Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.71492
BOTANY

Fruit and seed ontogeny and its taxonomic relevance for Vernonieae (Asteraceae)

Caroline Heinig Voltolini[1], Fernanda Maria Cordeiro Oliveira[2] and Luiz Antonio de Souza[1]*

[1]Programa de Pós-graduação em Biologia Comparada, Departamento de Biologia, Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. [2]Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brasil. *Author for correspondence. E-mail: lasouza@uem.br

ABSTRACT. In the Asteraceae the pappus and cypsela provide many important structural features that are of potential taxonomic significance. *Vernonia* is a revised genus that has recently undergone a rearrangement consisting of the genera *Vernonia* s.s., *Chrysolaena*, *Lepidaploa*, *Lessingianthus* and *Vernonanthura*. In the present investigation, the cypsela ontogeny of species of these genera and of the monospecific *Orthopappus* was analyzed. Our study is an attempt to show that the anatomical features of the fruit (pericarp and seed) have taxonomic value within the tribe. Flowers and fruits in development were analyzed using a light microscope and scanning electron microscope. The persistent fruit pappus is bristly and biseriate. The mature pericarp is usually composed of more or less collapsed exocarp and sclerenchymatous mesocarp. At this stage, the mature seed coat is unspecialized; the unlayered endosperm and collapsed endothelium persist. Some features regarding the pappus, cypselae, and seeds have provided support to separate the segregated species of Vernonieae, especially when they are compared to the *Orthopappus angustifolius*.

Keywords: cypsela; *Orthopappus;* pappus; seed; *Vernonia*.

Received on November 02, 2023
Accepted on June 05, 2024

Introduction

Vernonieae has approximately 1500 species distributed in the New and Old World, with the main diversity centers in Africa and South America (Mandel et al., 2019; Siniscalchi, Loeuille, Funk, Mandel, & Pirani, 2019; Keeley, Cantley, & Gallaher, 2021). Vernonieae shows uncertain delimitation between species and subtribes, and has more than 1000 similar-appearing species distributed across the globe (Keeley et al., 2021). According to these authors there are overlapping characters in both vegetative and reproductive morphologies that obscure species boundaries.

A historical solution by Vernonieae taxonomists was to include the taxa in a single genus *Vernonia* Schreb. This genus was revised by Robinson (1999), who presented an arrangement of the genus into *Vernonia* — which is a genus practically restricted to North America —, and other derived genera, such as *Chrysolaena* H. Rob., *Lepidaploa* (Cass.) Cass, *Lessingianthus* H. Rob (belonging to the subtribe Lepidaploinae), and *Vernonanthura* H. Rob (Vernoniinae) (Keeley & Robinson, 2009). Species from these genera were selected for study in the present work. Additionally, the monospecific genus *Orthopappus* Gleason was analyzed (subtribe Elephantopinae) (Keeley & Robinson, 2009).

Our conception of cypsela, that was adopted here for the fruits of Vernonieae, was based on the terminology used by Marzinek, De-Paula, and Oliveira (2008) who define it as a complex fruit, dry, indehiscent, unilocular, with a single seed, and originating from an inferior ovary. Even though it is not the most appropriate term, the achene has been widely used by many authors, such as Roth (1977), Spjut (1994) and Judd, Campbell, Kellogg, Stevens, and Donoghue (2009). Marzinek's conception of fruit for Asteraceae does not admit the term achene because this fruit originates from a superior ovary and it is a typical fruit of Plumbaginaceae.

Structural features of the pappus and cypsela play an important role as a useful character in Asteraceae taxonomy (Robinson, 2009), and they have been used in the characterization of tribes and species (Judd et al., 2009; Marzinek & Oliveira, 2010; Pallone & Souza, 2014; Batista & Souza, 2017a, 2017b). As pointed out by Robinson (2009) some cypsela features have revealed an interesting taxonomical value, as the presence of phytomelanin, the trichome diversity, and the distribution of idioblasts and raphides.

The fruit structure is used to a limited extent for the identification of various genera and species of Vernonieae (Freitas, De-Paula, Nakajima, & Marzinek, 2015). There are studies on Vernonieae cypselae, such as the discovery of new pattern of phytomelanin deposition in *Heterocoma*, and research on the structure and ontogeny of cypselae of *Elephantopus mollis* (Batista & Souza, 2017a) and *Vernonia stricto sensu* (Misra, 1972; Pandey & Singh, 1980; Mukherjee, 2001; Basak & Mukherjee, 2003). Shamso, Hosni, Ahmed, & Shaltout, (2021) recorded morphological characters on the cypsela (achene) of the species *Ethulia conyzoides* L. fil. subsp. *conyzoides*. In addition, there are studies of the fruits of *Chrysolaena* and *Lessinghianthus* (Angulo, Sosa, & Dematteis, 2015; Via-do-Pico, Veja, & Dematteis, 2016; Marques, Oliveira-Franca, Angulo, Via-do-Pico, Dematteis, & Marzinek, 2020), and *Lepidaploa* (Marques, Oliveira-Franca, Dematteis, & Marzinek, 2018; Marques et al., 2020), but an only few taxa were ontogenetically investigated.

