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ECOSYSTEMS

Comparative cypsela morphology in *Campuloclinium* DC. and contributions to Eupatorieae tribe (Asteraceae) systematics

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Abstract: Cypselae anatomical studies have helped to understand the evolution and classification of some groups within Asteraceae. In Eupatorieae, there are many uncertainties about the *Campuloclinium* circumscription. There are currently two classifications for the genus, and still no consensus for their delimitation. Since structural studies have contributed to the delimitation of groups in Asteraceae, we studied the cypselae of *Campuloclinium*, searching how the pericarpial taxonomic features could enlighten the genus classification. We studied the fruits of eleven species of this genus through morphological and anatomical observation. Our results showed relevant features to the classification of *Campuloclinium* and its closely related groups. The stipitate cypsela together with other diagnostic characters are relevant to delimitation of this genus within of Eupatorieae. The trichomes present in cypselae have taxonomic proved to be a possible diagnostic character for the genus, and the six-celled trichomes are essential to distinguish *C. campuloclinioides* and *C. hirsutum*. The combination of phylogenetic and structural studies may lead to future research on the delimitation of *Campuloclinium* and its clades and understand how the stipitate cypselae and the phytomelanin layer evolve in Eupatorieae.

Key words: anatomy, fruit, Gyptidinae, pericarp, phytomelanin layer, stipitate cypsela.

INTRODUCTION

Campuloclinium DC. is a genus in the Eupatorieae tribe within Asteraceae (King & Robinson 1972, 1987). It presents a Neotropical distribution and comprises 12 species, which occur in southeastern South America, especially in Brazil (Calvo & Roque 2018).

The genus was described by Candolle (1836), and since then, it has undergone several modifications in its generic classification. It has been recognized as a section within *Eupatorium* L. (Bentham 1873, Baker 1876, Robinson 1918), and at others as a separate genus (Candolle 1836, Gardner 1846). However, *Campuloclinium* is currently recognized at the generic level (King & Robinson 1972, 1987). According to King & Robinson (1972, 1987), the genus is characterized by its receptacle being hemispherical or conical, scrobiculate and glabrous, and by elongate cypsela with tapered base (stipitate).

Although King & Robinson (1987) allocated *Campuloclinium* within Gyptidinae, the genus position is still uncertain. Phylogenetic studies performed by Robinson et al. (2009) and Rivera et al. (2016a) differ in the subtribal positioning of the genus. While Robinson et al. (2009) agreed that *Campuloclinium* belongs in the subtribe Gyptidinae, Rivera et al. (2016a) postulated that this genus and its related genera should be circumscribed in a new subtribe.

As King & Robinson (1972, 1987) mentioned, reproductive structures have great importance to the classification of *Campuloclinium*. The fruits of Asteraceae, known as cypselae, arise from the inferior ovary (Marzinek et al. 2008). The cypselae have a high classification value in different hierarchical levels in the family (Angulo et al. 2015, Freitas et al. 2015, Via do Pico et al. 2016, Silva et al. 2018, Marques et al. 2018a, b, 2020, Bonifácio et al. 2019, Grossi et al. 2020) and the anatomical studies of these structures have contributed to solving taxonomy problems (Marzinek & Oliveira 2010, Freitas et al. 2015, Silva et al. 2018, Marques et al. 2018a, b, 2020, Bonifácio et al. 2019).

In Eupatorieae, anatomical studies with the cypselae have contributed to the resolution of taxonomic issues at the subtribal (Silva et al. 2018), generic (Marzinek & Oliveira 2010), and specific levels (Franca et al. 2015). Moreover, these studies have reevaluated the morphological terms of cypselae that sometimes generate confusion (Marzinek et al. 2010).

In this study, we evaluated the taxonomic value of cypselae for the classification of *Campuloclinium*. We compared the cypselae anatomy between species in the genus and then to other genera and subtribes within Eupatorieae to find features that would support some current classifications.

MATERIALS AND METHODS

The cypselae of 11 *Campuloclinium* species were studied using light microscopy and scanning electron microscopy (SEM). The material was obtained from herbaria of the Northeast Botany Institute (CTES, Argentina), the New York Botanical Garden Herbarium (NY), in NY, United States and the Federal Universities of Brasília (UB), Uberlândia (HUFU), and Bahia (ALCB), in Brazil. Details of the collection locations and the vouchers are available in Table I. For the SEM analysis, some samples were coated with gold and others were dehydrated in acetone and immersed in CO_2 to a critical point before gold coating. After that, all the cypselae were examined and photographed using the scanning electron microscopes JEOL 5800 LV or Zeiss EVO MA 100.

