

Embryology of *Ageratum conyzoides* L. and *A. fastigiatum* R.M. King & H. Rob. (Asteraceae)

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Received: April 29, 2014. Accepted: July 1, 2014

ABSTRACT

Ageratum has a complex circumscription, and recent studies have indicated its polyphyly. The genus has been placed in the tribe Eupatorieae whose embryology is not fully known. Embryological data are conservative and important indicators of phylogenetic relationships and can improve family relationships. This study presents, for the first time in Eupatorieae, embryological data for *Ageratum conyzoides* and *A. fastigiatum*. Both species have common features of the family such as a unitegmic anatropous ovule, basal placentation, secretory tapetum, *Polygonum* megagametophyte, and *Asterad* embryogenesis. The data obtained reinforce the heterogeneity of the family embryology and show, for the first time, the anther wall development of the monocotyledonous type for Asteraceae. The species studied show also differences between themselves. *A. conyzoides* has bisporangiate and introrse anthers, conspicuous pappus, and cypselae with trichomes on the ribs, whereas *A. fastigiatum* has tetrasporangiate and latrorse anthers, pappus absent at maturity, and glabrous cypselae. The data presented support recent phylogenetic molecular studies, suggesting the replacement of *A. fastigiatum* to another genus along with Gyptidinae.

Keywords: Compositae, embryogenesis, Eupatorieae, ontogeny, phytomelanin

Introduction

Ageratum comprises approximately 29 species (King & Robinson 1987) in the Americas and adjacent West Indies, and *A. conyzoides* is a pantropical introduced weed (Johnson 1971). The species name is derived from the Greek *a* (=not) and *geras* (=old age) due to the longevity of their flowers (Johnson 1971 therein). The genus is characterized can be recognized by a conical receptacle, leaves with large glandular punctuations, large anther appendages, and cypselae with distinct and contorted carpodia (King & Robinson 1987). *Ageratum* is the best known genus in the tribe Eupatorieae (King & Robinson 1987), and its members have been cultivated in Europe since the seventeenth century as an ornamental species, while some species are used in traditional medicine to treat a variety of diseases (Johnson 1971 therein).

The delimitation of the genus *Ageratum* is complex and it has been discussed since the seventeenth century, mainly based on pappus morphology. In the current circumscription, this genus comprises species with pappus that have a distinct five-dome or cup format and connate scales (Johnson 1971). Hattori (2013), studying the phylogeny of several subtribes inside Eupatorieae, observed *Ageratum* in two separate terminals, suggesting a new combination of *A. fastigiatum* to another genus.

The embryology of Asteraceae is heterogeneous and does not show a fixed structural pattern that separates the family from other angiosperms (Johri *et al.* 1992). Embryology in Eupatorieae is focused only on the fruit, seed (Pandey & Singh 1983; Marzinek & Oliveira 2010; Marzinek *et al.* 2010), and female gametophyte development (Holmgren 1919; Bertasso-Borges & Coleman 2005).

Embryological data are constant for genera, making may be useful in determining taxonomic relationships within families, genera, or species (Palser 1975; Stuessy 2009). Thus, this study aims to investigate the embryology of *A. conyzoides* and *A. fastigiatum*, thereby testing the phylogenetic hypothesis of Hattori (2013) with an ontogenetic approach.

Materials and methods

Flower buds, flowers, and fruits in various stages of development from *Ageratum conyzoides* (Gardner) R.M. King & H. Rob. and *Ageratum fastigiatum* L. were collected in Uberlândia, Minas Gerais, Brazil (19°10'942"S, 48°23'61"W and 19°11'026"S, 48°23'804"W). The voucher was incorporated into the *Herbarium Uberlandense* (HUFU) under accession numbers 20,142 for *A. conyzoides* and 57,943 for *A. fastigiatum*.

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Samples were fixed in FAA50 (Johansen 1940), stored in 50% alcohol (Berlyn & Miksche 1976), and embedded in methyl methacrylate-based. The material was 2–8µm thick on a rotary microtome. Sections were stained with toluidine blue at pH 4.7 with acetate buffer (O'Brien *et al.* 1964 modified), and mounted with synthetic mounting media. The slides produced were analysed and documented on Olympus BX51 light microscope.

The results were described following Marzinek & Oliveira (2010), while embryo development description was based on Johri *et al.* (1992).

Results

Microsporangium

In the early stages, the anther is formed by homogeneous meristematic cells surrounded by the protoderm (Fig. 1A). The cells of the primary parietal layer has hypodermic origin. They divide periclinally resulting in two secondary parietal layers. The outer secondary parietal layer differentiates directly into the endothecium. The cells of the inner secondary parietal layer undergo periclinal divisions forming the middle layer (externally) and tapetum (internally) (Fig. 1B).