A little attention has been given to the Asteraceae seed, especially the seed-coat, but some authors believe that the seed features may be useful in the separation of Asteraceae species (Corner, 1976; Pallone & Souza, 2014; Batista, Santos, Muller, & Souza, 2015). The exotestal with thick-walled cells seems to be relevant in the separation of some species of Asteraceae (Batista, Santos, Muller, & Souza, 2015).

We investigated the anatomical features of potential taxonomic and phylogenetic significance within the tribe. Our studies include the pappus structure and the cypsela/seed ontogeny of eight species belonging to some of the derived genera of *Vernonia s.l.* (Vernonieae) and an *Orthopappus* species of the same tribe (Table 1). In addition, the relative percentage of cypselae with embryos and those lacking embryos (abortive cypselae) found in the capitula. This data may enhance ecophysiological studies that include reproductive success of Asteraceae in several environments.

Table 1. Species studied, accession numbers and flower/fruit phases of the investigated plants of Vernonieae (Asteraceae). FB: Floral bud; PEF: Pre-anthesis flower; AF: Anthesis flower POF: Post-anthesis flower; IM: Immature fruit; MF: Mature fruit.

Species/Herbarium record numbers	City/State	Date	Flower/Fruit phases					
			FB	PEF	AN	POF	IM	MF
Chrysolaena flexuosa (Simns) H. Rob.	Ponta Grossa/ PR	Dec/2014	X	X	X			
HUEM 24524, HUEM 29440	Florianópolis/ SC	Jan/2015	X	X	X	X	X	X
Chrysolaena nicolackii H. Rob.	Tibagi/PR	Dec/2014	X	X	X	X	X	X
HUEM 30132, HUEM 24495, HUEM 24506	Tibagi/PR	Feb/2015	X	X	X	X	X	X
	Tibagi/PR	Jan/2017	X	X	X	X	X	X
	Tibagi/PR	Mar/2017			X	X	X	X
Lepidaploa chamissonis (Less.) H. Rob	Florianópolis/SC	Jan/2015	X	X	X	X	X	X
HUEM 24494, HUEM 29439	Tibagi/PR	Feb/2015	X	X	X	X	X	X
	Tibagi/PR	Mar/2017	X	X	X	X	X	
Lepidaploa psilostachya (DC.) H. Rob.	Ponta Grossa/ PR	Dec/2014	X	X	X			
HUEM 24504, HUEM 24569	Tibagi/PR	Dec/2014	X	X	X	X	X	
	Tibagi/PR	Nov/2016	X	X	X			
	Tibagi/PR	Jan/2017						X
Lessingianthus polyphyllus (Sch. Bip. ex-Baker) *H. Rob.*	Tibagi/PR	Feb/2015	X	X	X			
HUEM 24491, HUEM 24501, HUEM 24497	Ponta Grossa/PR	Mar/2015	X	X	X			
	Ponta Grossa/PR	May/2016						X
	Tibagi/PR	Mar/2017				X	X	X
Lessingianthus sanctipauli (Hieron.) Dematt.	Tibagi/PR	Feb/2015	X	X	X			
HUEM 30137, HUEM 2449	Tibagi/PR	Mar/2017			X	X	X	X
Orthopappus angustifolius (Sw.) Gleason	Navegantes/SC	Dec/2015	X	X	X	X	X	X
HUEM 30006, HUEM 30015	Ponta Grossa/PR	Jan/2016	X	X	X	X	X	X
	Ponta Grossa/PR	May/2016						X
Vernonanthura nudiflora (Less.) H. Rob	Ponta Grossa/PR	Mar/2015	X	X	X	X	X	
HUEM 24489, HUEM 30018	Ponta Grossa/PR	May/2016	X	X	X	X	X	X
	Ponta Grossa/PR	Jan/2016	X	X	X			
	Ponta Grossa/PR	Mar/2017			X	X	X	X
Vernonanthura oligolepis (Sch. Bip. ex-Baker) H. Rob.	Tibagi/PR	Feb/2015	X	X	X	X	X	X
HUEM 24493, HUEM 24499, HUEM 30019	Ponta Grossa/PR	Mar/2015	X	X	X	X	X	X
	Ponta Grossa/PR	Jan/2016	X	X	X			
	Tibagi/PR	Mar/2017						X

Material and methods

Floral buds, flowers and developing cypselae were collected from State Parks of Vila Velha (Ponta Grossa, Paraná state, Brazil) and Guartelá (Tibagi, Paraná state, Brazil) (Table 1).

The plant material was fixed in FAA (formalin-acetic acid- alcohol) for 72 hours and preserved in 70% ethyl alcohol, following the protocol of Johansen (1940). Fixed and dehydrated samples were embedded in historesin (hidroxietilmetacrilato, Jung's Historesin, Leica), sectioned (cross and longitudinal sections) with a rotary microtome, and stained with 0.25% Toluidine Blue (Ruzin, 1999). Light microscope photographs were taken with a Leica EZ4D digital camera and subsequently processed using the software Leica Application Suite version 1.8. Histochemical tests were conducted for lignin (phloroglucinol-hydrochloric acid) and lipids (Sudan IV) (Johansen, 1940). The cypsela fragments were dissociated in an acid solution (chromic acid x nitric acid, 1:1) (Johansen, 1940).

