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## Systematic consideration of floral microcharacters of the South American genus *Chrysolaena* (Vernonieae, Asteraceae)

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Floral microcharacters of the genus *Chrysolaena* H. Rob. (Vernonieae, Asteraceae) were analysed in detail for the first time in order to evaluate the taxonomic position of conflictive species in the group. The results were also compared with studies carried out in species of related genera. In addition to distinctive microcharacters previously studied in some species of the genus, other characters such as trichome types of the corolla, style, anthers and cypselae have been analysed for the first time. The presence of glandular apical appendage and cypselae are common characteristics among species *Chrysolaena*. In addition to these, this study shows that presence of glands on the style and corollas is another consistent characteristic in the genus. However, the absence of basal stylar node would not be a diagnostic character since this varies widely among species. The results indicate that most of the microcharacters of *Chrysolaena* analysed are quite consistent in the genus, but they are no more consistent than the pollen morphology (type 'C') and chromosome base number ( $x = 10$ ). Until now, these last two features would be most useful for separating *Chrysolaena* from the related genera *Lessingianthus* and *Lepidaploa*. At species level, the results show that related species can be distinguished by the different combinations of floral microcharacters. The value of microcharacters could be increased if they are combined with other morphological, cytological, and palynological data.

**Keywords:** anthers, crystals, cypselae, idioblasts, Lepidaploinae, style, taxonomy, trichomes

### Introduction

The Asteraceae comprise between 1600–2000 genera and 24,000–30,000 species divided into 13 subfamilies and 43 tribes (Panero et al., 2014). The family has presented continuing taxonomic problems as a result of its size and its recurring combinations of superficial characters (Robinson, 1999). The tribe Vernonieae is a clear example of one of the most complex and less elucidated groups. It is one of the largest within the Asteraceae with approximately 1500–1600 species distributed in America, Asia, and Africa (Keeley, Forsman, & Chan, 2007; Keeley & Robinson, 2009). Good taxonomic understanding of Vernonieae has been difficult to achieve, especially among certain New World taxa (Ekman, 1914; Gleason, 1906; Jones, 1979, 1981; Keeley & Jones, 1977). The source of much of this difficulty lies with the extensive variability inherent in the group, the occurrence of gradual morphologies and overlapping characters with numerous combinations of autoapomorphic states (Keeley & Jones, 1977; Keeley & Turner, 1990; Robinson, 1999).

With the emergence of 'The new synantherology' (King & Robinson, 1970), dozens of new genera have been split out of several traditional genera of Asteraceae on the basis of micromorphological characters. In subsequent years, several authors have used a cluster of micromorphological characters in taxonomic studies in large groups as Senecioneae, Astereae, Vernonieae, Eupatorieae, Inuleae (Dematteis, 2009; Faust & Jones, 1973; Isawumi, 1999; Isawumi, El-Ghazaly, & Nordenstam, 1996; Liu & Yang, 2011; Narayana, 1979; Pruski, 2012; Riva, Pozner, & Freire, 2009; Robinson, 1988a, 1988b, 1992, 1999). Some uses of microcharacters are, for example, species identification from small fragments of the plant, or the relocation or change of taxonomic position of taxa (Robinson, 2009).

The taxonomic history of the tribe Vernonieae has changed over time and the huge or core genus *Vernonia* Less. was suffering divisions, fragmentations, and rearrangements. Based on morphological, phytochemical, cytological, palynological, and molecular studies a total of 126 genera distributed in 21 subtribes, 15 in the New World, and six in the Old World are recognized for the tribe (Keeley & Robinson, 2009). Most of the emerged and resurrected new genera were established based on

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micromorphological characters. In taxonomic studies on American Vernoniae, Robinson (1999) attached great importance to the value of floral microcharacters for subtribal and generic delimitations (e.g., the style base, the anther appendages, the carpodium shape, and the shape of cypselae wall crystals). Some of these characters were used as major distinguishing features in the segregation of genera such as *Chrysolaena* H. Rob., *Lessingianthus* H. Rob., *Vernonanthura* H. Rob., and *Lepidaploa* H. Rob., previously members of the core genus *Vernonia* (Robinson, 1988a, 1988b, 1990, 1999). Other characters taken into account were the morphology of the pollen grains, chromosome number, chemical composition, and inflorescence shape (Dematteis, 2007, 2009; Robinson, 2009).