The protoderm differentiates in the epidermis persisting at maturity and its cells are periclinally elongated. Endothecium cells are also elongated and radially thickened (Fig. 1C). The middle layer is ephemeral. The tapetum has cells with dense cytoplasm and many fused nuclei (Fig. 1D). During development, the tapetum is projected toward the anther locule involving the microspores (Fig. 1D–F). Styloid crystals are observed in all layers of the developing anther (Fig. 1F). At the stage of pollen dispersal, only the epidermis and endothecium remain. Anther dehiscence of *A. fastigiatum* is latrorse (Fig. 1G) and is introrse in *A. conyzoides* (Fig. 1H).

Ageratum conyzoides has two functional abaxial sporangia, whereas the adaxial sporangia are rarely presented (Fig. 1B) and *A. fastigiatum* has four functional sporangia (Fig. 1F).

Microsporocytes undergo simultaneous meiosis generating tetrahedral tetrads (Fig. 1D–E) and each microspore produces a tricellular pollen grain with elongated gametes (Fig. 1I) and evident exine (Fig. 1J).

Ovary

The ovary is inferior bicarpelar, syncarpous, and unilocular (Fig. 2A–B). The outer epidermis is uniseriate with trichomes on the ribs only in *A. conyzoides* (Fig. 2C–D).

The mesophyll has two regions (Fig. 2E–F). The outer two layers of mesophyll have bulky (Fig. 2E), slightly longitudinally elongated cells (Fig. 2F). The internal mesophyll has four to six layers of varying diameters (Fig. 2E) and longitudinally elongated cells (Fig. 2F). Five procambial bundles accompany the ribs (Fig. 2B–D). The inner epider-

mis is uniseriate (Fig. 2E–F). At the base of the ovary, there is a protuberance with parenchyma cells that have various shapes constituting the carpogonium. In both species, the carpogonium is asymmetric (Fig. 2A, G, H).

Ovule

The ovule is anatropous, unitegmic, and tenuinucellate with basal placentation (Fig. 2A). The outer integument epidermis is uniseriate with cuboidal, juxtaposed cells and an evident nucleus. The mesophyll comprises approximately nine layers of cells (Fig. 2I–J). A procambial bundle crosses through the raphe and extends to the middle portion of the anti-raphé. The inner epidermis has one to two layers of cells with dense cytoplasm, large nuclei, and distinct nucleoli constituting the endothelium (Fig. 2J).

The nucellus has only one archesporial cell differentiating directly into the megaspore mother cell, just below the epidermis (Fig. 2K). The megaspore mother cell undergoes meiosis, giving rise to a linear tetrad of megaspores (Fig. 2L), and only the chalazal megaspore remains functional (Fig. 2M). The embryo sac is monosporic and 7-celled or a *Polygonum* type megagametophyte. The nucellus remnants occur around the embryo sac (Fig. 2N).

Fertilization, embryo, and endosperm

Fertilization is micropylar with pollen tube entering into the one synergid (Fig. 3A). The egg cell and one of the gametes join to form the zygote (Fig. 3B). The other gamete merges with the fused nuclei of the middle cell forming the first cell of the endosperm (Fig. 3A). The first cell of the endosperm divides forming walls between the nuclei (Fig. 3B–D). The zygote undergoes a transverse division generating basal and apical cells (Fig. 3C). Basal cell undergo transverse division and each gives rise to two daughter cells (*ci* and *m*) (Fig. 3D). The *ci* cell is transversely divided into *n* and *n'* and divides again giving rise to *n*, an *o*, and *p* cells, resulting in the suspensor (Fig. 3E–H). The *m* cell undergoes two longitudinal divisions giving rise to the quadrant *m* that will in turn give rise to the hypocotyl and radicle (Fig. 3D–G). The apical cell undergoes three longitudinal divisions forming the octant *q*, responsible for formation of cotyledons, epicotyl, and plumule (Fig. 3D–G). After successive divisions the embryo has a globular shape (Fig. 3H). The mature embryo is axial and occupies the whole seminal chamber (Fig. 3I). The embryo axis is straight, and the plumule is poorly differentiated.

Fruit and seed

During fruit development, the outer epidermis develops the exocarp, the mesophyll develops in mesocarp, and endocarp originates from the inner epidermis of the ovary.