Micromorphological analyses (Bozzola & Russel, 1992) were performed with FAA 50 fixed cypselae. After washing in 0.1 M sodium cacodylate buffer, the samples were dehydrated in a graded acetone series, critical-point dried with CO_2, mounted on aluminum stubs, gold coated and, subsequently, examined using scanning electron microscopy (SEM) (Shimadzu SS-550 Super scan).

Embryo formation of each species was studied in 100 sectioned cypselae, which were analyzed under a stereoscopic microscope, following the procedure conducted by Galastri & Oliveira (2010).

Results

Pappus structure-The persistent fruit pappus is bristly and biseriate or double (Figure 1A, B). The outer series consists of bristles of larger length than the inner series (Figure 1A). The inner bristles are made up of a laminar base and the remaining has a cylindrical shape; the outer bristles are laminar (Figure 1A). By comparing with other species, both series have equal size in *Orthopappus angustifolius* (Figure 1B).

The pappus consists of a uniseriate epidermis with elongated and narrow cells, which have free and tapered ends (Figure 1C–F), and the multistratified mesophyll (Figure 1E, F). In floral buds, the pappus mesophyll is parenchymatous, and in the developing fruit the cells undergo wall thickening (Figure 1E, F). The pappus is devoid of the vascular system, but a more detailed analysis of the bristles revealed the presence of rudimentary vascular cells in some segments.

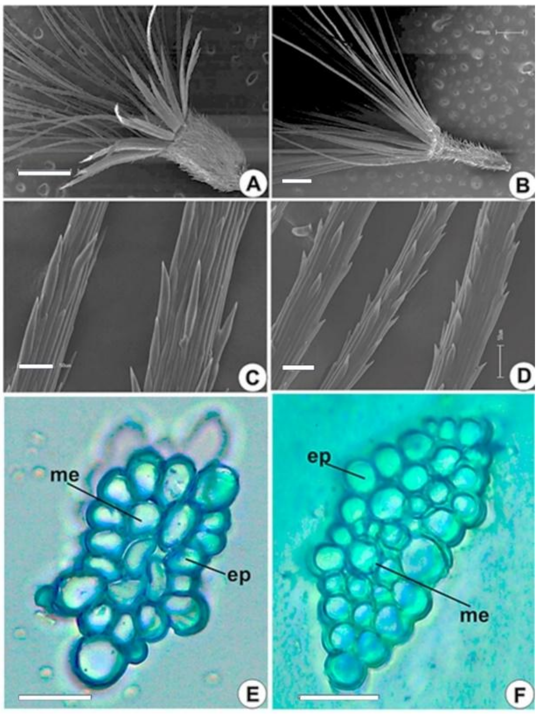

Figure 1. Pappus structure of *Chrysolaena flexuosa* (A), *Orthopappus angustifolius* (B, D, F), *Chrysolaena nicolackii* (C) and *Vernonanthura nudiflora* (E). A, B. SEM micrographs of cypselas showing biseriate pappus (outer and inner pappus). C, D. SEM micrographs of bristles showing epidermal cells with tapered ends. E, F. Bristles in cross-section with epidermis (ep) and mesophyll (me). Scale bars: 1mm (A), 500 µm (B), 20 µm (C, D), 30 µm (E, F).

In the apical plate (Figure 2A) of the flower, the region that supports the pappus, cells are conspicuously lignified and suberized; idioblasts with prismatic crystals may also be identified in this plate in all species, except *Lepidaploa psilostachya* and *Orthopappus angustifolius*. As seen at anthesis stage, the pappus cells are sclerified, and during fruit development these cells undergo lignification.

Fruit development—Cypselae develop from bicarpellary, syncarpous, unilocular inferior ovary (Figure 2A). All species have the outer ovary epidermis with non-glandular twin hairs (Figure 2B), and the glandular twin hairs with unicellular secretory apex also occur in the epidermis (Figure 2C-E), but not in *Lepidaploa psilostachya*. However, the secretory apex differs by its large size in derived species of *Vernonia* and reduced dimension in the glandular trichome of *Orthopappus angustifolius*. The outer epidermal cells may have two sizes in *Orthopappus angustifolius* (Figure 2E); the shorter cells occur mostly in the regions of vascular bundles.

The ovary mesophyll is parenchymatous 6–9 cell-layers thick consisting of two tissue regions, the outer parenchyma, devoid of intercellular spaces, and the inner spongy parenchyma (Figure 2D, E). Idioblasts with prismatic crystals are present in the mesophyll, but *Vernonanthura nudiflora* also exhibits cubic crystals. It has been noted that the mesophyll of *Orthopappus angustifolius* is devoid of crystals. The inner ovary epidermis is uniseriate (Figure 2D, E) and it collapses at anthesis. The ovary vasculature is made up of various collateral bundles.