For observation of microcharacters, cypselae were softened in boiling water with a drop detergent, mounted in Hoyer's solution (Anderson 1954, King & Robinson 1970), and then analyzed under a Zeiss Axioplan light microscope.

For the anatomical analysis, fruits were rehydrated with a solution of 5M NaOH for half an hour (Anderson 1963, modified), dehydrated in an ethanol series, and embedded in historesin (Leica Microsystems, Heidelberg, Germany). The samples were sectioned in a rotating microtome using 10µm thickness. The cuts were stained with 0.05% toluidine blue in acetate buffer, pH 4.7 (O'Brien et al. 1964, modified), and mounted with synthetic resin. The slides were analyzed under a light microscope and images from three regions of the cypsela (basal, median, and apical) were acquired under the microscope Olympus BX51.

For the distribution of trichomes, three cypselae of each specimen were analyzed. The term rare was used when the trichomes were present in up to 10% of the surface of the cypsela, the frequent term was used between 11% and 50% and the term abundant when the trichomes were present above 50% of the surface of the cypsela.

Trichome terminology was based on Metcalfe & Chalk (1950, 1979). The description of the pericarp and the trichome distribution were carried out in an ontogenetic way, as proposed by Marzinek & Oliveira (2010).

Table I. **Voucher information for species of *Campuloclinium* used in present study. * samples submitted to critical point drying. ** samples not submitted to critical point drying.**

Species	Voucher	Country and Locality
C. alternifolium Gardner**	J. Calvo & E. Ross-Nadie 7521 (ALCB)	Brazil, Tocantins, Arraias, Fazenda Sapé
C. burchellii (Baker) R.M. King & H. Rob.*	A. Krapovickas 25302 (CTES)	Argentina, Corrientes, Depto. Santo Tomé, Ruta 40,18 km NE de Santo Tomé
C. burchellii (Baker) R.M. King & H. Rob.*	A. Schinini et al. 11.200 (CTES)	Argentina, Corrientes, Depto. Ituzaungó
C. campuloclinioides (Baker) R.M. King & H. Rob.**	H.S. Irwin et al. 22823 (UB)	Brazil, Minas Gerais, Diamantina
C. campuloclinioides (Baker) R.M. King & H. Rob.**	H.S. Irwin et al. 21871 (UB)	Brazil, Minas Gerais, Diamantina
C. chlorolepis (Baker) R.M. King & H. Rob.*	G.E. Farco et al. 49 (CTES)	Brazil, Goiás, Serra Dourada. Reserva Biológica Prof. José Angelo Rizzo
C. chlorolepis (Baker) R.M. King & H. Rob.**	M. Brandão 28483 (HUFU)	Brazil, Minas Gerais, Poços de Caldas, UC do Rio das Antas
C. hirsutum Gardner*	G.E. Farco et al. 69 (CTES)	Brazil, Minas Gerais, Reserva do Clube Caça e Pesca Itororó
C. hirsutum Gardner**	R. Romero et al. 4208 (HUFU)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. hirsutum Gardner**	J.N. Nakajima et al. 2377 (HUFU)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. irwinii R.M. King & H. Rob.*	H.S. Irwin et al. 28326 (NY)	Brazil, Minas Gerais, Serra do Espinhaço
C. irwinii R.M. King & H. Rob.*	W.R. Anderson 8543 (NY)	Brazil, Minas Gerais, Serra do Espinhaço
C. irwinii R.M. King & H. Rob.*	H.S. Irwin et al. 27695 (NY)	Brazil, Minas Gerais, Serra do Espinhaço
C. macrocephalum DC.**	R. Záchia 6052 (HUFU)	Brazil, Rio Grande do Sul, Santana do Livramento
C. megacephalum R.M. King & H. Rob.**	R. Pacheco et al. 952 (HUFU)	Brazil, Goiás, Serra dos Pireneus
C. megacephalum R.M. King & H. Rob.*	J. Paula-Souza et al. 4166 (CTES)	Brazil, Goiás, Serra dos Pireneus
C. megacephalum R.M. King & H. Rob.*	J. Paula-Souza et al. 4417 (CTES)	Brazil, Goiás, Alto Paraíso de Goiás
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	P.L. Roth 1803 (HUFU)	Brazil, Minas Gerais, Belo Horizonte
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.*	J.N. Nakajima et al. 1004 (CTES)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	R. Romero & J.N. Nakajima 645 (UB)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.*	J.N. Nakajima & R. Romero 1732 (CTES)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra

Table I. Continuation.

