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Structure and ontogeny of the pericarp of six Eupatorieae (Asteraceae) with ecological and taxonomic considerations

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ABSTRACT

The ontogeny of cypselae and their accessory parts were examined using light and scanning electron microscopy for the species *Campuloclinium macrocephalum*, *Chromolaena stachyophylla*, *Mikania micrantha*, *Praxelis pauciflora*, *Symphyopappus reticulatus*, and *Vittetia orbiculata*, some of these being segregated from the genus *Eupatorium*. A layer of phytomelanin observed in the fruit appears to be secreted by the outer mesocarp into the schizogenous spaces between the outer and inner mesocarp; its thickness was observed to vary among the different species examined. The bristles of the pappus are vascularized, except in *M. micrantha*, and have cells that are superficially projected and arranged acropetally; in *S. reticulatus* some of the projections are retrorse and a fracture line on the floral disk that is only seen in this species may indicate a double dispersal process. Numerous differences observed among the cypselae examined here reinforce earlier segregations of the genus *Eupatorium sensu lato*.

Key words: : anatomy, Asteraceae, carpopodium, fruit, pappus, phytomelanin.

INTRODUCTION

The tribe Eupatorieae (Asteraceae) comprises 190 genera and 2,000 species (Anderberg et al. 2007) that are encountered primarily in Mexico and Central and South America, with some representatives from North America, but with few species in the Old World (King and Robinson 1987). *Eupatorium* is a very complex genus and has experienced numerous segregations, as compiled by King and Robinson (1987).

Wagenitz (1976) suggested that anatomical studies of the fruits of the Asteraceae might aid in elucidating its systematics. Characteristics of the indumentum (Ritter and Miotto 2006), pappus (Bean 2001), carpopodium (Haque and Godward 1984), and the anatomy of the pericarp itself (Bruhl and Quinn 1990), or a combination of all of the characters cited above (Leszek et al. 1997), have been used to delimit tribes, genera, and even species of this family.

In spite of the global occurrence of the family, the fruits of the Asteraceae have not been intensively studied, as can be confirmed by the discordance seen in the names attributed to its fruits. Marzinek et al. (2008) took into consideration many anatomical and historical aspects of Asteraceae fruits, especially their complex origin, and reaffirmed their true nature as cypselae.

One character that stands out in descriptions of the cypselae of the Heliantheae *s.l.* (which includes Eupatorieae) is their dark appearance (Anderberg et al. 2007). This aspect can be attributed to the deposition of a rigid layer of phytomelanin, an organic material that fills the schizogenous space of the pericarp during the development of the cypsela after fertilization (Pandey and

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Dhakal 2001). Sometimes this phytomelanin layer is referred to as a "carbon layer" or black pigment layer (see Roth 1977). Panero (2007) indicates the occurrence of phytomelanin as a synapomorphy of the Asteraceae. The Phytomelanin Cypsela Clade (PCC) is composed of more than 5,000 species that produce this substance.

Ontogenetic studies of the reproductive organs of Eupatorieae are generally scarce, and are almost exclusively limited to the publications of Pandey and Singh (1983, 1994). In spite of the importance of the Eupatorieae within the Asteraceae, no single study has focused simultaneously on the ontogeny, indumentum, pappus, and carpopodium of the species within this taxon.

The present work analyzed the development of the pericarp and the accessory parts of the cypselae of six species of Eupatorieae from Brazil (some of them segregated from *Eupatorium s.l.*), emphasizing the most relevant structures to taxonomic and ecological questions related to this tribe.

MATERIALS AND METHODS

Specimens of *Campuloclinium macrocephalum* (Less.) DC., *Chromolaena stachyophylla* (Spreng.) R. King and H. Robinson, *Mikania micrantha* H.B.K., *Praxelis pauciflora* (H.B.K.) R. King and H. Robinson, *Symphyopappus reticulatus* Baker, and *Vittetia orbiculata* (DC.) R. King and H. Robinson were collected in *cerrado* vegetation fragments in the municipality of Botucatu, São Paulo State, Brazil. Reference plant material was prepared and deposited in the BOTU Herbarium (Holmgren et al. 1990) as collections 25,552 to 25,557.

Micromorphological analyses of the surfaces of the cypselae were performed with material fixed in glutaraldehyde (2.5% in 0.1M phosphate buffer, pH 7.3, maintained at 4°C). Samples were processed and, then, mounted on aluminum stubbs, gold coated, and subsequently examined using a scanning electron microscopy (Quanta 200, FEI Company) to generate digital images.

Anatomical studies were performed on ovaries and cypselae in different phases of development that had been fixed in FAA 50 for 48 hours (Johansen 1940) and, then, conserved in 70% ethanol (Jensen 1962). The fixed material was dehydrated in an ethanol series and embedded in methacrylate (Leica$^{\text{TM}}$) following the manufacturer's recommendations. Both transversal and longitudinal sections 6 to 10μm thick were prepared using a rotary microtome, stained with 0.05% toluidine blue at pH 4.7 (O'Brien et al. 1964), and subsequently mounted in synthetic resin. The permanent slides were observed under an Olympus BX41 optical microscope and the images captured digitally.

The following histochemical tests were performed: ruthenium red to indicate the presence of polysaccharides and pectins (Jensen 1962); phloroglucinol with hydrochloric acid to stain lignified cell walls (Sass 1951); Sudan IV to stain lipidic substances; Lugol solution for starch; ferric chloride with sodium carbonate to indicate the presence of phenolic compounds (Johansen 1940); Dragendorff reagent to detect alkaloids (Yoder and Mahlberg 1976); and bromophenol blue for proteins (Mazia et al. 1953).

The results are described in ontogenetic terms. Considering the inferior origin of the ovary, the *lato sensu* definition of the pericarp was adopted, in which the exocarp is produced by the outer epidermis of the inferior ovary, the endocarp by the inner epidermis, and the mesocarp by the ground region where the vascular tissues are inserted.