*Chrysolaena* belongs to the subtribe Lepidaploinae S. C. Keeley & H. Rob. and comprises 18 species geographically concentrated in the central region of South America, with the main center of diversity in southern Brazil and northeast of Argentina (Dematteis, 2009; Robinson, 1988b). Most species have a wide geographic distribution, although there are some endemic entities. The species of genus are perennial herbs or erect shrubs with well developed xylopodia. They are characterized by sericeous or velutine indumentum, mainly in stems, leaves and phyllaries, style without basal node, and glandular anthers and cypselae (Robinson, 1988b). However these features are only known for seven species: *C. flexuosa* (Sims) H. Rob., *C. lithospermifolia* (Hieron.) H. Rob., *C. obovata* (Less.) Dematt., *C. oligophylla* (Vell.) H. Rob., *C. platensis* (Spreng.) H. Rob., *C. propinqua* (Hieron.) H. Rob., and *C. verbascifolia* (Less.) H. Rob. (Robinson, 1988b). Other distinctive characteristics of the genus are the presence of pollen grain type 'C' (tricolporate, echinolophate with 29 lacunae) and the base chromosome number  $x = 10$  (Dematteis, 2009; Oliveira, Forni-Martins, & Semir, 2007a, 2007b; Oliveira, Semir, & Forni-Martins, 2012; Via do Pico & Dematteis, 2012, 2013a, 2013b, 2014).

*Chrysolaena* is closely related to *Lessingianthus* and *Lepidaploa*, with which it shares some characteristics. With *Lepidaploa*, for example, it shares the presence of glands in anthers and cypselae, elongated crystals of cypselae wall, and pollen grains type 'C'. However, the last feature is not exclusive to *Lepidaploa*, since pollen type 'D' and 'G' can also occur. By contrast, with *Lessingianthus* it shares the absence of basal style node (Dematteis, 2007, 2009; Robinson, 1990). Recent molecular studies suggest that *Chrysolaena* is more closely related to *Lepidaploa*, with *Lessingianthus* as sister group (Keeley et al., 2007).

Based on this background, the aims of this study are to: (1) characterize floral microcharacters of the genus *Chrysolaena*; (2) evaluate the taxonomic position of the

conflictive species of the genus; and (3) determine the systematic value of these microcharacters in specific segregation of the taxa.

## Materials and methods

The specimens for this study were obtained from living material and the herbarium of Instituto de Botánica del Nordeste (CTES). A complete voucher list is given in the Appendix.

For the micromorphological observations with a light microscope (LM), florets and fruits were removed from herbarium specimens. Samples were rehydrated in warm soapy water and dissected under a stereoscopic microscope. They were then mounted in Hoyer's solution (Anderson, 1954; King & Robinson, 1970). Observations were done with a LM equipped with a digital photographic camera (Canon Power Shot A 640). Trichome types were drawn with a camera lucida microscope.

Observations with a scanning electron microscope (SEM) were performed on herbarium and living material. Herbarium samples were rehydrated with warm soapy water and fixed with FAA. Disc florets were dehydrated in a graded series of acetone and critical point-dried with liquid CO<sub>2</sub>. Dried samples were partially dissected, mounted on stubs, coated with gold/palladium and observed with SEM (JEOL 5800 LV).

For the description of microcharacters, terminology proposed by Metcalfe and Chalk (1979), Narayana (1979), Ramayya (1962), and Robinson (1988a, 1988b, 1990, 1999) was used.

## Statistical analyses

A data matrix with 59 OTUs  $\times$  13 characters was made. The size, the presence of idioblasts and crystals on the fruit were not considered because these showed great intraspecific variation. Also taxa with incomplete information were not considered. The characters were coded in binary (Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at <http://dx.doi.org/10.1080/14772000.2015.1134699>). The homogeneous characters among all species were not considered as variables, and nor were the presence of idioblasts and calcium oxalate crystals, since they showed high intraspecific variation. A cluster analysis UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and a principal coordinate analysis (PCoA) using Dice distance index (Nei & Li, 1979) were performed. The program InfoStat 2012 version was used for both analyses (Di Rienzo et al., 2012).

## Results

Micromorphological data of all the *Chrysolaena* species are presented in Tables 1 and 2.

### Trichomes

Glandular and non-glandular trichomes on the anther appendages, the corolla lobes, the cypselae, and the

corolla tube (in a few species) were observed. We found four types of trichomes based on the glandular or non-glandular nature and cellular structure, as follows.

**Type I: simple filiform trichome** (Figs 2, 17, 20, 40): non-glandular, unicellular formed by a single elongated cylindrical cell of acute apex; or multicellular with a foot formed by 1 or 2 basal cells and a cylindrical elongated terminal cell of acute apex. The side walls are thin or

**Table 1.** Qualitative and quantitative data of the floral microcharacters of *Chrysolaena*. The “x” indicates the presence of character. The dash indicates missing or immature material.