The exocarp remains unchanged. The outer mesocarp exhibits increased cells with large vacuoles. Between the

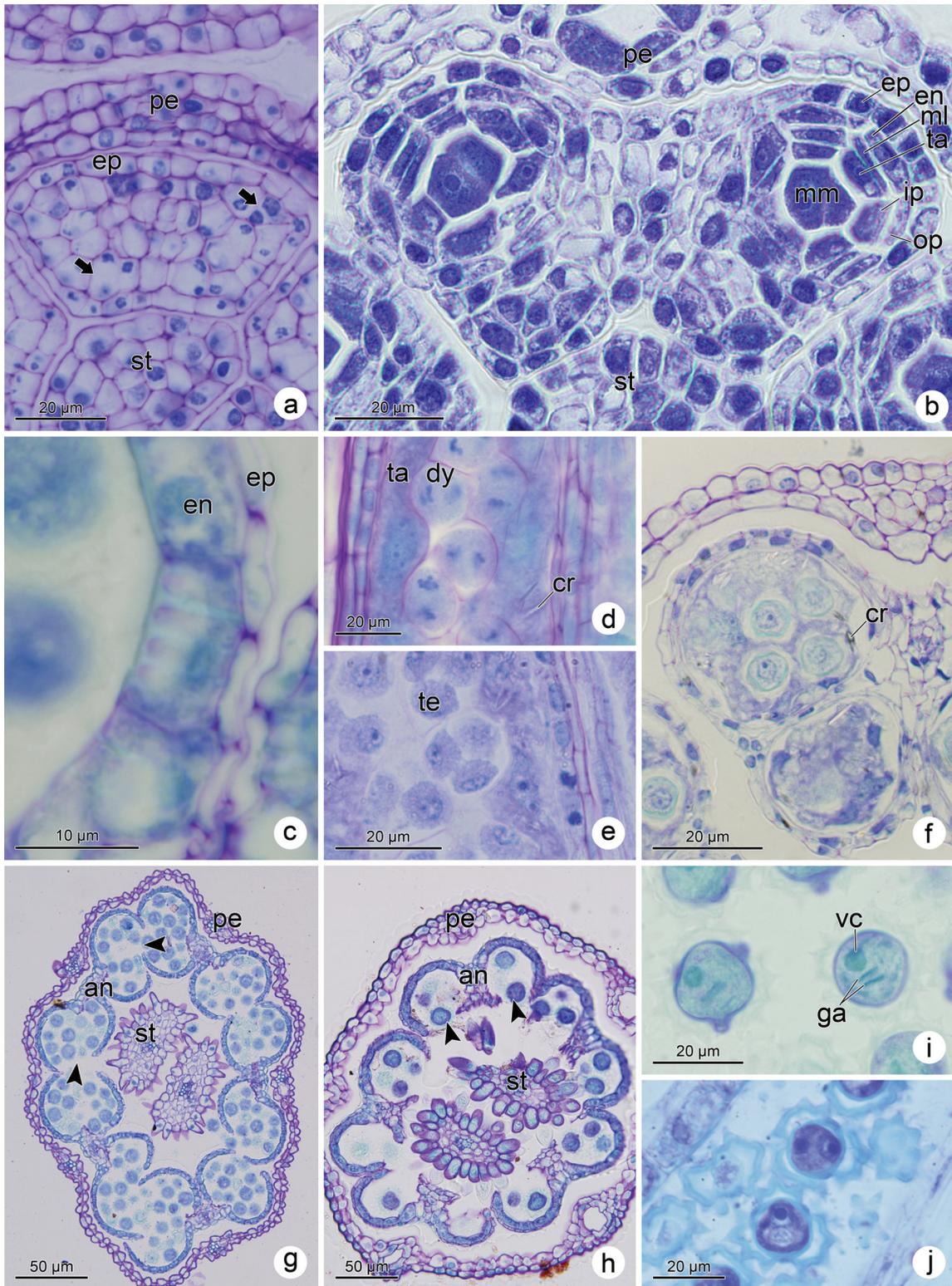


Figure 1. Transverse (A–C, F–H) and longitudinal sections (D–E, I, J) of the floral buds and flowers at anthesis of *Ageratum*. **A** Young anther of *A. fastigiatum*, showing the divisions of the primary parietal layer. **B** Anther of *A. conyzoides*, note two abaxial sporangia and two remnants of adaxial sporangia. **C** Detail of the anther wall of *A. fastigiatum* with persistent epidermis and radial thickening of the endothecium. **D–E** Simultaneous cytokinesis and tetrahedral tetrad of the microspores of *A. fastigiatum*. **F** Anther of the *A. fastigiatum* under polarized light showing styloid crystals. **G** General view of the anther of *A. fastigiatum* showing lateral anther dehiscence. **H** General aspect of the anther of *A. conyzoides* showing introrse dehiscence. **I–J** Detail of the pollen grains of *A. fastigiatum* with the vegetative cell and two elongated gametes. *an* anther, *arrow*: periclinal division of the primary parietal layer, *arrowhead* stomium, *mm* microspore mother cell, *cr* styloid crystal, *dy* dyad, *en* endothecium, *ep* epidermis, *ga* gamete, *ip* inner secondary parietal layer, *ml* middle layer, *op* outer secondary parietal layer, *pe* petal, *st* stigma, *ta* tapetum, *te* tetrad of microspores *vc* vegetative cell