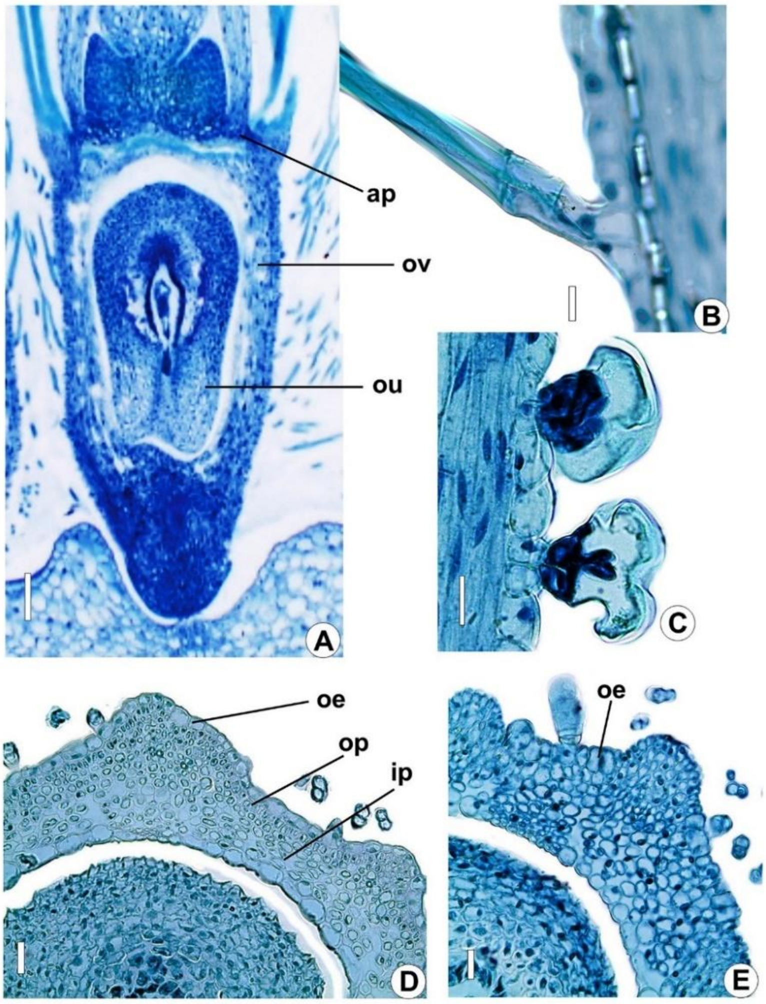

Figure 2. Ovary structure of *Lepidaploa psilostachya* (A), *Chrysolaena nicolackii* (B), *Vernonanthura oligolepis* (C), *Lepidaploa chamissonis* (D) and *Orthopappus angustifolius* (E). A. Inferior ovary in longitudinal section. B, C. Outer epidermis showing non-glandular twin hair and glandular twin hair, respectively. D, E. Ovaries in cross-sections. (ap= apical plate; ip=inner parenchymatous mesophyll; oe=outer epidermis; op=outer parenchymatous mesophyll; ou=ovule; ov=ovary). Scale bars: 50 mm (A), 20 mm (B-E).

During the ripening process of the cypsela, the epidermal exocarp remains uniseriate and pubescent. At maturity, some exocarpic cells collapse, whereas the non- glandular twin hairs (Figure 3A, D) and glandular twin hairs (Figure 3C) are very similar in structure to the ovary, but with lignified apical cells in the non-glandular twin hairs. Idioblasts (Figure 3B) containing lipids are present in the mature exocarp. Sclerification affects the outer parenchyma of the mesophyll (Figure 4A), except for the developing fruit of *Lepidaploa* *psilostachya* (Figure 4B), which is formed with 1–3 layers of fibers. The inner parenchyma and the inner epidermis of the ovary collapse during the cypsela development (Figure 4A, B).

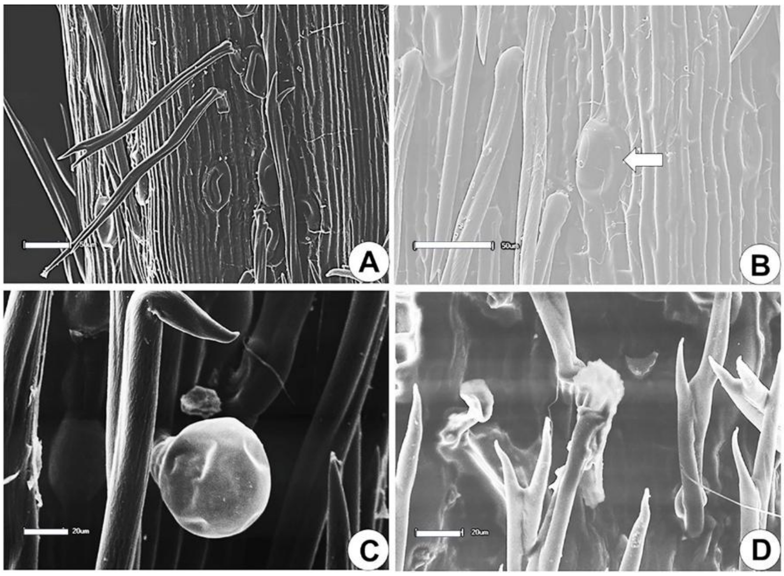

Figure 3. SEM micrographs of the exocarp of *Lessingianthus sactipauli* (A), *Lessingianthus polyphyllus* (B), *Chrysolaena nicolackii* (C), and *Orthopappus angustifolius* (D) showing non-glandular biseriate trichomes (A, D), idioblasts (B, white arrow), and glandular biseriate trichomes (glandular twin hairs) (C). Scale bars: 20 μm (C, D), 50 μm (A, B).