Species and voucher herbarium	Trichomes type of corolla					Anthers					Style	
	I	II	III A	IIIB	IV	Apical appendage		Trichome types		Glandular trichomes (Type IIIA)	Basal node	
						Shape	Size (mm)	III A	I		Shape	Developed
<i>C. campestris</i> 8707	x	x	x			ovate	0.6–0.8	x	x	sagittate	x	x
<i>C. candelabrum</i> 45761	x		x			lanceolate	0.7–0.8	x		sagittate	x	x
<i>C. cognata</i> 46948	x		x			ovate	0.6–0.8	x		cleft	x	x
<i>C. cognata</i> 3399	x		x			ovate	0.5–0.7	x		cleft	x	
<i>C. cognata</i> 3053			x			ovate	0.5–0.6	x		cleft	x	
<i>C. cognata</i> 6			x			ovate	0.6–0.8	x		sagittate	x	
<i>C. cognata</i> 3040	x		x			ovate	0.5–0.6	x		sagittate	x	
<i>C. cordifolia</i> 24905	x		x			lanceolate	1.0–1.1	x	x	sagittate	x	
<i>C. cordifolia</i> 3975			x			lanceolate	0.9–1.0	x		sagittate	x	
<i>C. cristobaliana</i> 882			x			ovate-lanceolate	0.4–0.5	x		sagittate	x	x
<i>C. cristobaliana</i> 34544			x			ovate-lanceolate	0.4–0.6	x		sagittate	x	x
<i>C. cristobaliana</i> 41			x			ovate-lanceolate	0.5–0.6	x		sagittate	x	x
<i>C. desertorum</i> 23783	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. desertorum</i> 5840			x			ovate	0.4–0.5	x		cleft	x	
<i>C. desertorum</i> 35640	x		x			ovate	0.5–0.6	x		sagittate	x	
<i>C. desertorum</i> 24369		x				ovate	0.3–0.6	x		sagittate	x	x
<i>C. dusenii</i> 4688	x		x			ovate	0.7–1.0	x		sagittate	x	x
<i>C. dusenii</i> 2093			x			lanceolate	0.8–1.0	x		sagittate	x	x
<i>C. dusenii</i> 1286			x			lanceolate	0.7–0.9	x		sagittate	x	x
<i>C. dusenii</i> 12012			x			lanceolate	0.7–1.0	x		sagittate	x	x
<i>C. flexuosa</i> 45			x			ovate-lanceolate	0.7–0.8	x	x	cleft	x	
<i>C. flexuosa</i> 5			x			ovate	0.5–0.7	x		sagittate	x	
<i>C. flexuosa</i> 79091	x		x			ovate	0.6–0.7	x		cleft	x	
<i>C. flexuosa</i> 44199			x			ovate	0.6–0.7	x		sagittate	x	x
<i>C. guaranitica</i> 12524	x					orbicular	0.5–0.7			sagittate		
<i>C. lithospermifolia</i> 9			x			ovate	0.5–0.6	x		cleft	x	
<i>C. lithospermifolia</i> 15			x			ovate	0.5–0.6	x		cleft	x	x
<i>C. lithospermifolia</i> 42055			x			ovate-lanceolate	0.5–0.6	x		cleft	x	
<i>C. lithospermifolia</i> 5884			x			ovate	0.7–0.8	x		sagittate	x	
<i>C. nicolackii</i> 5533			x	x		lanceolate	0.8–0.9	x		sagittate	x	x
<i>C. nicolackii</i> 12023			x	x		ovate-lanceolate	0.4–0.7	x		cleft	x	x
<i>C. nicolackii</i> 6214			x	x		lanceolate	0.9–1.1	x		sagittate	x	
<i>C. nicolackii</i> 682			x	x		ovate-lanceolate	0.8–0.9	x		sagittate	x	x
<i>C. obovata</i> 3475	x	x	x			ovate-lanceolate	0.4–0.6			sagittate		x
<i>C. obovata</i> 1736			x			lanceolate	0.5–0.6	x		sagittate	x	x

(continued)

Table 1. (Continued)