C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.*	J.N. Nakajima et al. 805 (CTES)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	R. Romero et al. 843 (UB)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	R. Romero & J.N. Nakajima 3385 (HUFU)	Brazil, Minas Gerais, São Roque de Minas
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	J.N. Nakajima et al. 810 (HUFU)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	R. Romero et al. 3850 (HUFU)	Brazil, Minas Gerais, Parque Nacional Serra da Canastra
C. parvulum (Glaz. Ex B.L. Rob.) R.M. King & H. Rob.**	R. Romero & J.N. Nakajima 3341 (HUFU)	Brazil, Minas Gerais, São Roque de Minas
C. purpuracens (Baker) R.M. King & H. Rob.**	A.A. Barbosa et al. 3544 (HUFU)	Brazil, Minas Gerais, Uberlândia
C. purpuracens (Baker) R.M. King & H. Rob.**	A.A.A. Barbosa & C.P. Coelho. 3544 (HUFU)	Brazil, Minas Gerais, Uberlândia
C. purpuracens (Baker) R.M. King & H. Rob.*	H.F. Leitão-Filho 407 (CTES)	Brazil, São Paulo, Estância Santa Elisa
C. purpuracens (Baker) R.M. King & H. Rob.*	S.G. Tressens et al. 2068 (CTES)	Argentina, Corrientes, Depto. San Miguel
C. purpuracens (Baker) R.M. King & H. Rob.*	G. Hatschbach & E. Barbosa 60629 (CTES)	Brazil, Rio Grande do Sul, São Francisco de Paula
C. riedelii (Baker) R.M. King & H. Rob.*	D. Sucre 10574 (CTES)	Brazil. Mato Grosso do Sul, Maracaju
C. riedelii (Baker) R.M. King & H. Rob.*	D. Sucre 10404 (CTES)	Brazil, Mato Grosso do Sul, Campo Grande
C. riedelii (Baker) R.M. King & H. Rob.*	A. Pott 4562 (CTES)	Brazil, Mato Grosso, Corumbá
C. riedelii (Baker) R.M. King & H. Rob.*	G. Hatschbach 25217 (CTES)	Brazil, Mato Grosso, Rio Brilhante
C. riedelii (Baker) R.M. King & H. Rob.*	D. Sucre 10574 (CTES)	Brazil, Mato Grosso do Sul, Maracaju

RESULTS

External morphology

All cypselae studied are stipitate and prismatic, with evident ribs and trichome throughout the pericarp (Fig. 1a-k, Table II). Biseriate tector trichomes are restricted to the ribs and are composed of four cells (Table II). In the apical and basal portion of cypsela of *C. campuloclinioides* (Fig. 2b) and *C. hirsutum* is found biseriate tector trichome with six cells (Table II). Glandular trichomes were observed in the interrib region, with four to six basal cells and a biseriate head with eight cells (Fig. 2c-d, Table II). In Table II, it is also possible to observe the distribution pattern of the trichomes which have been categorized as rare, frequent and abundant in all species.

In the basal region is located the carpopodium, which presented annular shape in all species (Fig. 2e-h). The apical region has a uniseriate pappus with bristles connate on the base (Fig. 2i-l).

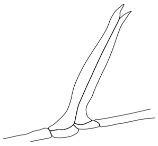
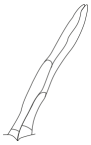
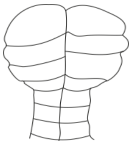
Figure 1. SEM of cypselae of *Campuloclinium* in the general view (a-k). a) *C. alternifolium*. b) *C. burchellii*. c) *C. campuloclinioides*. d) *C. chlorolepis*. e) *C. hirsutum*. f) *C. irwinii*. g) *C. macrocephalum*. h) *C. megacephalum*. i) *C. parvulum*. j) *C. purpuracens*. k) *C. riedelii*. ap: apex; ca: carpopodium; gt: glandular trichome; pa: pappus; tt: tector trichome; double arrowhead: ribs.

Anatomy

Almost all species presented five evident ribs (Fig. 3a). However, in some specimens of *C. chlorolepis,* (Fig. 3b) the cypsela has six ribs and in specimens *C. parvulum,* the cypsela can present eight ribs (Fig. 3c).

The exocarp is uniseriate with juxtaposed ordinary cells (Fig. 3d-f). The outer mesocarp is compound by the outer parenchymatic region; more internally by a schizogenous space filled with phytomelanin (Fig. 3d-f). The inner mesocarp is formed by a sclereid layer and a variable number of parenchymatic cells (Fig. 3d-f). This parenchymatic layer is found with five or six layers in *C. parvulum*; two to five in *C. burchellii*; one in *C. alternifolium*, *C. campuloclinioides*, *C.*

Table II. Types and distribution of trichomes on the cypselae of the *Campuloclinium* (A: apex, M: middle region, B: base; - absent, +: rare, ++: frequent, +++: abundant).