The mature pericarp is composed of a more or less collapsed exocarp and sclerenchymatous mesocarp (Figure 4A), but in *Lepidaploa psilostachya* the mesocarp is parenchymatous (Figure 4B), usually with a single cell-layer thick. Conspicuous ribs may be seen on the cypsela surface of *Lessingianthus* (Figure 4A) and *Vernonanthura* species, but they are poorly developed in *Lepidaploa chamissonis* and *Orthopappus angustifolius*, and always absent in the other species.

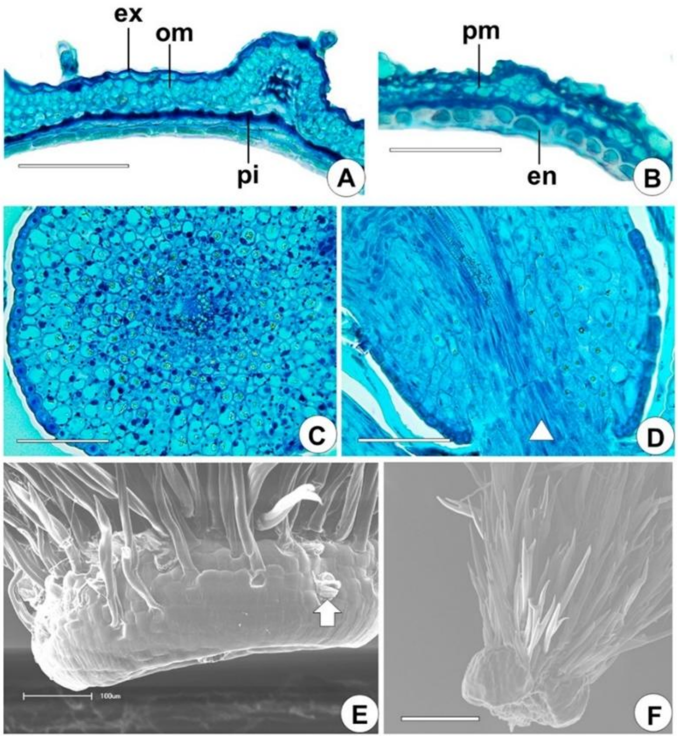

Figure 4. Fruit structure of *Vernonanthura nudiflora* (A, C), *Lepidaploa psilostachya* (B), *Lessingianthus sactipauli* (D), *Chrysolaena nicolackii* (E) and *Orthopappus angustifolius* (F). A, B. Pericarp in cross-section. C, D. Carpopodium in cross-section and longitudinal section (white arrowhead indicates vascular bundle), respectively. E, F. SEM micrographs of the carpopodium continuous (white arrow indicates glandular twin hair) and discontinuous, respectively. (en=endosperm; ex=exocarp; om=sclerified outer mesocarp; pi=collapsed integument, endocarp and inner mesocarp; pm=outer parenchymatous mesocarp). Scale bars: 80 μm (A-D), 100 μm (E, F).

Carpopodium structure—The ovary carpopodium consists of uniseriate epidermis (Figure 4C, D), with glandular twin hairs only in *Chrysolaena* species (Figure 4E). Parenchyma underlies the epidermis with idioblasts which contain either druses or prismatic crystals (Figure 4C, D). A prominent collateral vascular bundle runs though the carpopodium (Figure 4C, D).

In the carpopodium of the mature cypsela, the cell walls of the parenchyma are thicker than those of the ovary carpopodium. The carpopodium is continuous in the derived species of *Vernonia* (Figure 4E), whereas it is discontinuous in *Orthopappus angustifolius* (Figure 4F).