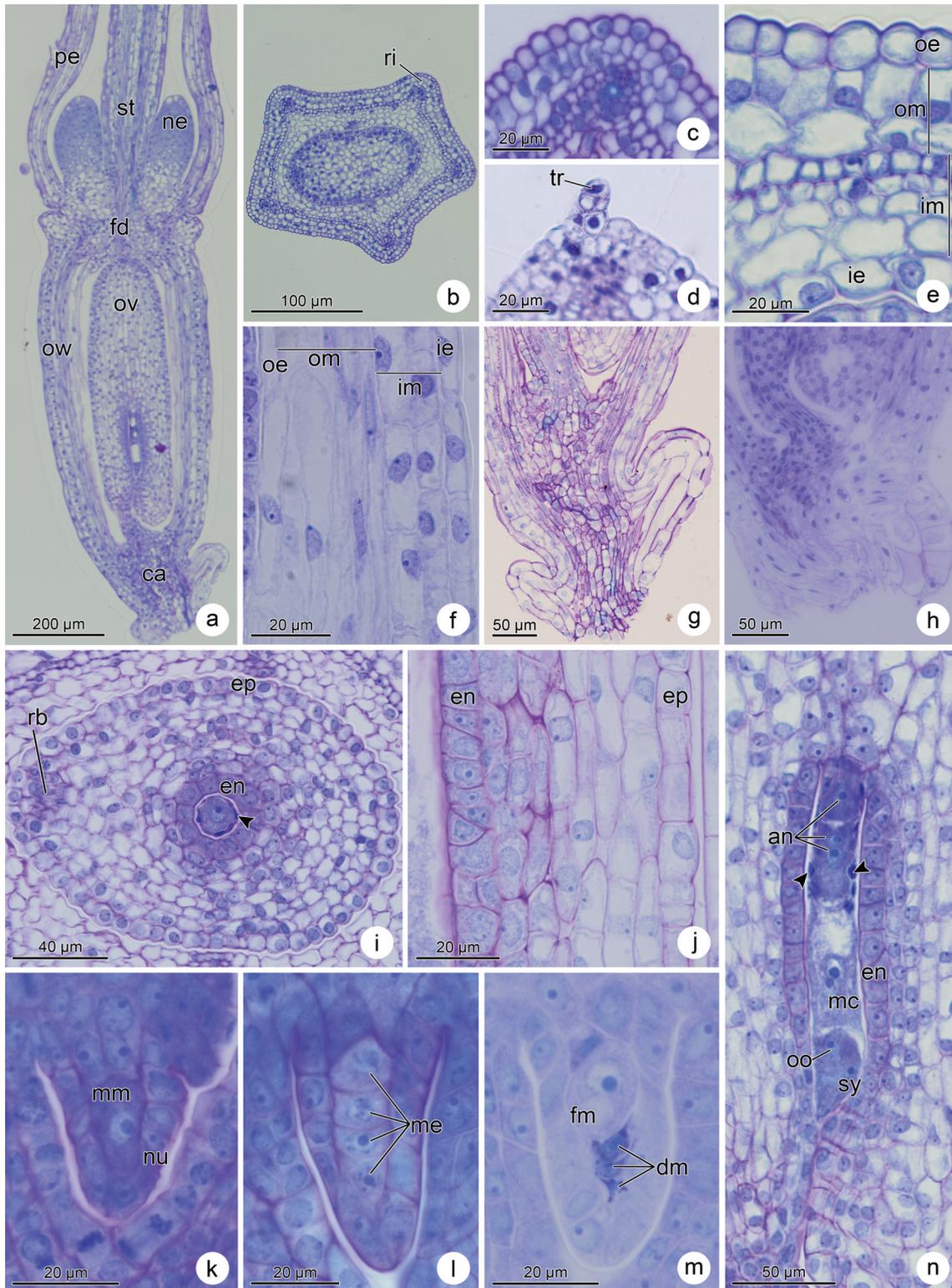


Figure 2. Transversal (B–E, I) and longitudinal sections (A, F–H, J–N) of the floral buds and flowers at anthesis of *Ageratum*. **A–B** Overview of the flower bud of *A. fastigiatum*. **C** Glabrous ribs of *A. fastigiatum*. **D** Ribs with trichomes of *A. conyzoides*. **E–F** Ovary of *A. fastigiatum*. **G** Detail of the asymmetric carpogonium of *A. fastigiatum*. **H** Detail of the asymmetric carpogonium of *A. conyzoides*. **I–J** Ovule of *A. fastigiatum* with differentiated endothelium. **K** Ovule, note early development of the megaspore mother cell below the nucellar epidermis of *A. fastigiatum*. **L** Ovule showing the linear tetrad of megaspores of *A. fastigiatum*. **M** Detail of the ovule of *A. fastigiatum*, note chalazal functional megaspore and three microspores degenerating. **N** Megagametophyte of *A. fastigiatum*. *an* antipodes, *ca* carpogonium, *dm* degenerated megaspore, *en* endothelium, *ep* epidermis, *fd* floral disk, *fm* functional megaspore, *ie* inner epidermis, *im* inner mesophyll, *mc* middle cell, *me* megaspore, *mm* megaspore mother cell, *ne* nectary, *nu* nucellus, *oe* outer epidermis, *om* outer mesophyll, *oo* egg cell, *ov* ovule, *ow* ovary wall, *pe* petal, *rb* raphe bundle, *ri* rib, *st* style, *sy* synergids, *tr* trichome