Trichome										
		tector, biseriate - 4 cells			**tector biseriate - 6 cells**			**glandular, biseriate**		
Species	**Distribution**	**A**	**M**	**B**	**A**	**M**	**B**	**A**	**M**	**B**
C. alternifolium	rib	+++	+++	+++	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	+	-	+
C. burchellii	rib	-	-	-	-	-	-	++	++	++
	interrib	++	++	++	++	-	++	-	-	-
C. campuloclinioides	rib	+++	+++	+++	++	-	++	-	-	-
	interrib	-	-	-	-	-	-	+	+	+
C. chlorolepis	rib	++	++	++	-	-	-	-	-	-
	interrib	++	++	++	-	-	-	+++	++	++
C. hirsutum	rib	++	++	++	++	-	++	-	-	-
	interrib	-	-	-	++	-	++	++	++	++
C. irwinii	rib	++	++	++	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	++	++	++
C. macrocephalum	rib	++	++	++	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	+++	+++	+++
C. megacephalum	rib	++	++	++	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	-	-	-
C. parvulum	rib	++	++	++	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	++	++	++
C. purpuracens	rib	+	+	+	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	++	+	+
C. riedelii	rib	-	-	-	-	-	-	-	-	-
	interrib	-	-	-	-	-	-	++	++	++

irwinnii (Fig. 3d), *C. macrocephalum* (Fig. 3e), *C. purpuracens* (Fig. 3f), and absent in *C. chlorolepis*, *C. hirsutum*, *C. megacephalum,* and *C. riedelii* consumed during seed development. In the rib region, we observed a collateral vascular bundle involved by fibers (Fig. 3g-i). The distribution of phytomelanin is internal to the bundles (Fig. 3g-i). In all species, the endocarp is also consumed by seed development.

The carpopodium has a uniseriate exocarp and lignified, with cells slightly elongated in the periclinal sense (Fig. 3j); in *C. purpuracens* and *C. hirsutum* are observed glandular trichome. The mesocarp presents isodiametric to elongated cells, which can be parenchymatic or lignified