Trichomes distribution was analyzed using both scanning electron and optical microscopy. Ten cypselae (n=10) of each species mounted in glycerin were also observed. The terms scarce (when trichomes were present on up to 10% of the cypsela surface), frequent (between 11% and 50%), and abundant (>50%) were used.

The terminology used to designate the outward projected portions of the pappus fibers was adapted from Hickey (1979), considering the angle of divergence between the distal portion of the projected cells and the apex of the bristles. These angles were classified as narrow (>45°) or moderate (between 45° and 65°) acute. The nomenclature proposed by Barthlott et al. (1998) was used to describe the bristle surfaces.

RESULTS

The ovaries of the floral buds of the species examined were all inferior (Fig. 1), bicarpelar, and unilocular (Fig. 2), oblong shaped, and had variable numbers of longitudinal ridges that were visible during the development of the pericarp.

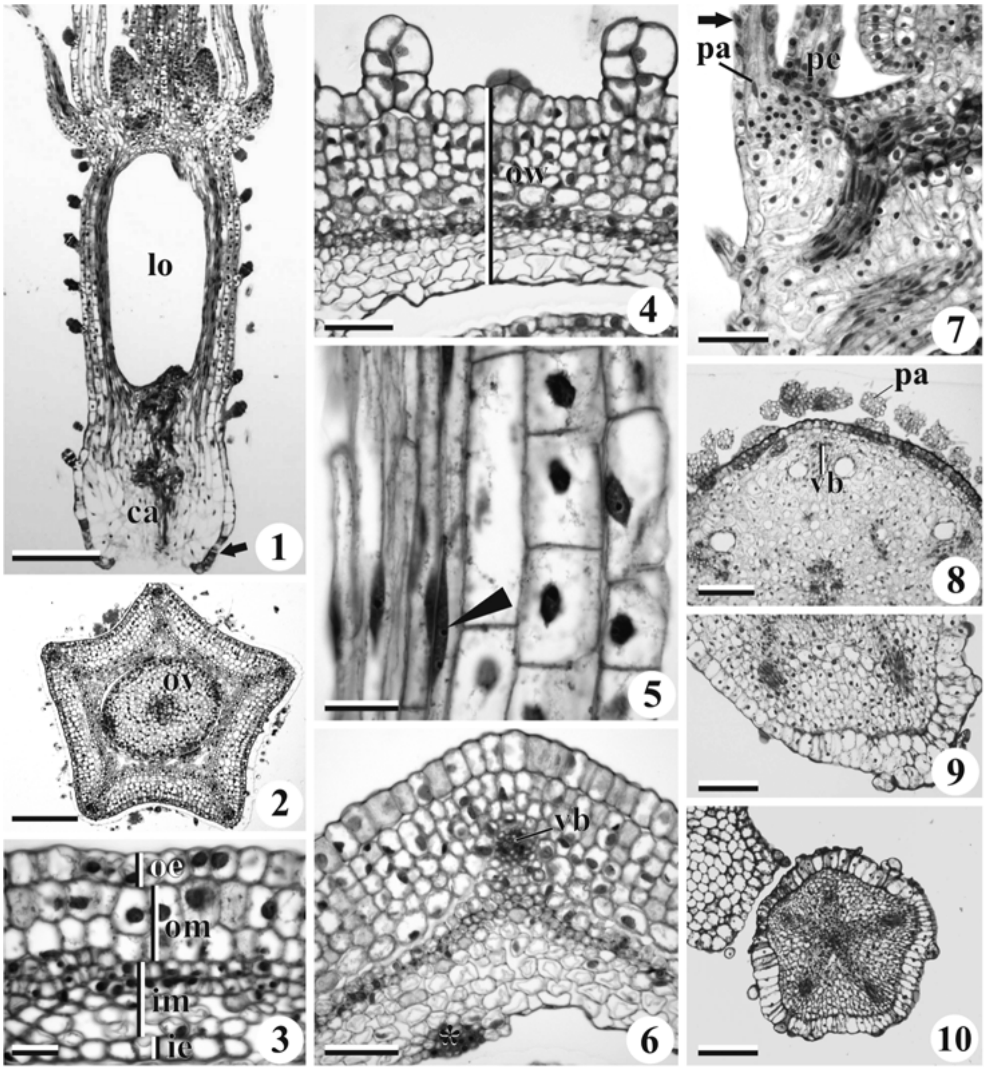


Fig. 1 – Ovary of the floral bud. Figs. 1, 5, 7, 9 – Longitudinal sections. Figs. 2-4, 6, 8, 10 – Transversal sections. Fig. 1 – *Campuloclinium macrocephalum*; note lignified basal epidermal cells (arrow). Fig. 2 – *Mikania micrantha*. Figs. 3-4 – Detail of the ovarian walls of *Campuloclinium macrocephalum* and *Symphyopappus reticulates*, respectively. Fig. 5 – *Campuloclinium macrocephalum*; note fusiform nuclei of the inner mesophyll cells (arrowhead). Fig. 6 – Detail of the ovarian wall of *Mikania micrantha* showing the transmission tissue (*). Fig. 7 – Detail of the floral disk of *Praxelis pauciflora*; note salient external cells of the pappus with evident nuclei (arrow). Fig. 8 – Detail of part of the floral disk of *Mikania micrantha* demonstrating secretory cavities near the vascular bundles. Figs. 9-10 – Carpopodium of *Symphyopappus reticulatus*. (ca – carpopodium; ie – inner epidermis; im – inner mesophyll; lo – locule; oe – outer epidermis; om – outer mesophyll; ov – ovule; ow – ovarian wall; pa – pappus; pe – petal; vb – vascular bundle). Bars – 250μm (1); 150μm (2, 10); 20μm (3, 5); 50μm (4,6-7,9); 100μm (8).

The outer ovarian epidermis is uniseriate (Fig. 3) with cubical cells, slightly longitudinally elongated (Fig. 5) and vacuolated, with visible nuclei; biseriate tector (Fig. 4), as well as uni- and biseriate glandular trichomes, were observed with variable cell numbers and densities, and will be described in more details below.