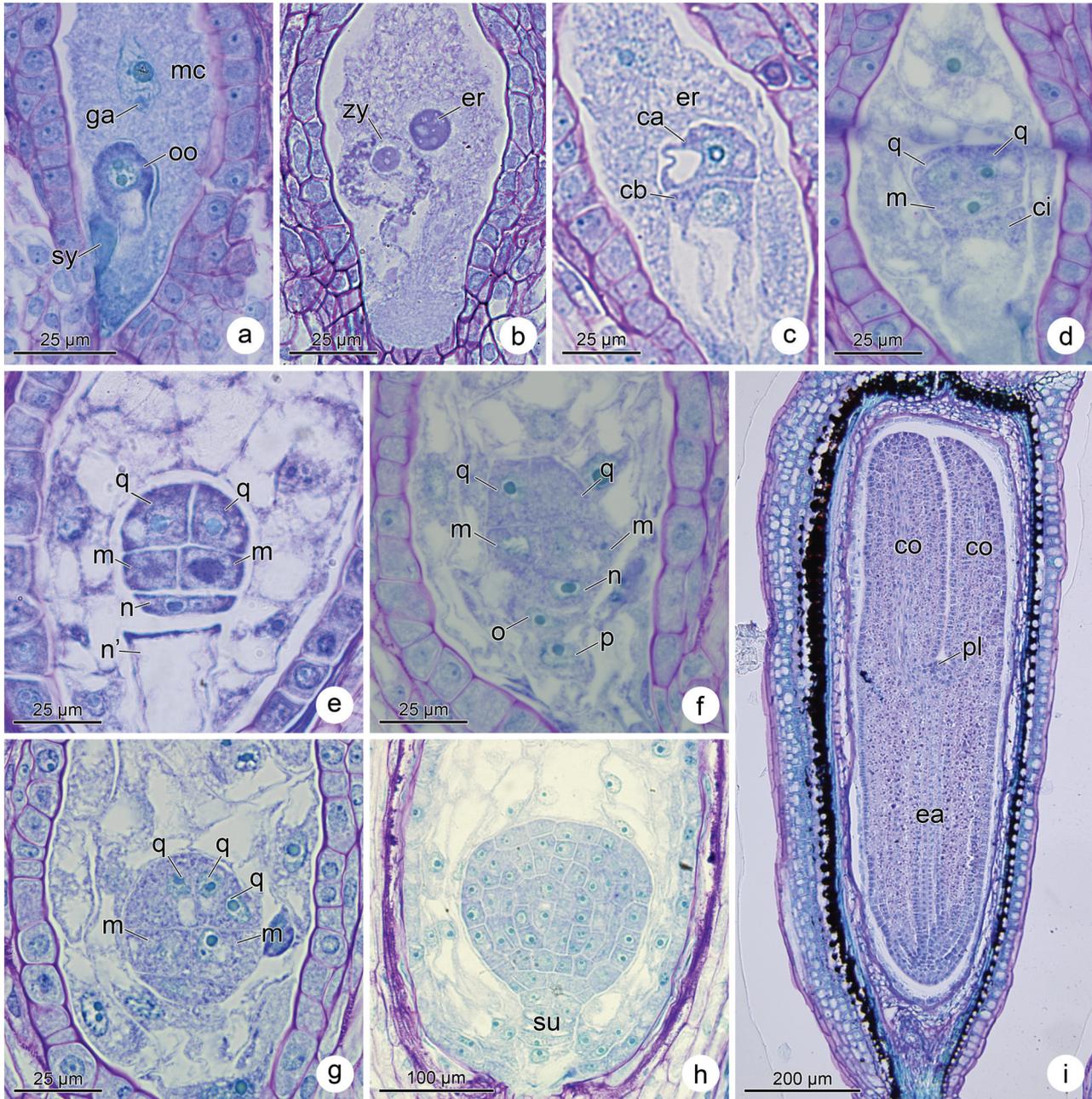


Figure 3. Longitudinal sections of the seeds of *Ageratum fastigiatum* (A–D, F–I) and *A. conyzoides* (E). A–H Immature seeds. A Fertilization of egg cell and middle cell, noted synergid increased density of the cytoplasm and polar nuclei of middle cell fused (arrow). B Early zygote and first cell of the endosperm. C Proembryo resulting from the transversal division of the zygote resulting in an apical cell (*ca*) and basal cell (*cb*). D Embryo with four cells, noted *q* cells originated from a longitudinal division of the apical cell (*ca*), *m* and *ci* resulting from a transverse division of the basal cell (*cb*). E–F Embryo with 8 cells showing two cells *q*, two *m* cells, *n* and *n'* derived from a transverse division of *ci*. G Embryo with 16 cells, which 8 *q* cells, 4 *m*, 2 *n*, *o* and *p* result of transversal division of *n'* cell. H Globular embryo with suspensor. I Mature seed showing embryo with embryo axis straight and two convex planes cotyledons. *co* cotyledon, *ea* embryo axis, *er* endosperm, *ga* gamete, *mc* middle cell, *oo* egg cell, *pl* plumule, *su* suspensor, *sy* synergid, *zy* zygote

outer and inner mesocarp, there is a partial separation of the cells forming a space schizogenous with projections of inner mesocarp connecting the two layers. The schizogenous space is filled by the secretion of phytomelanin. The inner mesocarp is formed of only one fibre layer, while the other layers were crushed by the seed growth (Fig. 4A–E).

At maturity, both the carpodium (Fig. 4F–G) and the floral disk (Fig. 4H–I) show lignification. The pappus is lacking in *A. fastigiatum* and persistent in *A. conyzoides* (Fig. 4H–I). The exotesta shows no modifications. The internal mesotesta and endotesta are crushed (Fig. 4C–E). The endosperm cells are persistent, with one to three cell layers surrounding the embryo (Fig. 4E).