Species and voucher herbarium	Trichomes type of corolla					Anthers					Style		
	I	II	III A	III B	IV	Apical appendage		Trichome types			Glandular trichomes (Type III A)	Basal node	
						Shape	Size (mm)	III A	I	Shape		Developed	Ring of cells
<i>C. obovata</i> 36				x		lanceolate	0.4–0.6			sagittate	x	x	
<i>C. obovata</i> 17361				x		lanceolate	0.4–0.5	x		sagittate	x	x	
<i>C. oligophylla</i> 270				x		ovate	0.8–0.9	x		sagittate	x		
<i>C. oligophylla</i> 1729				x		ovate	0.5–0.7	x		sagittate	x		
<i>C. oligophylla</i> 5455				x		ovate	0.4–0.6	x		sagittate	x		
<i>C. oligophylla</i> 2953	x	x		x		ovate	0.5–0.7	x		sagittate	x		x
<i>C. platensis</i> 3083				x		lanceolate	0.6–1.0	x		sagittate	x		
<i>C. platensis</i> 5123				x		lanceolate	1.0–1.1	x		sagittate	x	x	
<i>C. platensis</i> 609				x		lanceolate	0.7–0.8	x		sagittate	x		
<i>C. platensis</i> 1478		x		x		lanceolate	0.7–0.8	x		sagittate	x		
<i>C. propinqua</i> 913	x		x			ovate	0.6–0.8	x		cleft	x		
<i>C. propinqua</i> 3038	x		x			ovate	0.6–0.7	x		sagittate	x		x
<i>C. propinqua</i> 20	x		x			ovate	0.4–0.6	x		sagittate	x		x
<i>C. propinqua</i> 25	x		x			ovate	0.3–0.5	x		sagittate	x		
<i>C. propinqua</i> 35815	x		x			ovate	0.3–0.4	x		sagittate	x		x
<i>C. propinqua</i> D y O	x		x			ovate	0.5–0.6	x		sagittate	x		x
<i>C. sceptrum</i> 76914	x		x			ovate	0.7–0.8	x		sagittate	x		
<i>C. sceptrum</i> 30442	x		x			ovate	0.6–0.7	x		sagittate	x		
<i>C. sceptrum</i> 3307	x		x			ovate	0.5–0.7	x		sagittate	x		
<i>C. simplex</i> 547	x				x	orbicular	0.6–0.7			sagittate			
<i>C. simplex</i> 5704	x				x	orbicular	0.5–0.7			sagittate			
<i>C. simplex</i> 69630	x	x			x	orbicular	0.7–0.8			sagittate			
<i>C. simplex</i> 23722	x				x	orbicular	0.6–0.7			sagittate			
<i>C. simplex</i> 6057	x	x			x	orbicular	0.6–0.7			sagittate			
<i>C. verbascifolia</i> 12031				x		ovate-lanceolate	0.5–0.6	x		sagittate	x		
<i>C. verbascifolia</i> 34	x		x		x	lanceolate	0.7–0.9	x		sagittate	x		x

slightly thickened. Trichome type II differs from papillae in that the latter are short and rounded emergences of the membrane of epidermal cells.

**Type II: twin hair** (Figs 13, 18, 21, 41): non-glandular, multicellular, biseriate, formed by four cells, two basal cells and two 'hair cells', cylindrical, sharp ends and thick walls; arranged parallel to each other. The latter may have the same length as each other, or one may be longer; they may be completely joined together or separate at the ends or at the base. Trichomes type II of the anther appendages and the corollas usually have small variations of the typical morphology of twins hairs present on fruits. They may have from 3 to 8 cells arranged in two rows, with the two terminal cells cylindrical and with sharp ends. These atypical morphologies were observed in few specimens.

**Type III: short-stalked capitate glands: Type III A** (Figs 1, 10, 19, 29, 30, 42): short trichomes; narrow foot formed by 1 or 2 cells. The biseriate body has 2–4 cells

of thin walls in each row. It has a cuticular vesicle in the terminal apex that in frontal view usually presents bilobed appearance. This may persist or collapse. **Type III B** (Figs 3, 11, 43): simple or compound foot, biseriate body formed by 4–7 cells on each row, differentiated in stalk and head. The basal cells of the rows are generally longer and of different size; towards the apex are of smaller size and homogeneous with each other. The cell walls are thin. The cuticular vesicle can be persistent or collapse.

**Type IV: long-stalked glandular trichome** (Figs 12, 23, 44): foot formed by 1 or more cells. The body is biseriate, elongated and differentiated into stalk and head. The stalk is formed by 3–4 long cells in each row, more or less uniform in width. Cells of 2 rows are arranged alternately and can be of different sizes. The head is formed by 1–2 cells on each row, sharply demarcated with the stalk, or continuous. The cells have thin walls or slightly thickened in certain parts. In the early stages of development are seen as glandular biseriate trichomes.