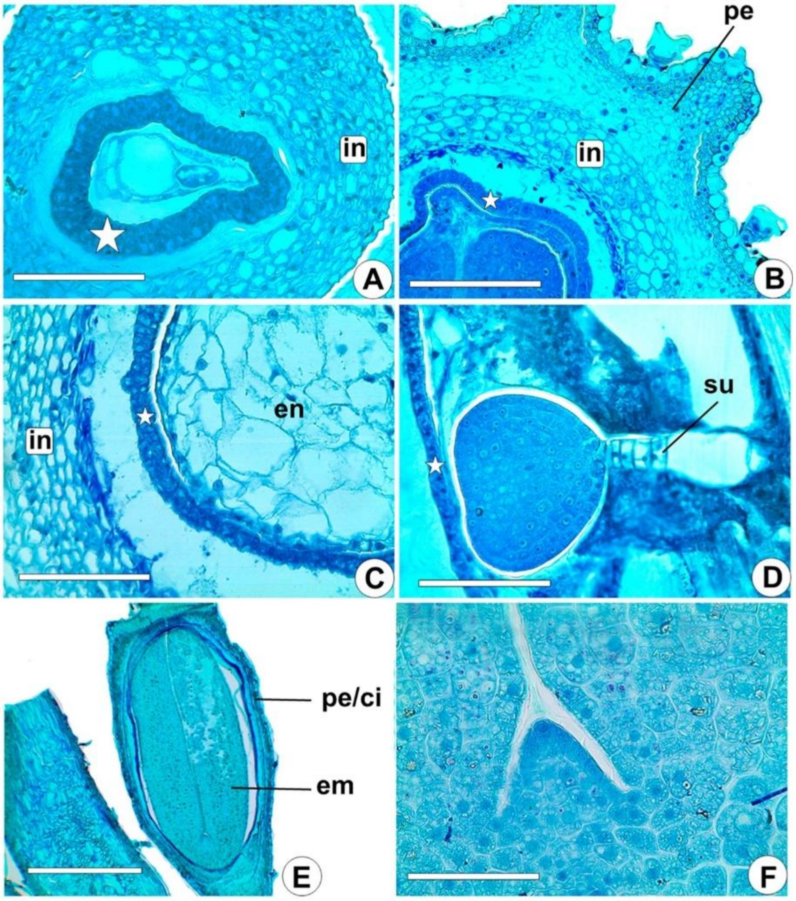

Figure 5. Developing seed structure of *Lepidaploa chamissonis* (A), *Vernonanthura nudiflora* (B), *Lessingianthus polyphyllus* (C, D) and *Chrysolaena nicolackii* (E, F). A-D. Seed sections in different stages of differentiation, showing endothelium (white star), outer portion of the integument (in), endosperm (en) and cordiform embryo with suspensor (su). E, F. Mature cypsela/seed in longitudinal section, showing pericarp and integument colapsed (pe/ci), right embryo (em) and detail of the conical plumule (F). Scale bars: 60 µm (A-F), 200 µm (E).

Seed development-Seeds developed from anatropous, unitegmic and tenuinucellate ovules. The ovule and immature seed integument consist of glabrous and uniseriate epidermis, parenchymatous mesophyll and endothelium (Figure 5A). In the mesophyll, two parenchymas are distinguished, the outer compact parenchyma, and the inner parenchyma with conspicuous intercellular spaces (Figure 5B, C). The endothelium, whose cells are more heavily stained, is uniseriate. It is a remarkable fact that some ovules or immature seeds have pluriseriate endothelium (Figure 5C). The vascular supply of the ovule consists of a single vascular strand, extending from the funicle to the chalaza.

During seed development, the exotesta and outer mesotesta cells are compressed, and the inner mesotesta undergoes cellular dissolution (Figure 5A-C). The endothelium remains surrounding the developing embryo (Figure 5A-C). Endosperm has cellular formation (Figure 5C). The cordate embryo (Figure 5D) exhibits a multicellular and uniseriate suspensor with a reasonably large basal cell.

The mature seed coat is unspecialized, and the endothelium collapses (Figure 5E). One cell-layer thick of endosperm (Figures 4A, B; 5E) remains in the seed with thick-walled cells and rich content in lipid substances. The proembryo exhibits a uniseriate suspensor with a basal cell relatively wide (Figure 5D). The embryo is straight with a short hypocotyl-radicle axis, large cotyledons of the same size, and plumule (Figure 5E) as a conical protuberance (Figure 5F). Lipid substances were found in the embryos, but *Vernonanthura* species are devoid of them.

Embryo formation—Subsequently, the analysis of cypselae with normal embryos (Figure 5E) showed a high percentage of abortive seeds, especially in the derived species of *Vernonia*. It is important to highlight that the *Orthopappus* was the only genus with more than 50% of cypselae with completely formed embryo (Table 2).

Table 2. Seed percentage with embryo formed and devoid of embryo in selected Vernonieae taxa (Asteraceae).

Species	Seeds with embryo (%)	Seeds devoid of embryo (%)
Chrysolaena flexuosa	28	72
Chrysolaena nicolackii	31	69
Lepidaploa chamissonis	14	86
Lepidaploa psilostachya	7	93
Lessingianthus polyphyllus	38	62
Lessingianthus sanctipauli	27	73
Vernonanthura nudiflora	6	94
Vernonanthura oligolepis	11	89
Orthopappus angustifolius	65	35

Discussion

The biseriate pappus of the Vernonieae investigated is interpreted as the floral calyx according to the studies of Judd et al. (2009) and Mandel et al. (2019). Regarding their structure, Roth (1977) interpreted the pappus members as reduced, less differentiated forms of foliage leaves with mostly undifferentiated mesophyll or transformed into sclerenchyma, and reduced or rudimentary vascular system. We may also accept this interpretation for the fruit pappus of the *Vernonia s.l.* and *Orthopappus angustifolius*, which have a relatively simple structure, with epidermis and sclerenchymatous mesophyll.

According to the results, the Vernonieae species have some bristles of the pappus devoid of the vascular system and others with few rudimentary vessels. So far, the various species of this tribe, such as *Elephantopus mollis* Kunth, *Chrysolaena platensis* (Spreng.) H. Rob., *Lessingianthus brevifolius* (Less.) H. Rob. and *Chrysolaena herbacea* (Vell.) H. Rob. (Martins & Oliveira, 2007; Galastri & Oliveira, 2010; Batista & Souza, 2017b), have also a pappus without vascularization. On the other hand, vascularization of bristles in *Lepidaploa* species was recorded by Marques et al. (2018). As pointed out by Misra (1972), *Vernonia anthelmintica* (L.) Willd. seems to be the only species belonging to the tribe Vernonieae that has vascularized pappus. We therefore infer that the absence of vascular tissue or the rudimentary nature of the vascular system in the pappus may be a character quite constant on the Vernonieae tribe.