The ovarian mesophyll is composed of two regions. The outer mesophyll region demonstrates juxtaposed and longitudinally elongated cells; in transversal section, the subepidermal cells appear isodiametric (Figs. 3-4). These cells are vacuolated and have very evident nuclei.

Campuloclinum macrocephalum (Fig. 3), *Ch. stachyophylla*, *P. pauciflora*, and *V. orbiculata* have two layers in the outer mesophyll, while five to six cell layers are observed in *M. micrantha* (Fig. 6) and *S. reticulatus* (Fig. 4). Procambial strands vascularize the ovary in the outer mesophyll, but differentiated vascular elements are only rarely observed (Fig. 6).

The inner mesophyll demonstrates from four to five cylindrical layers of small-diameter cells arranged longitudinally, with evident fusiform nuclei (Fig. 5). The more internal layers have large intercellular spaces and irregular shapes in comparison with the external layers (Figs. 3-4, 6).

The inner epidermis in *Ch. stachyophylla*, *P. pauciflora*, *S. reticulatus* (Fig. 4), and *V. orbiculata* is uniseriate and little evident, while in *Ca. macrocephalum* (Fig. 3) and *M. micrantha* these cells are elongated longitudinally, juxtaposed, with evident nuclei, and of large diameters in comparison with the cells of the inner mesophyll. The transmission tissue in all of the species studied can be seen in the inner epidermis where the carpels are joined, and it is composed of a multiseriate region of small diameter cells with pectin-containing cell walls and evident nuclei (Fig. 6).

The floral disk has an uniseriate epidermis that covers varying layers of elongated parenchyma cells disposed obliquely along the periphery; these cells form a protuberance where the pappus is inserted (Fig. 7); there are vascular traces of other floral parts immersed in this same region. In *M. micrantha*, two lateral secretory cavities were observed near the bundles that irrigate the corolla (Fig. 8).

A pappus is present in all of the species studied and is connate at its base. Each bristle is composed of a multiseriate group of cells that are rounded in transversal section and longitudinally elongated. The distal extremities of the external cells are projected towards the exterior, and evident nuclei are seen in the cells in this region (Fig. 7).

A protuberance that constitutes the carpopodium and is composed of cubic parenchyma cells with thin walls can be observed at the basal region of the ovary. The walls of the epidermal cells of the carpopodium are lignified in the floral bud of *Ca. macrocephalum* (Fig. 1) and *Ch. stachyophylla*; *S. reticulatus* has an asymmetrical carpopodium (Fig. 9), with epidermal cells of different sizes that are larger than the internal cells and arranged in a single layer (Fig. 10). The other species have indistinct carpopodium at the median section of the ovary. The ovule trace is single and robust, reaching to the base of the locule (Fig. 1).

The exocarp remains unaltered during development (Figs. 11, 13, 17, 19, 21), except in *M. micrantha*, where it develops an ornamented cuticle (Fig. 15). The cellular density of the outer mesocarp distinctly increases in all of the species, and these cells have large nuclei (Figs. 13, 17).

The most significant events that occur in the young pericarp take place in the inner mesocarp, with the most external cells forming numerous anticlinal projections in the direction of the outer mesocarp, and producing schizogenous spaces that are evident between the two mesocarpic regions (Figs. 11-22). These spaces are most ample in *S. reticulatus* (compare Figs. 19 and 20 with the other species). The lobed contour of the internal periclinal wall of the outer mesocarp (which is in contact with the numerous anticlinal projections referred to above) produces large intercellular spaces in *M. micrantha* and *S. reticulatus*, (Figs. 16, 20). The external peripheral layer of the inner mesocarp becomes lignified during the later stages of development in all of the species examined, forming sclereids.

The deposition of phytomelanin is initiated in the intercellular spaces described above, forming a continuous, thick, dark layer that is evident in all of the species, but thickest in *M. micrantha* and *S. reticulatus* (Figs. 23-34). Phytomelanin occupies the whole space between the outer and inner mesocarp, but did not give a positive reaction in any of the histochemical tests applied.

Lignification of the walls of the external cells of the inner mesocarp occurs rather late in the development of *M. micrantha*. The schizogenous spaces between the two mesocarpic regions in this species are contiguous with the intercellular spaces of the outer mesocarp, resulting in an irregular deposition of phytomelanin so that it occupies only part of that space (Fig. 28). In *Ca. macrocephalum* and *S. reticulatus* (Fig. 35), as well as *V. orbiculata*, phytomelanin is deposited in intercellular spaces that are external to the vascular bundles, while this layer is interrupted near the vascular bundles in *Ch.*

