylogeny and evolution. Sinauer, Sunderland, MA
- Suessenguth K (1920) Beiträge zur Frage des systematischen Anschlusses der Monokotylen. *Beih Bot Centralbl* 38:1–79
- Tobe H, Kimoto Y, Prakash N (2007) Development and structure of the female gametophyte in *Austrobaileya scandens* (Austrobaileyaaceae). *J Plant Res* 120:431–436
- Van Miegroet F, Dujardin M (1992) Cytologie et histologie de la reproduction chez le *Nymphaea heudelotii*. *Can J Bot* 70:1991–1996
- Wang JB, Chen JK, Li RQ (1997) Embryological Studies on *Sagittaria guayanensis* HBK Subsp. *lappula* (D. Don) Bojin (Alismataceae). *J Syst Evol* 35(4):297
- Ward HM (1880) A contribution to our knowledge of the embryo sac in Angiosperms. *Jour Linn Soc Bot de Londres* 17:519–546
- Williams JH, Friedman WE (2004) The four-celled female gametophyte of *Illicium* (Illiciaceae; Austrobaileyales): implications for understanding the origin and early evolution of monocots, eumagnoliids, and eudicots. *Am J Bot* 91(3):332–351
- Zini LM, Galati BG, Ferrucci MS (2015) Ovule and female gametophyte in representatives of *Nymphaea* subgenus *Hydrocallis* and *Victoria* (Nymphaeaceae; Nymphaeoidaeae). *Aquatic Botany* 120:322–332

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