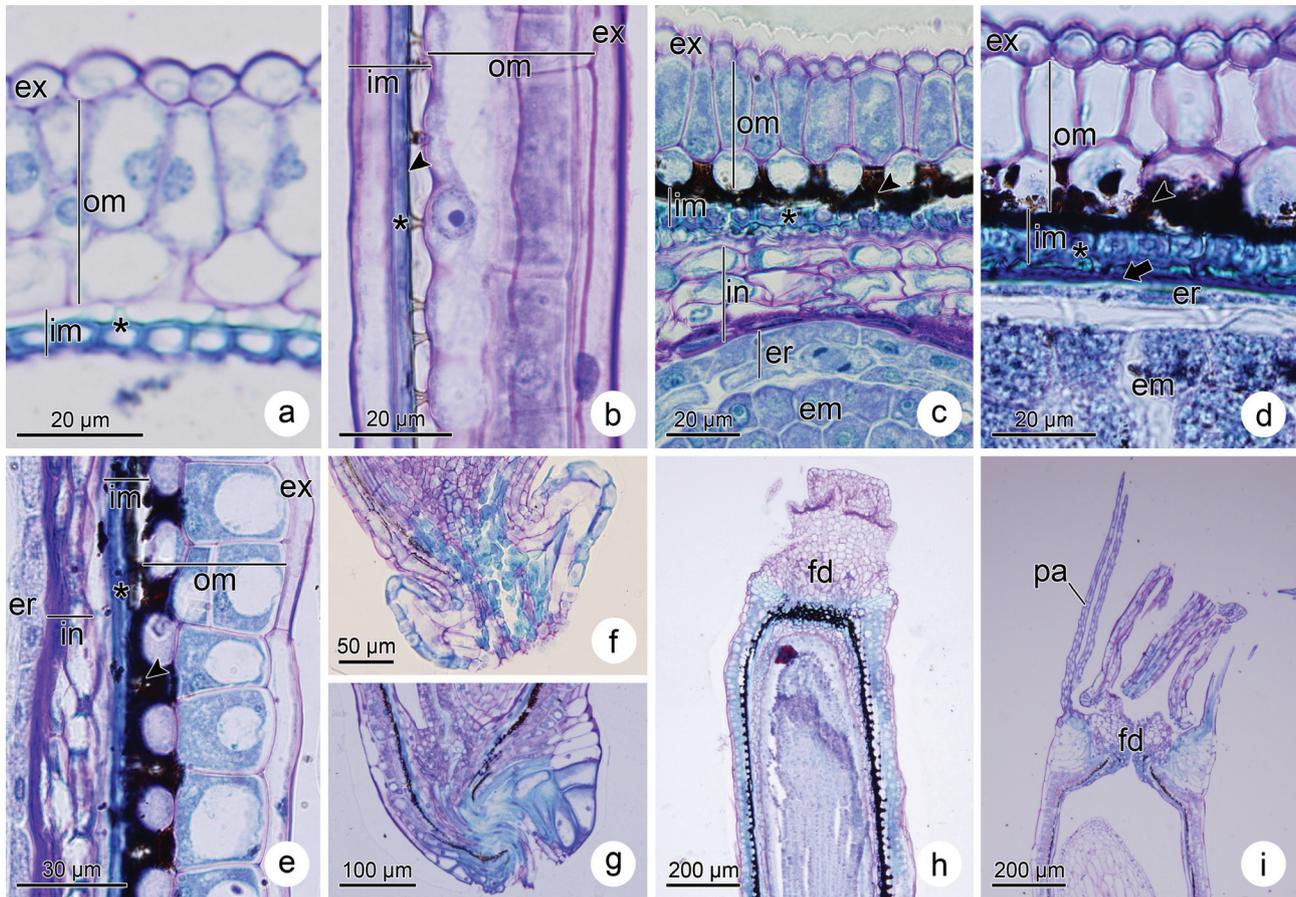


Figure 4. Transversal (A, C–D) and longitudinal sections (B, E–I) of the floral buds, flowers, and fruits of *Ageratum*. A–B Immature fruit of *Ageratum* showing the early formation of schizogenous space. A *A. fastigiatum*. B *A. conyzoides*. C–E Immature fruit of *Ageratum*, note schizogenous space filled by phytomelanin. C *A. conyzoides*. D–E *A. fastigiatum*. F–G Carpopodium of *Ageratum*. F *A. fastigiatum*. G *A. conyzoides*. H–I Floral disk of *Ageratum*. H *A. fastigiatum*, note persistent pappus. I *A. conyzoides*. arrow crashed layer (part of the inner mesocarpm, endocarpm, and integument), arrowhead phytomelanin, asterisk fibre layer, em embryo, er endosperm, ex exocarpm, fd floral disk, im inner mesocarpm, in integument, om outer mesocarpm, pa pappus

Discussion

In *A. fastigiatum* and *A. conyzoides*, the outer secondary parietal layer originates directly into the endothecium and the inner develops a middle layer and tapetum. This pattern of anther wall development is the monocotyledonous type and is the first record for Asteraceae. In the family, the pattern described until now has been dicotyledonous (Davis 1966; Johri *et al.* 1992; Gotelli *et al.* 2008; Liu *et al.* 2011).

Both species of *Ageratum* present an endothecium with radial thickening. In Eupatorieae, the same type of thickening was observed as in *Eupatorium cannabinum* (Dormer 1962). The presence of crystals in the tapetum, as found in the studied species, is poorly explored in embryological studies of the Asteraceae