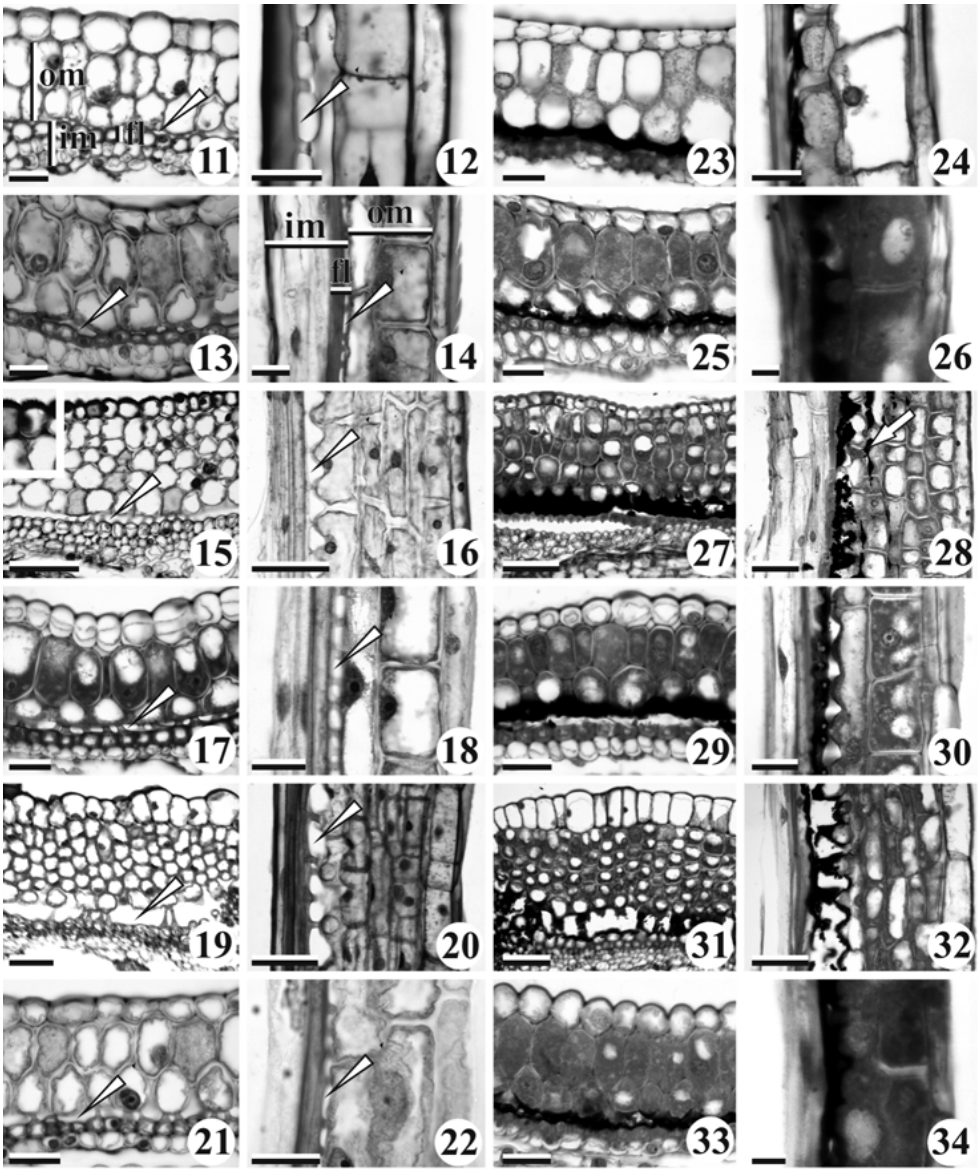


Fig. 2 – Pericarp during development. From left to right, first and third columns – Transversal sections. Second and fourth columns – Longitudinal sections. Figs. 11-22 – Immature pericarp initiating the formation of schizogenous spaces (arrowhead). Figs. 23-34 – Pericarp during maturation, with phytomelanin deposits. Figs. 11-12, 23-24 – *Campuloclinium macrocephalum*. Figs. 13-14, 25-26 – *Chromolaena stachyophylla*. Figs. 15-16, 27-28 – *Mikania micrantha*; note phytomelanin deposits on the outer mesocarp (arrow). Figs. 17-18, 29-30 – *Praxelis pauciflora*. Figs. 19-20, 31-32 – *Symphyopappus reticulatus*. Figs. 21-22, 33-34 – *Vittetia orbiculata*. (fl – fibrous layer; im – inner mesocarp; om – outer mesocarp). Bars – 20μm (11-14, 17-18, 21-26, 29-30, 33-34); 50μm (15-16, 19-20, 27-28, 31-32).

stachyophylla (Fig. 36), *M. micrantha*, and *P. pauciflora*. The inner mesocarp collapses at this time and is partially reabsorbed (Fig. 24).

Macroscopic observations indicated that phytomelanin deposition in *Ca. macrocephalum* initiates in the basal region of the fruit and, subsequently, proceeds towards the apex. All the other species examined have small cypselae, making this analysis much more difficult.

At maturity, the cypselae take on the final forms that are characteristic of each species, as can be seen in Figure 37. Two different processes can occur in the