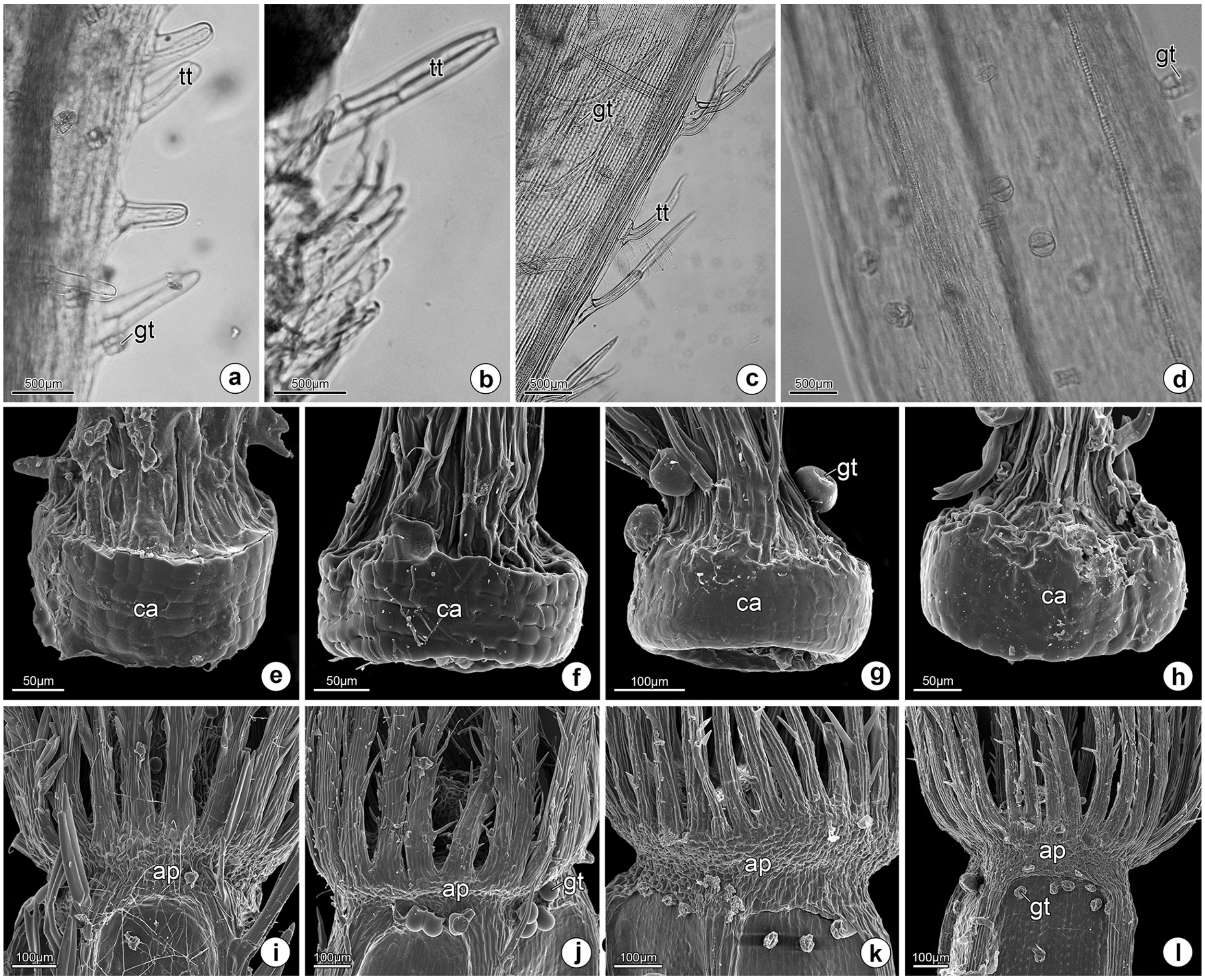


Figure 2. Microcharacters (a-d) and SEM (e-l) of *Campuloclinium* in detail. a-d. Detail of the trichomes. a-b. *C. campuloclinioides*. a) Note the biseriate trichomes is 4-celled. b) Note the biseriate tector trichome is 6-celled. c) Middle region of *C. hirsutum*, with 4-celled biseriate trichomes composed and glandular trichomes with 4–6 basal cells and 8-celled biseriate head. d) *C. riedelli* note glandular trichomes with 4–6 basal cells and 8-celled biseriate head. e-h) Annular carpopodium. e) *C. burchelli*. f) *C. campuloclinioides*. g) *C. chlorolepis*. h) *C. hirsutum*. i-l) Apex of the cypsela. Note the fused base of the pappus. i) *C. campuloclinoides*, j) *C. irwinii*, k) *C. purpurascens*, l) *C. riedelli*. ap: apex; ca: carpopodium; gt: glandular trichome; tt: tector trichome.

(Fig. 3j). In the central region is observed vascular bundles (Fig. 3j).

The pappus presents one series of lignified bristles connate in the base in all species (Fig. 3k).

In the apical region of fruit, the exocarp presents cells with a primary wall, flattened in the periclinal sense (Fig. 3l). More internally lignified cells are found elongated in the periclinal sense (Fig. 3l).

DISCUSSION

External morphology

As King & Robinson (1987) mentioned, all these cypsela features are important to delimitate this genus once these traits and the receptacle shape are diagnostic to *Campuloclinium*. Our study is in agreement with these authors and, again, the detailed study of cypselae corroborated the