family and has been previously reported only in *Helianthus* (Meric & Dane 2004). The presence of crystals does not necessarily represent patterns in different taxonomic levels (Buss Jr & Lersten 1972; Prychid & Rudall 1999; De-Paula & Sajo 2011).

According to Davis (1966), tetrasporangiate anthers are common in the family. *A. fastigiatum* presented tetraspo-

rangiate anthers, and *A. conyzoides* presented bisporangiate anthers. The position of the anther opening also differs between the two species. In *A. fastigiatum*, the anther is latrorse but is introrse in *A. conyzoides*.

The tapetum cells of both *Ageratum* species protrude toward the anther locule and possess fused nuclei. Multinucleated amoeboidal tapetum is the most common type in Compositae (Johri *et al.* 1992); it has also been observed in Carduoideae (Yeung *et al.* 2011), Senecioneae (Pullaiah 1983; Lakshmi & Pullaiah 1987), Gnaphalieae (Davis 1962a; Pullaiah 1979), Astereae (Davis 1968), Anthemideae (Davis 1962b; Li *et al.* 2010), Inuleae (Pullaiah 1979), and Heliantheae (Gotelli *et al.* 2008). Secretory tapetum occurs in Pertyeae (Kapil & Sethi 1962), Cichorieae (Sood *et al.* 2000; Yurukova-Grancharova *et al.* 2006), and Vernoniae (Tiagi & Tamni 1963).

Simultaneous cytokinesis was recorded for both species of *Ageratum* and according to Davis (1966), is considered the main type of cytokinesis in the family.

As previously described for the family, both species have an anatropous and unitegmatic ovule with basal placentation (Davis 1966; Johri *et al.* 1992).