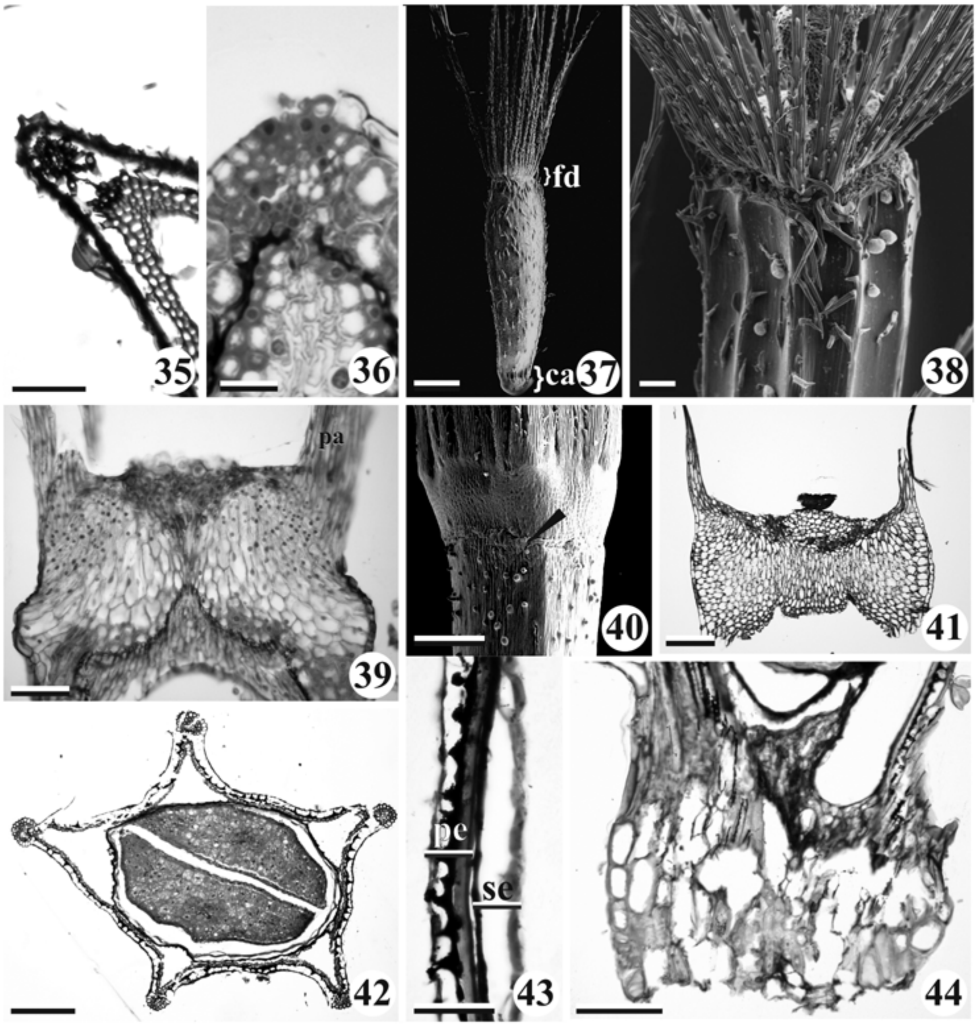


Fig. 3 – Cypselae during maturation. Figs. 35-36, 42-43 – Transversal sections. Figs. 39, 41, 44 – Longitudinal sections. Fig. 35 – Phytomelanin deposit in the external intercellular spaces of the vascular bundle of *Symphyopappus reticulatus*. Fig. 36 – Interruption of phytomelanin layer in the proximity of a vascular bundle in *Chromolaena stachyophylla*. Fig. 37 – Mature cypsela of *Praxelis pauciflora*. Figs. 38-39 – Detail of the micromorphology and anatomy of the floral disk region of *Chromolaena stachyophylla* respectively. Figs. 40-41 – Detail of the micromorphology (arrowhead – start of the fracture line) and anatomy of the pappus crown of *Symphyopappus reticulatus*. Figs. 42-43 – Mature pericarp of *Campuloclinium macrocephalum*. Fig. 44 – Carpopodium of *Vittetia orbiculata* showing lignified surface. (ca – carpopodium; fd – floral disk; pa – pappus; pe – pericarp; se – seed). Bars – 75μm (35); 20μm (36); 50μm (37); 100μm (38-39, 44); 30μm (40); 150μm (41-43).

upper region of the fruit: after lignification of the most internal cell walls of the floral disk (Fig. 39), the peripheral parenchyma cells dehydrate and collapse, thereby reducing their volume in most species (Fig. 38) and increasing the distance among the pappus bristles. Only *S. reticulatus* demonstrated lignification of the cell walls at the base of the floral disk, generating a fracture line at maturity (Fig. 40) that facilitates the abscission of the floral disk together with the pappus (Fig. 41).

The bristles of the pappus also become lignified during fruit development, but remain alive throughout this phase and show conspicuous nuclei (Fig. 39).

Mature pericarps undergo intense dehydration mainly in their furrows, and most of the cell layers collapse so that only some exocarp cells, fibers, vascular bundles, and the phytomelanin layer can still be distinguished (Figs. 42-43). The carpopodium demonstrates a lignified surface (Fig. 44).

The pappus is trichomic at maturity, with a persistent, bristly, and isomorphous aspect; the bristles are ordered into a whorl. The pappus has from 14 to 43 bristles, depending on the species: 14-16 in *M. micrantha*; 16-19 in *P. pauciflora*; 17-20 in *Ch. stachyophylla*; 22-25 in *V. orbiculata*; 25-27 in *S. reticulatus*; and 40-43 in *Ca. macrocephalum*. The distal extremities of the cells projecting from the surface of the bristles have a consistently acute divergence angle that ranges from narrow in *Ca. macrocephalum* (Fig. 45), *Ch. stachyophylla* (Fig. 46) and *M. micrantha* (Fig. 47), to moderate in *P. pauciflora* (Fig. 48), *S. reticulatus* (Fig. 49) and *V. orbiculata* (Fig. 50). These extremities have sharp apices, except in *M. micrantha* (Fig. 47) where it is rounded; the cell extremities in *S. reticulatus* are also sometimes rounded.

Micromorphological examination of the bristle surfaces reveals variable patterns: reticulated in *Ca. macrocephalum* (Fig. 51); short longitudinal threads in *Ch. stachyophylla* (Fig. 52); finely striated and with projections demonstrating slight apical compression in *M. micrantha* (Fig. 53); and more finely striated on the surface of *P. pauciflora* (Fig. 54), while smooth in *S. reticulatus* (Fig. 55) and *V. orbiculata* (Fig. 56). Some of the bristle projections on the pappus of *S. reticulatus* are retrorsely flexed (see detail in Fig. 49).

The pappus bristles of the species examined here demonstrate completely lignified cell walls (Figs. 57-62). Most bristles are vascularized, with the exception of those of *M. micrantha*, but the extension of the vascular bundles varied among all the species. Only 1.7% of the total length of the bristles are vascularized in *Ch. stachyophylla*; only 2% in *Ca. macrocephalum*; 4% in *P. pauciflora*; 10% in *S. reticulatus*; and in *V. orbiculata* 37% of the length of each bristle has a small vascular bundle.

The carpopodium is lignified and symmetrical at maturity in most of the species examined (Figs. 63-65, 69), being asymmetrical only in *P. pauciflora* (Fig. 66) and *S. reticulatus* (Figs. 67-68). The carpopodium surface ranged from: undifferentiated in *M. micrantha*, having a thickness of approximately two cell layers in *Ch. stachyophylla*; having from one to three differentiated cell layers in *P. pauciflora*; approximately four layers in *Ca. macrocephalum*; four to six layers in *S. reticulatus*; and approximately eight layers in *V. orbiculata*.

The indumentum was observed to be variable among the cypselae evaluated, as can be seen by comparing Figure 37 (*P. pauciflora*), 38 (*Ch. stachyophylla*), and 40 (*S. reticulatus*). Five types of trichomes were observed: two biseriate tectors and three glandular, the latter ranging from uni- to biseriate. Table I lists the five trichome types observed among the species examined, specifying the variations in their horizontal (in the ribs and furrows) and vertical (considering the proximal third, median, and distal regions) distributions on the cypsela.