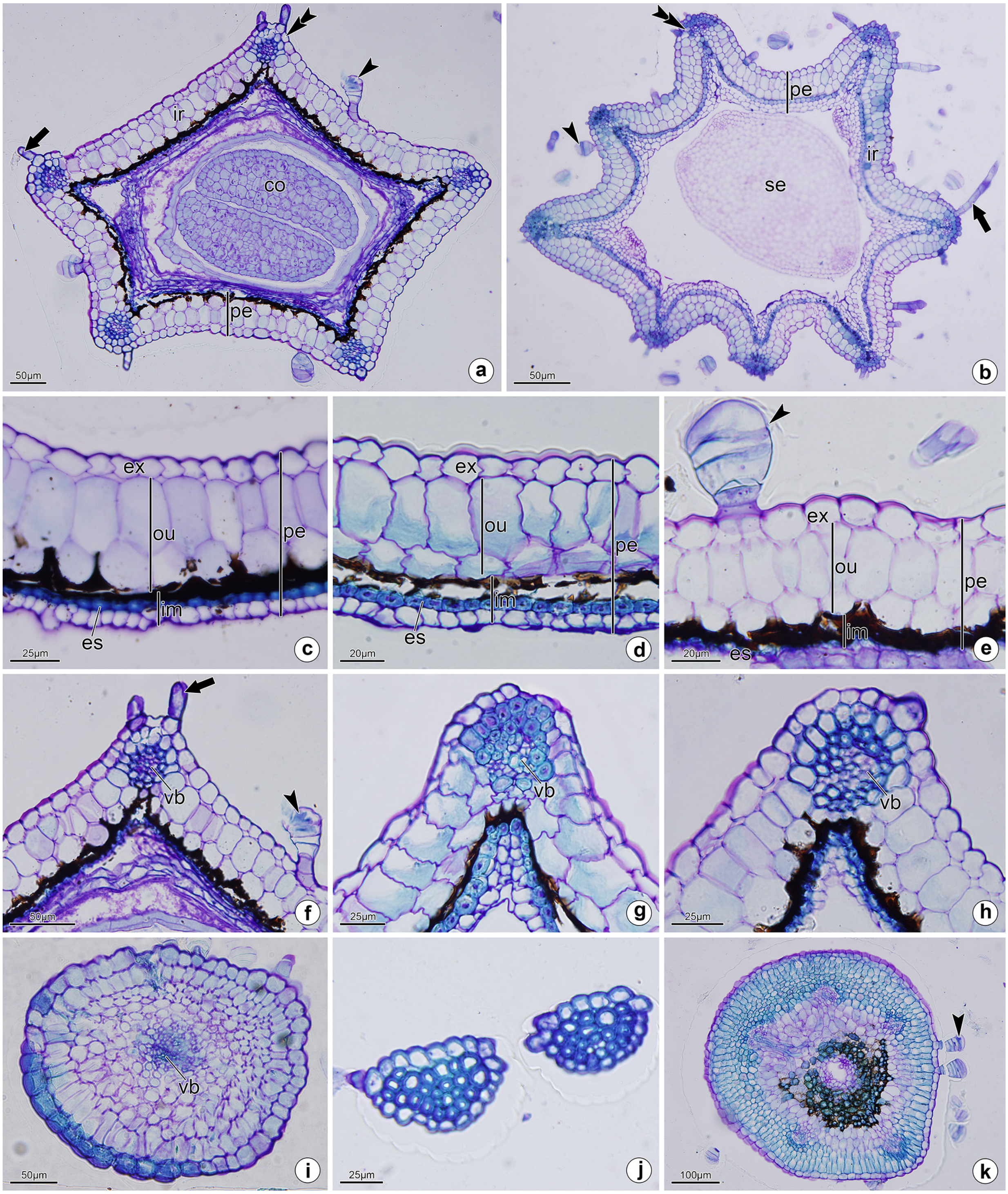


Figure 3. Transversal sections of the *Campuloclinium* cypsela. a–b) General view. a) *C. irwinnii* with five ribs. b) *C. chlorolepis* with six ribs. c) *C. parvulum* with eight ribs. d-f) Pericarp detail. d) *C. irwinnii*. e) *C. macrocephalum*. f) *C. purpuracens*. g-i) Detail of the ribs. g) *C. irwinnii*. h) *C. macrocephalum*. i) *C. purpuracens*. Note the phytomelanin is a continuous layer internal to the vascular bundle. j) Carpopodium of *C. burchelli*. k) Lignified pappus of *C. irwinnii*. l) Apex in *C. purpuracens*. co: cotyledon; ex: exocarp; gt: glandular trichome; im: inner mesocarp; ir: interrib; ou: outer mesocarp; pe: pericarp; sc: sclereids; se: seed; tt: tector trichome; vb: vascular bundle; double arrowhead: ribs.

importance of this reproductive organ for the classification of Asteraceae.

The presence of stipitate cypsela is important for generic delimitation in the two current possible phylogenetic relationships of this genus, one proposed by Robinson et al. (2009) and the second by Rivera et al. (2016a). In the first classification, *Campuloclinium* is closely related to *Trichogonia* (DC.) Gardner, with both genera sharing the presence of stipitate cypsela (Robinson et al. 2009, Roque et al. 2012). On the other hand, in the studies performed by Rivera et al. (2016a), *Campuloclinium* appears phylogenetically related to *Macropodina* R.M.King & H.Rob., which most species presents stipitate cypsela (Nakajima et al. 2017). In Eupatorieae, this cypsela type is found in some genera and species of the different subtribes (Grossi et al. 2020), but it is commonly found in genera in the Gyptidinae subtribe (Funk et al. 2009, Robinson et al. 2009) and some others Eupatorieae genera, as *Trichogoniopis* R.M.King & H.Rob., *Platypodanthera* R.M.King & H.Rob., *Trichogonia*, *Campuloclinium* and *Macropodina* (Hind & Robinson 2007, Ferreira 2010, Nakajima et al. 2017, Grossi et al. 2020). Based on molecular data, Rivera et al. (2016b) describe a new subtribe, named Trichogoniinae, including the genera *Platypodanthera*, *Trichogonia*, and *Trichogoniopsis*. According to Rivera et al. (2016b), in addition to the molecular data, the presence of a subplumose to plumose pappus and stipitate cypsela supports the classification of these genera into this new subtribe. In both analyses by Rivera et al. (2016a, b), a clade formed by *Campuloclinium*, *Macropodina*, and other genera in the Ayapaninae subtribe emerge as a sister group of a clade that contains Trichogoniinae and others subtribes. So, given these new phylogenetic analyses, is relevant to emphasize the importance of stipitate cypsela in the classification of *Campuloclinium* and possible related groups. Nevertheless, further research involving phylogenetic and ontogenetic studies is still necessary for understanding the evolution of the stipitate cypsela within these groups, once the delimitation of *Campuloclinium* within Eupatorieae is yet unclear.