The pappus of *Vernonia s.l.* consists of bristles that have the same shape, but different size, with the exception of *Orthopappus angustifolius* as previously noted. While the pappus morphology has been shown to be useful in the separation of species and genera of some Asteraceae (Bremer, 1994; Roque & Bautista, 2008; Marzinek & Oliveira, 2010; Pallone & Souza, 2014) this type does not vary within Vernonieae. The apical plate of the flower, the region that supports the pappus, was found to contain suberized and lignified cells in most species of Vernonieae. These features may have diagnostic value, when added to prior studies of morphological characters (Robinson, 1999).

Among the Vernonieae, the fruit (cypsela) features that can be successfully employed for distinguishing various species from each other, include the surface ribs. The well-defined ribs are observed in the fruits of *Lessingianthus polyphyllus*, *L. sanctipauli*, *Vernonanthura nudiflora* and *V. oligolepis*. Poorly developed ribs have been found in the cypselae of *Lepidaploa psilostachya* and *Orthopappus angustifolius*, and are absent in *Chrysolaena flexuosa*, *C. nicolackii* and *Lepidaploa chamissonis*. The absence of ribs may be an interesting feature for the phylogeny of Vernonieae because the genera *Chrysolaena* and *Lepidaploa* belong to the same clade (sister-group relationship) according to the phylogenetic hypothesis of Keeley, Forsman, and Chan (2007).

All species of *Vernonia s.l.* and *Orthopappus angustifolius* have an outer ovary epidermis and exocarp with non-glandular twin hairs. Glandular twin hairs were also found in the taxa, but they are absent from the ovary and cypsela of *Lepidaploa psilostachya*. Knowledge of hair types is uneven both within and among tribes, and therefore, indumentum characters have been of limited use at the suprageneric level (Bremer, 1994); however, Ciccarelli, Garbari, & Pagni, (2007) have reported the structural differences in the glandular trichomes of Asteroideae, which are significant in the subfamily taxonomy. It has also been noted that the glandular twin hairs of Vernonieae may contribute to the separation of species, where they differ in shape and size in the derived genera of *Vernonia* when compared with the *Orthopappus angustifolius*. In *Orthopappus angustifolius* epidermal cells differ greatly in size (two different sizes), whereas the species belonging to the segregated genera of *Vernonia s.l.* have practically the same size emphasizing their phylogenetic proximity.

According to Angulo et al. (2015) and Via do Pico, Veja, and Dematteis, (2016), until recently little diagnostic value has been attributed to the crystals in the taxonomy and phylogeny of Vernonieae. However, they may be useful in the tribe as an indicator of subtribal affiliation. For example, here crystalliferous idioblasts are found in the ovary mesophyll in all species of *Vernonia s.l.* indicating a likely close relationship among those genera that have been segregated from *Vernonia* using other morphological and molecular characters (Keeley & Robinson, 2009; Keeley et al., 2021). Most of the species have rectangular crystals, as is the case with *Chrysolaena* and *Lepidaploa* (Ângulo & Demateis, 2014), but cubic crystals are also present in *Vernonanthura nudiflora* and species of *Lessingianthus* (Ângulo & Demateis, 2014). Marques et al. (2018) found crystals in the mesocarp of the 21 *Lepidaploa* species investigated. Crystals are not present in the ovary mesophyll of *Orthopappus angustifolius*, supporting its generic independence from the segregated genera of *Vernonia s.l.*

Developing fruits of Vernonieae show the tissue collapse, especially the endocarp and inner mesocarp. The persistent outer mesocarp is sclerified in the eight investigated species, but it is parenchymatous in the cypsela of *Lepidaploa psilostachya*. There is increasing evidence that the tissue collapses, and sclerification are common within Vernonieae (Martins & Oliveira, 2007; Galastri & Oliveira, 2010; Freitas et al., 2015; Batista & Souza, 2017a).

The carpopodium morphology is often taxonomically useful in Asteraceae (Robinson, 1981; Haque & Godward, 1984; Roque & Bautista, 2008). Marques et al. (2018, 2020) report lignification in the carpopodium of *Lepidaploa* species and considered this character unprecedented in the genus. Our results show that it has a homogenous structure, but some details may assist in distinguishing certain Vernonieae species. For instance, there are only capitate glandular twin hairs in the *Chrysolaena* carpopodium. Moreover, the carpopodium is discontinuous in *Orthopappus angustifolius* and continuous in all segregated species of *Vernonia s.l.* The similarities and differences of the carpopodium within Vernonieae found here support the findings of Martins & Oliveira (2007) and Galastri & Oliveira (2010), which identified similarities in the carpopodium among species of *Chrysolaena* and *Lessingianthus*.

Our results also showed that all species of *Vernonia s.l.* and *Orthopappus angustifolius* have ovules in which the funicular vascular system terminates in the chalaza, supporting the works of Tiagi & Taimni (1960) for *Vernonia cinerascens* Sch. Bip., *Chysolaena platensis* (Galastri & Oliveira, 2010), *Lessingianthus brevifolius* and *Chysolaena herbacea* (Martins & Oliveira, 2007). While two states of vascular supply have been reported in Vernonieae, the first with the vascular supply extending from funicle to the anti-raphe in *Vernonia anthelmintica* (Misra, 1972), and the other with postchalazal course found in *Elephantoppus mollis* by Batista et al. (2015). The overall usefulness of these different vasculature patterns is not yet established, although Corner (1976) and Werker (1997) used them to make evolutionary assumptions in Asteraceae.