DISCUSSION

The species examined here demonstrated ovarian structures typical of the Eupatorieae, including an uniseriate epidermis with trichomes, outer mesophyll with closely juxtaposed cells, inner mesophyll composed of parenchyma cells with ample intercellular spaces, and an inner epidermis with differentiated cells in two regions that form the transmission tissue. This data corroborates the only previously published work concerning this tribe (Pandey and Singh 1983, 1994).

The dark color of the cypselae observed in some groups of Asteraceae is due to the presence of phytomelanin, and this substance is deposited in the schizogenous spaces between the outer and inner mesocarp in the species studied. According to Hanausek (1910) *apud* Roth (1977), there are three possible origins of the "carbon or phytomelan layer": "intracellular secretion", by the accumulation of a black pigment within the cells, especially the epidermis cells; by an "intercellular secretion" that is formed in lysigenous intercellular spaces; or the disintegration of entire cells or cell layers, with subsequent carbonization of their walls. The species examined in the present study did not demonstrate cellular disintegration, while the outer mesocarp cells have secretory characteristics, such as dense cytoplasm and evident nuclei. This layer apparently synthesizes and/or polymerizes phytomelanin, which is then deposited in the space created by the cellular projections between the inner and outer mesocarp. According to Pandey et al. (1989), phytomelanin precursors are synthesized in the endoplasmic reticulum of hypodermic cells (outer mesocarp) and then migrate to the schizogenous spaces where they are subsequently polymerized.

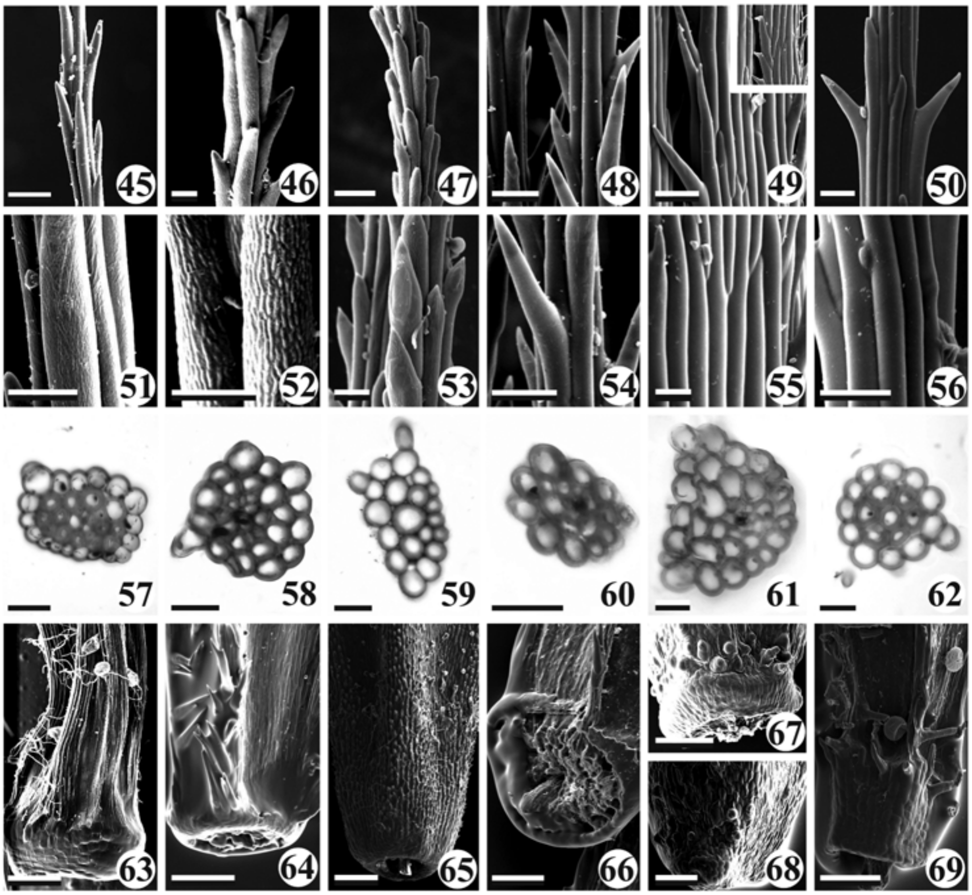
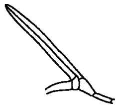
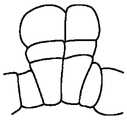
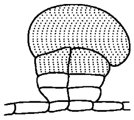
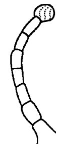
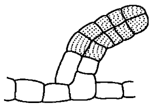


Fig. 4 – Micromorphology (first and second rows) and transversal sections of the pappus (third row), and micromorphology of the carpopodium (fourth row). Figs. 45, 51, 57, 63 – *Campuloclinium macrocephalum*. Figs. 46, 52, 58, 64 – *Chromolaena stachyophylla*. Figs. 47, 53, 59, 65 – *Mikania micrantha*. Figs. 48, 54, 60, 66 – *Praxelis pauciflora*. Figs. 49, 55, 61, 67, 68 – *Symphyopappus reticulatus*. Figs. 50, 56, 62, 69 – *Vittetia orbiculata*. Bars – 50μm (45, 47, 49); 20μm (46, 48, 50-62); 150μm (63-69).

There were distinct differences among the thicknesses of the phytomelanin layers among the various species studied. *Mikania micrantha* and *S. reticulatus* have thicker phytomelanin layers than other species have, which may be related to the greater numbers of secretory layers in the outer mesocarps of these species. According to Hanausek (1910, 1912) *apud* Roth (1977), phytomelanin may serve as a protective screen against excessive insolation or in mechanical protection of the pericarp. As the studied species occur in the *cerrado* biome, protection against excessive insolation could be very important. Pandey and Dhakal (2001) reviewed the available literature concerning phytomelanin in the Asteraceae, and pointed out that this substance has a deterrent effect on insect predators, which was confirmed in experiments with sunflower seeds (*Helianthus* spp). However, J. Marzinek and D.M.T. Oliveira (unpublished data) analyzed the cypselae of *M. micrantha* and *S. reticulatus* and found insect larvae in 10% and 22% of the fruits, respectively. As these are the species with the thickest recorded phytomelanin layers, the deterrent effect of this substance cannot be confirmed, and the role of phytomelanin in the cypselae still needs to be elucidated.