Ageratum conyzoides and *A. fastigiatum* present the monosporic *Polygonum* megagametophyte type, which has been observed in *Eupatorium* (Holmgren 1919; Bertasso-Borges & Coleman 2005) and for most Asteraceae species (Davis 1962a; Johri *et al.* 1992). The bisporic *Allium* type was found in Gnaphalieae, Anthemideae, Astereae, and Heliantheae: the *Adoxa* type in Heliantheae, the *Drusa* type in Gnaphalieae, Anthemideae, and Astereae, and the *Fritillaria* type in Heliantheae (Davis 1966).

The cellular endosperm was observed in the species studied and is consistent with findings for the tribe (Pandey & Singh 1983; Marzinek & Oliveira 2010), it has also been found in Senecioneae (Pullaiah 1983; Lakshmi & Pullaiah 1987), Gnaphalieae (Davis 1961; 1962a), Anthemideae (Davis 1962b), Inuleae (Pullaiah 1979), and Tageteae (Misra 1964). Nuclear endosperm was found in Mutisieae (Devi 1957), Cichorieae (Yurukova-Grancharova *et al.* 2006), and Vernoniaceae (Sharma & Murty 1978). The Astereae tribe presents both types of endosperm development: nuclear in *Erigeron bonariensis* and cellular in *Felicia bergeriana* (Sharma & Murty 1978) and *Brachycome ciliaries* (Davis 1964).

The embryogenesis of both species is the *Asterad* type, in which basal and apical cells participate in the formation of the embryo as previously described for Compositae (Davis 1966; Johri *et al.* 1992).

The pericarp of *A. conyzoides* and *A. fastigiatum* follows the pattern of the development observed in Eupatorieae, with the outer mesocarp presenting cells and large vacuoles and a phytomelanin layer and fibres (Marzinek & Oliveira 2010; De-Paula *et al.* 2013). Phytomelanin was observed in some tribes of Asteraceae such as Cardioideae (Pandey & Singh 1982), Vernoniaceae (Basak & Mukherjee 2003; Loeuille *et al.* 2013), Gnaphalieae (Davis 1962a), Anthemideae (Aguado *et al.* 2011), Heliantheae (Maheshwari & Srinivasan 1944), and Eupatorieae (Pandey & Singh 1983; Marzinek & Oliveira 2010). According to De-Paula *et al.* (2013), the fibres of the pericarp are directly responsible for secreting phytomelanin in *Praxelis diffusa*; this fact will probably also be true in *Ageratum*, where phytomelanin is observed primarily near the fibres.

In both species studied, vascular bundles correspond to the salient region of fruit called ribs in Eupatorieae, and both species are able to have bundles without ribs. Reinforcing the taxonomic importance for this feature, the ribs of *A. conyzoides* possess trichomes, and *A. fastigiatum* has glabrous ribs. Another important feature is the pappus, present in *A. conyzoides* and lacking at maturity in *A. fastigiatum*. The presence or absence of the pappus shows an important feature for a new circumscription of the genus.

Carpopodium promotes fruit abscission at the dispersion. Haque & Godward (1984) associated the asymmetry of carpopodium with presence of the pappus, but this was not observed in this study in which *A. fastigiatum* features a symmetrical carpopodium and the pappus is absent; *A. conyzoides* has a weakly asymmetric carpopodium and persistent pappus.

The results of this study confirm the heterogeneity of embryological processes in the Asteraceae family. Common features to the family, but not exclusive, have been observed as the unitegmic anatropous ovule, basal placentation, secretory tapetum, *Polygonum* megagametophyte type, and embryogenesis of the *Asterad* type. Other features expressed the homogeneity of embryological data within the tribes, such as the radial thickening of the endothecium and development of the pericarp typical for Eupatorieae. The monocotyledonous anther development type reported for the *Ageratum* species studied here, and not yet described for Asteraceae, is emerging as an important feature for the tribe. Because this was the first study to describe this type of development for the family, more studies are needed to assess the true potential of this feature for Eupatorieae. Both species showed distinguishing features: *A. fastigiatum* with a tetrasporangiate and latrorse anther and *A. conyzoides* with a bisporangiate and introrse anther. In addition to the anther, the presence of the pappus and trichomes in the ribs can separate *A. conyzoides* from *A. fastigiatum*, corroborating the phylogenetic hypothesis of Hattori (2013) that suggests that *Ageratum* should be segregated into two distinct clades: one with *A. conyzoides*, which is widely distributed in the Americas, and one with *A. fastigiatum*, which, along with Gyptidinae, is exclusively distributed in Brazil.

Acknowledgements

The authors thank Jimi N. Nakajima and Eric K. O. Hattori for identifying the plants, FAPEMIG (process no. APQ 02127-09) for financial support, and two anonymous reviewers for their constructive comments.

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