In the cypsela, the biseriate tector trichome (twin hairs) are common in most Asteraceae genera (Hess 1938, Robinson 2009), but glandular trichomes can also be found (Marzinek & Oliveira 2010, Angulo et al. 2015, Via do Pico et al. 2016, Silva et al. 2018, Marques et al. 2018a, b, 2020, Grossi et al. 2020). The presence or absence of tector and glandular trichomes has successfully been used for the delimitation of genera and species in several groups within Asteraceae, including Eupatorieae (Marzinek & Oliveira 2010, Angulo et al. 2015, Freitas et al. 2015, Via do Pico et al. 2016, Silva et al. 2018, Marques et al. 2018a, b, 2020, Grossi et al. 2020). In *Campuloclinium*, all species present tector trichomes in ribs and glandular trichomes in interribs. Marzinek & Oliveira (2010) had already reported the trichome distribution pattern in *Campuloclinium macrocephalum*. This distribution pattern in *Campuloclinium* cypsela in conjunction with other characteristics can assist in genera identification at a specific level, type of trichomes and its distribution is very important to identification of species. The tector trichome with six cells is unique to *C. campuloclinoides* and *C. hirsutum*, and it can be used to differentiate both species within the genus. Distribution and trichome type are very important in *Campuloclinium* to species delimitation. The tector trichome with six cells is unique to *C. burchelli*, *C. campuloclinoides* and *C. hirsutum*, and this type of trichome could be a synapomorphy for these last two species taking into account the phylogeny performed by Rivera et al. (2016a). However, *C. hirsutum* is unique species with six cells tector trichome presents in the interrib region. *Campuloclinium riedelii* is a unique species that has cypsela with only glandular trichomes, while *C. macrocephalum* has only tector trichome, such characteristics differentiate both species from the others of the genus. Finally, *C. hirsutum* and *C. purpuracens* are the only species of the genus that present glandular trichome in the carpopodium.

The carpopodium is an abscission zone between the cypsela and the receptacle (Roque et al. 2009). According to Haque & Godward (1984), this region can delimitate genera in Asteraceae. The carpopodium symmetry is of great taxonomic value to some tribes (Freitas et al. 2015, Silva et al. 2018, Grossi et al. 2020). In Eupatorieae, studies performed by Silva et al. (2018) demonstrated that all species within of Disynaphiinae have a symmetric carpopodium, except *Disynaphia praeficta* (B.L.Rob.) R.M.King & H.Rob. In these studies, the assymetric carpopodium and other cypsela characteristic found in *D. praeficta* supported the exclusion this species from the subtribe, as well as had been demonstrated by molecular data (Silva et al. 2018). The carpopodium in Eupatorieae, in general, is conspicuous, but in some species this structure can be undistinguishable, as presented in recent studies performed by Silva et al. (2018) and Grossi et al. (2020). However, in *Campuloclinium*, the carpopodium is present and conspicuous in all species, therefore it is a structure with no taxonomic value for species delimitation.

Anatomy

Most of the analyzed species of *Campuloclinium* presented five ribs. This result agrees with those of Grossi et al. (2020), who reported that having five ribs is a feature present in most Eupatorieae. However, according to Marzinek et al. (2010), species with broader capitula had cypselae with a more uniform number of ribs while species of narrower capitula caused greater pressure between the cypselae, changing their shape. In our studies, we corroborate the hypothesis of Marzinek et al. (2010), because the cypselae of *C. chlorolepis* and *C. parvulum* as previously reported by Calvo & Roque (2018) they are narrower, and in our results these fruits have a variable number of ribs.

Campuloclinium species present the phytomelanin layer positioned internally to the vascular bundles, the same position found in most Eupatorieae subtribes (Marzinek & Oliveira 2010, Franca et al. 2015, T.D.G. Silva, unpublished data). This position of phytomelanin is also found in *Ageratum conyzoides* L., *Ageratum fastigiatum* R.M.King & H.Rob. (Ageratinae subtribe) (Franca et al. 2015), *Mikania micrantha* H.B.K (Mikaniinae subtribe) (Marzinek & Oliveira 2010), *Vittetia orbiculata* (DC.) R.M.King & H.Rob. (Gyptidinae subtribe), and in the Praxelinae subtribe (Marzinek & Oliveira 2010, T.D.G. Silva, unpublished data). However, in Disynaphiinae, the phytomelanin layer is positioned externally to the vascular bundles (Silva et al. 2018). Until now, all anatomical studies with Eupatorieae cypsela (Marzinek & Oliveira 2010, Franca et al. 2015, T.D.G. Silva, unpublished data, Silva et al. 2018, present work), has been corroborated that the phytomelanin layer observed externally to the vascular bundles is a synapomorphy to Disynaphiinae, as already mentioned by Silva et al. (2018).