Phytomelanin was observed here to be deposited in an acropetal direction in *Ca. macrocephalum*, contrary to the direction reported by Vries (1948) *apud* Hegnauer (1977) for *Tagetes patula*, a species of Helenieae (*sensu* Bremer 1994). No other published references were encountered concerning this aspect of phytomelanin deposition, and further observations were limited by the small size of the cypselae of the other species examined

TABLE I
Types* and distribution of trichomes on the cypselae of the examined species.
(A: apex; M: median region; B: base; –: absent; +: rare; ++: frequent; +++: abundant).

Type of trichoma																
		Type I			Type II			Type III			Type IV			Type V		
Species	Distribution	A	M	B	A	M	B	A	M	B	A	M	B	A	M	B
Campuloclinium macrocephalum	ribs	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	furrows	–	–	–	–	–	–	+	+	+	–	–	–	–	–	–
Chromolaena stachyophylla	ribs	++	+	+	–	–	–	++	–	–	–	–	–	–	–	–
	furrows	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–
Mikania micrantha	ribs	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	furrows	–	–	–	–	–	–	+	–	–	+	+	+	–	–	–
Praxelis pauciflora	ribs	+++	+++	++	–	–	–	–	–	–	–	–	–	–	–	–
	furrows	++	++	+	–	–	–	–	–	–	–	–	–	–	–	–
Symphyopappus reticulatus	ribs	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
	furrows	–	–	–	++	++	++	–	–	–	–	–	–	–	–	–
Vittetia orbiculata	ribs	++	++	++	–	–	–	–	–	–	–	–	–	–	–	–
	furrows	+	+	+	–	–	–	+++	++	+	–	–	–	++	–	–

* Type I: tector, biseriate, 4-cells; Type II: tector, biseriate, 4-6 cells of hyaline content; Type III: glandular, biseriate, peduncle with 4-6 hyaline cells, distal 2-4 cells globoid to spatulate with dense content; Type IV: glandular, uniseriate, peduncle with 6-7 cells, secretory portion unicellular; Type V: glandular, biseriate, peduncle with 2-4 hyaline cells, distal 8-10 cells with dense content.

in this study, and by the velocity with which deposition occurred.

The indumenta of the cypselae of the Asteraceae have been examined by numerous authors (e.g. Drury and Watson 1966, Wetter 1983, Blanca and Guardia 1997, Mukherjee and Sarkar 2001), and this character has a significant taxonomic value. Roth (1977) observed that biseriate trichomes are typically found on the pericarp in this family, and they apparently aid in fixing dispersed fruits to the soil. Types I and III trichomes referred to in the present study (biseriate non-glandular with four cells, and biseriate glandular with peduncle composed of from four to six hyaline cells, respectively) were common among the Eupatorieae studied. Among the Asteraceae of the *cerrado* vegetation studied by Castro et al. (1997), type IV trichomes (glandular and uniseriate with peduncle composed of from six to seven cells and unicellular secretory portion) occurred exclusively between the Eupatorieae and Heliantheae (*sensu* Bremer 1994). In their study of the micromorphology of species of *Mikania*, Ritter and Miotto (2006) reported that some simple, pluricellular and uniseriate trichomes, as well as many glandular trichomes, were observed on the surface of the fruits of *M. micrantha*. However, type IV trichomes that are found in the present examination of this species were not reported by those authors.

An analysis of the occurrence of trichomes among the different species revealed some interesting results (see Table I): *P. pauciflora* demonstrated a relatively homogeneous distribution of those structures, but has only biseriate tector trichomes known as twin hairs, the most characteristic type of trichome in the Asteraceae; *V. orbiculata* demonstrated the most heterogeneous distribution of trichomes (including the biseriate tector type, as well as two additional secretory types), all of them relatively abundant in the furrows of the cypselae; variations in trichomes between the ribs and furrows are only distinguishable in *Ca. macrocephalum*. Considering the distribution of trichomes among the apical, median, and basal regions of the fruits, all of the species demonstrated a greater density of trichomes near their apices.

Roth (1977) observed that the anatomy of the cypselae of different Asteraceae were quite characteristic. Their internal structure reveals certain characteristics that are taxonomically useful, mainly the arrangement of the support tissues that are preserved even after dehydration of the cypselae. Studies of the ontogeny of dry pericarps are likewise very informative among the Asteraceae, for few defined layers persist at maturity. However, only phytomelanin and residues of cell walls and vascular bundles remain after drying in the Eupatorieae, making comparisons among the different *taxa* very difficult. Pandey and Singh (1983, 1994) studied ontogenetic aspects of Eupatorieae fruits, but these authors cited only a few characteristics related to development.

Haque and Godward (1984) observed that the carpopodium (the abscission zone of the cypsela) sometimes demonstrated structures that facilitated separation from the inflorescence axis and the subsequent dispersal of the fruit. According to King and Robinson (1987), carpopodium anatomy is very variable in Eupatorieae. Among the species studied, *M. micrantha* had an indistinct carpopodium, while this structure is clearly asymmetrical in both *P. pauciflora* and *S. reticulatus*. *Vittetia orbiculata* had the most distinct carpopodium of all the species studied, followed then by *Ca. macrocephalum*. Haque and Godward (1984) examined the carpopodium and its usefulness in the taxonomy of the Asteraceae. These authors reported that *Ca. macrocephalum* had the most differentiated carpopodium among the Asteroideae (*sensu* Bremer 1994) studied. This finding is not in conflict with the present work, however, because these authors did not examine any species of *Vittetia* that had a more conspicuous carpopodium than *Ca. macrocephalum*.