The collapsed endothelium occurs in the mature seeds of *Vernonia* s.l. and *Orthopappus angustifolius*, similar to what was observed for *Flaveria repanda* Lag. by Misra (1964), where it loses its identity during seed development, and remains as a cuticle closely adhering to the persistent layer of endosperm. In some developing seeds of Vernonieae, the endothelium may be multiseriate and it appears to be correlated with the formation of abortive seeds. Our consideration is based on the suggestion formulated by Marzinek (J. Marzinek, unpublished data) who believes that multilayered endothelium may have some influence on the formation of abortive seeds. Certainly, we do not discard other factors that may be associated with the seed abortion, such as the failure in endosperm development (Chican & Palser, 1982), and the occurrence of self-incompatibility, which is common in Asteraceae (Heenan, Smissen, & Dawson, 2005).

The remnants of the endosperm remain in the ripe seed of *Vernonia s.l.* and *Orthopappus angustifolius* as a single layer of thick-walled cells. Mature seeds of other species of Asteraceae also have similar endosperm (Pandey & Singh, 1980; Pallone & Souza, 2014; Batista et al., 2015). Thick-walled endosperm is rigid and can provide physical protection to the embryo (Werker, 1997; Souza & Paoli, 2009), particularly in Asteraceae with unspecialized seed coat (Pallone & Souza, 2014; Batista et al., 2015) which appears to be the case of the species of Vernonieae investigated in this study.

Abortive seeds are relatively common in the segregated species of *Vernonia* s.l. and within the Asteraceae, especially in Brazilian savannas species (Sassaki, Rondon, & Zaidan, 1999). In a few cases, such as in the *Chysolaena platensis* (Galastri & Oliveira, 2010) and Eupatorideae (J. Marzinek, unpublished data), the embryo formation in seeds is also low. To explain the low formation of embryos in Asteraceae, Sassaki et al. (1999) and Galastri & Oliveira (2010) postulated a hypothesis that this condition occurs due to the capacity of vegetative propagation and to the presence of reserve substances in the underground systems of Asteraceae. However, commonly this condition is not so simple. Questions still remain unanswered regarding the reproductive success of the *Vernonia* species, despite the low number of embryos formed by the plant and the high investment in cypsela formation. It still is not known if the potential for vegetative propagation, genetic variability, or even the reproductive system of Vernonieae species contribute to this. The degree of correlation between abortive seeds and reproductive success in savanna Vernonieae needs to be better explored and it may be useful in ecophysiological studies, especially those on species in danger of extinction, such as *Chrysolaena nicolackii* that is on the Red List (Centro Nacional de Conservação da Flora [CncFlora], 2017).

The ontogeny of fruits and seeds provides important structural features which are of taxonomic value at the specific level in Vernonieae, as shown in Table 3.

Table 3. Significant features of fruits of Vernonieae.

Species	Pappus	Epidermis/ exocarp	Mesocarp	Carpopodium - hair/continuity
Chrysolaena flexuosa	Different length	Glandular twin hair present Cells of the same size	Sclerified Devoid of ribs	Glandular twin hair present Continuous
Chrysolaena nicolackii	Different length	Glandular twin hair present Cells of the same size	Sclerified Devoid of ribs	Glandular twin hair present Continuous
Lepidaploa chamissonis	Different length	Glandular twin hair present Cells of the same size	Sclerified Devoid of ribs	Glandular twin hair absent Continuous
Lepidaploa psilostachya	Different length	Glandular twin hair absent Cells of the same size	Non-sclerified Ribs poorly defined	Glandular twin hair absent Continuous
Lessingianthus polyphyllus	Different length	Glandular twin hair present Cells of the same size	Sclerified Ribs well defined	Glandular twin hair absent Continuous
Lessingianthus sanctipauli	Different length	Glandular twin hair present Cells of the same size	Sclerified Ribs well defined	Glandular twin hair absent Continuous
Vernonanthura nudiflora	Different length	Glandular twin hair present Cells of the same size	Sclerified Ribs well defined	Glandular twin hair absent Continuous
Vernonanthura oligolepis	Different length	Glandular twin hair present Cells of the same size	Sclerified Ribs well defined	Glandular twin hair absent Continuous
Orthopappus angustifolius	Same length	Glandular twin hair present Cells of the different size	Sclerified Ribs poorly defined	Glandular twin hair absent Continuous

Conclusion

In conclusion, it may be stated that certain features concerning the pappus, cypselae, and seeds have offered support for the separation of the segregated species within *Vernonia s.l.*, particularly compared to *Orthopappus angustifolius*. These characteristics also contribute to understanding the relationship within the Vernonieae, indicating that the derived genera of *Vernonia s.l.* share a close phylogenetic relationship.

Acknowledgements

We thank CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*, Brazil) for the support granted for the accomplishment of this study; IAP (Environmental Institute of the Paraná, Brazil) for the authorization of collection of plant material; and Dr. Jimi Naoki Nakajima and Dr. Danilo Marques for species identification.

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