The position of *Campuloclinium* remains uncertain within Eupatorieae. King & Robinson (1987) placed *Campuloclinium* in Gyptidinae, and Robinson et al. (2009) later elucidated the relations between *Campuloclinium* and other genera. According to Robinson et al. (2009), *Campuloclinium* closely relates to *Trichogonia* (DC.) Gardner (Gyptidinae) and *Acritopappus* R.M.King & H.Rob. (Ageratinae subtribe). On the other hand, Rivera et al. (2016a) in their studies they expand the sampling of Brazilian species of Eupatorieae suggest that *Campuloclinium* is closely related to *Macropodina*, *Heterocondylus* R.M.King & H.Rob., *Alomiella* R.M.King & H.Rob., *Monogereion* G.M.Barroso & R.M.King, and *Ayapanopsis* R.M.King & H.Rob. Indeed, the classification of several Eupatorieae subtribes is uncertain, with eight of them not monophyletic, including Gyptidinae and its genera (Rivera et al. 2016a). The uniformity of the pericarp structure (exocarp, outer mesocarp, middle mesocarp, inner mesocarp, and position of phytomelanin in respect to vascular bundles) of *Campuloclinium* and other genera within Eupatorieae demonstrated that several subtribes have the same pericarp pattern. Therefore, there is a need for further evolutionary studies to understand the infratribal classification of Eupatorieae. As Grossi et al. (2020) pointed out that Eupatorieae has several tribes and genera whose circumscriptions need reevaluation. In Vernonieae, studies on anatomical cypsela performed by Marques et al. (2018a, 2020) display that the genera *Chrysolaena* H.Rob, *Echinocoryne* H.Rob, *Lepidaploa* (Cass.) Cass and *Lessingianthus* H.Rob. also have similar pericarps. In the same way, as raised for Eupatorieae by Grossi et al. (2020), Marques et al. (2018a, 2020) reinforce the importance of more morphological and phylogenetic studies, as the current circumscription of these genera should be revised (Marques et al. 2020).

A lignified pappus has been found in Eupatorieae (Marzinek & Oliveira 2010, Silva et al. 2018), Vernonieae (Marques et al. 2018a, 2020), Tageteae, and Millerieae (Frangiote-Pallone & Souza 2014). In *Campuloclinium*, all species present lignified pappus and this feature is not relevant to its taxonomy. In general, the cypsela apex varies little in Asteraceae (Pandey & Singh 1980, Galastri & Oliveira 2010, Marzinek & Oliveira 2010, Silva et al. 2018, Marques et al. 2020), with no difference found between the apex of *Campuloclinium* species.

Until now, the carpopodium anatomy has shown little variation within Asteraceae (Marques et al. 2020). In some cases, as reported by Silva et al. (2018) and Marques et al. (2018a, 2020), the carpopodium exocarp lignification can be useful for species separation, but in *Campuloclinium* this character does not present taxonomic value because it occurs in all species.

CONCLUSION

The studies presented here have shown that the morphology and anatomy of cypselae can bring new perspectives to the cypselae evolution in Eupatorieae, as well as to the subtribal and generic classifications of this tribe. The morphological studies presented here corroborate the importance of the stipitate form of the cypsela together with other diagnostic characters (such as, hemispherical or conical, scrobiculate and glabrous receptacle) to delimit *Campuloclinium* within Eupatorieae, that is, stipitate cypsela, such as this feature could have evolved independently within the Trichogoniinae and *Campuloclinium* + *Macropodina*. However, phylogenetic studies with a larger sampling of *Campuloclinium* species must be carried out to understand their relation to these other clades. A new possible diagnostic trait presented to *Campuloclinium* is the trichome distribution in the cypsela, which is characterized by tector trichomes in the ribs and glandular trichomes in the interribs. The external position of the phytomelanin layer regarding the vascular bundles is also important for understanding the subtribal classification in Eupatorieae. So far, all studied subtribes have the phytomelanin layer internal to the vascular bundles, except for Disynaphiinae. The position of this layer in *Campuloclinium*, which is a group close to

Disynaphiinae. Finally, the finding of six-celled biseriate trichomes only in *C. campuloclinoides* and *C. hirsutum* reinforces the importance of the cypselae features at a specific level.

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