An analysis of the floral disk of *M. micrantha* demonstrated the existence of two cavities associated with each vascular bundle. Reports of internal secretory structures are rare for the cypselae of the Asteraceae, being more frequently observed in vegetative organs. Castro et al. (1997) reported the presence of ducts in the leaves of *M. officinalis*, and Oliveira (1972) observed secretory elements in the leaves, flowers, and fruits of *M. hirsutissima* var. *hirsutissima*. These authors did not specify, however, what type of secretions were produced, in much the same way as it was not possible to detect secretions in the cavities in *M. micrantha*.

Stuessy and Garver (1996) cited the pappus as one of the most important characteristics of the capitulum. According to Mukherjee and Sarkar (2001), the pappi encountered in the cypselae of the Asteraceae represent a classic source of taxonomic information and, depending on the degree of specificity of these characteristics, these structures can be used to identify genera or even species.

It is important to note that the vascular system of the pappus may be differentiated or not. The vasculature of the pappus has been described for some representatives of *Chaenactidinae* belonging to the tribe Heliantheae (Robinson 1981), as well as for some species of *Helogyne* (Eupatorieae), where the vascular bundle may extend for up to half of the length of the bristle (King and Robinson 1987). The bristles examined in the present work were all vascularized, with the exception of *M. micrantha*. Variations in the lengths of the bristle vascular bundles clearly demonstrates structural reduction of the calyx among the Asteraceae, reinforcing the classical affirmation of Cronquist (1955, 1981) and Ramiah and Sayeeduddin (1958) that the pappus is in fact a modified calyx. King and Robinson (1987) affirmed that the pappus is formed by a series of capillary bristles in most species of Eupatorieae, a condition that is considered primitive for the tribe. The pappus may even be reduced to five to ten bristles within this group, as in *Hofmeisteria*, or to just a few bristles as in *Fleischmannia*, or they may be totally absent as in *Ageratum* and some other genera. The presence of reduced vascularized lengths in the bristles of Eupatorieae is an indication of the evolutionary tendency to reduce the number of bristles, even to the point of completely suppressing the pappus. It has been well established that the vascular system is very conservative in plants, and that its reduction commonly precedes a reduction of the organ being served (Eames and MacDaniels 1947). This phenomenon has been previously reported for some species of Eupatorieae.

The pappus has an important role in fruit dispersal, including the performance of hygroscopic movements that regulate the moment of dispersal (Pijl 1982). In an aerodynamic sense, the pappus increases the resistance between the air and the fruit, thus prolonging the time required for it to fall and increasing its chances of being carried for longer distances during dispersal (Haberlandt 1914) *apud* Sheldon and Burrows (1973). The dehydration of the fibers of the floral disk apparently results in a retraction of the more peripheral fibers and the opening of the pappus bristles, thus favoring anemochoric dispersal.

Stuessy and Garver (1996) suggested that the pappus has a double function over time: it first has a defensive role in the flower, mainly against insects, while it is essential for dispersal in the fruit. This double function of the pappus may be one of the factors responsible for the great evolutionary success of the Asteraceae. The suggestion that the pappus has a role in the defense of the flowers was not supported in the species examined in the present study, however, because field observations revealed the existence of capitula that had been completely destroyed by insect predation (J. Marzinek, unpublished data).

Although the Asteraceae typically have a persistent calyx, some cypselae have a rupture line near their apex that seems to direct all of the bristles of the pappus into a single crown-like structure. This structure was observed only in *S. reticulatus* in the present study, but had been reported for *Carduus arvensis* by Dandeno (1905), who noted that the rupture line facilitates germination because water absorption can occur through the apical region of the cypsela. Shmida (1985) reported that a non-deciduous pappus promotes the dispersal of cypselae far from the mother plant. Experiments are needed to more accurately evaluate water uptake by seeds enclosed in the cypselae, and will be important in defining the role of the rupture line in germination.

It is interesting that the pappus is associated with two very distinct dispersal modes in the Asteraceae: biotic dispersal by epizoochory (in which the pappus bristles take on the form of hooks or become reflexed and able to attach to passing animals); and abiotic dispersal by anemochory (in which the extremities of the bristles tend to be erect). Curiously, diaspores of *S. reticulatus* demonstrate both modes, as the cypsela first escapes from the inflorescence axis by anemochory and, then, after initial dispersal, the backward-reflexed bristle extremities favor biotic dispersal.

Capillary pappi composed of thin and flexible bristles, as observed in *Emilia fosbergii*, favor wind transport, but due to their great flexibility they do not facilitate fixation to the ground (J. Marzinek, O.C. De-Paula and D.M.T. Oliveira, unpublished data). In the case of *S. reticulatus*, if the thick and rigid bristles of the pappus did not separate from the cypsela during dispersal, contact with the substrate would be much more difficult. The separation of the pappus crown may also facilitate water uptake and germination of the seeds of this species, but this hypothesis still needs to be tested.

The taxonomic characteristics of the species of Eupatorieae here examined (including the anatomy of the pappus, carpopodium, indumentum, and cypsela before maturation) are all very variable and support the segregation of the genus *Eupatorium* as proposed by King and Robinson (1987).

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RESUMO

A ontogênese das cipselas e de suas partes acessórias em *Campuloclinium macrocephalum*, *Chromolaena stachyophylla*, *Mikania micrantha*, *Praxelis pauciflora*, *Symphyopappus reticulatus* e *Vittetia orbiculata*, parte delas segregadas do gênero *Eupatorium*, foi estudada em microscopia de luz e eletrônica de varredura. A camada de fitomelanina presente no fruto aparentemente é secretada pelo mesocarpo externo e possui espessura variável entre as espécies, depositando-se em espaço esquizógeno entre o mesocarpo externo e interno. As cerdas dos pápus são vascularizadas, exceto em *M. micrantha*, e possuem células projetadas superficialmente, dispostas acropetamente; em *S. reticulatus*, algumas projeções são retrorsas e a presença de linha de fratura sob o disco floral, observada apenas nesta espécie, pode indicar processo duplo de dispersão. As numerosas diferenças registradas entre as cipselas estudadas reforçam segregações anteriores do gênero *Eupatorium sensu lato*.

Palavras-chave: anatomia, Asteraceae, carpopódio, fruto, papus, fitomelanina.

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