**Table 2.** Qualitative and quantitative data of cypselae of *Chrysolaena*. The “x” indicates the presence of character.

Species and voucher	Shape	Size (mm)	Trichomes		Crystals			
			type IIIA	Idioblasts	Cubic	Estiloid	Prismatic	Druses
<i>C. campestris</i> 8708	turbinate	1.7–1.9	x		x	x	x	
<i>C. candelabrum</i> 45761	turbinate	1.6–1.7	x		x	x	x	x
<i>C. cordifolia</i> 24905	turbinate	1.3–1.5	x			x	x	
<i>C. cordifolia</i> 3975	turbinate	1.5–1.7	x				x	
<i>C. cognata</i> 46948	cylindrical-turbinate	2.9–3.2	x	x			x	
<i>C. cognata</i> 3399	cylindrical-turbinate	1.9–2.0	x		x	x	x	x
<i>C. cognata</i> 3053	cylindrical-turbinate	2.5–2.8	x	x			x	
<i>C. cognata</i> 6	cylindrical-turbinate	1.6–1.9	x	x	x		x	
<i>C. cognata</i> 3040	cylindrical-turbinate	2.4–2.6	x	x	x		x	
<i>C. cristobaliana</i> 882	turbinate	1.7–2.1	x	x	x	x	x	
<i>C. cristobaliana</i> 34544	turbinate	2.0–2.3	x	x				
<i>C. cristobaliana</i> 41	turbinate	2.0–2.2	x	x	x			
<i>C. desertorum</i> 23783	cylindrical-turbinate	2.9–3.1	x	x			x	
<i>C. desertorum</i> 35640	cylindrical-turbinate	2.0–2.1	x	x			x	
<i>C. desertorum</i> 24369	cylindrical-turbinate	1.9–2.0	x	x				
<i>C. desertorum</i> 5840	cylindrical-turbinate	2.3–2.5	x					
<i>C. dusenii</i> 4688	turbinate	2.2–2.9	x		x	x		
<i>C. dusenii</i> 2093	turbinate	2.9–3.1	x			x	x	
<i>C. dusenii</i> 12012	-	-	-	-	-	-	-	-
<i>C. dusenii</i> 1286	turbinate	2.0–2.4	x			x	x	x
<i>C. flexuosa</i> 45	cylindrical-turbinate	2.0–2.3	x	x		x	x	
<i>C. flexuosa</i> 5	cylindrical-turbinate	2.0–2.2	x			x	x	
<i>C. flexuosa</i> 79091	turbinate	2.0–2.1	x	x		x	x	
<i>C. flexuosa</i> 44199	turbinate	2.0–2.2	x	x		x	x	
<i>C. guaranitica</i> 12524	turbinate	2.0–2.3		x	x	x	x	
<i>C. lithospermifolia</i> 9	turbinate	2.0–2.1	x	x		x	x	
<i>C. lithospermifolia</i> 15	turbinate	1.8–2.0	x		x	x	x	
<i>C. lithospermifolia</i> 42055	turbinate	2.0–2.3	x	x	x	x	x	
<i>C. lithospermifolia</i> 5884	turbinate	2.0–2.3	x	x	x		x	
<i>C. nicolackii</i> 5533	cylindrical-turbinate	1.8–2.0	x			x	x	x
<i>C. nicolackii</i> 12023	cylindrical-turbinate	2.6–2.9	x				x	
<i>C. nicolackii</i> 6214	cylindrical-turbinate	1.7–2.0	x			x	x	x
<i>C. nicolackii</i> 682	cylindrical-turbinate	2.0–2.2	x			x	x	
<i>C. oligophylla</i> 270	turbinate	2.0–2.2	x		x	x		
<i>C. oligophylla</i> 1729	turbinate	1.7–1.8	x			x		
<i>C. oligophylla</i> 5455	turbinate	2.0–2.2	x	x	x	x	x	
<i>C. oligophylla</i> 2953	turbinate	1.8–2.0	x		x		x	
<i>C. obovata</i> 3475	cylindrical-turbinate	1.9–2.1	x			x	x	
<i>C. obovata</i> 1736	cylindrical-turbinate	1.8–2.0	x	x	x		x	
<i>C. obovata</i> 36	cylindrical-turbinate	2.3–2.5		x	x		x	
<i>C. obovata</i> 17361	cylindrical-turbinate	2.0–2.2			x	x	x	
<i>C. platensis</i> 3083	turbinate	2.5–2.7	x	x	x	x	x	
<i>C. platensis</i> 5123	turbinate	1.9–2.1	x		x	x	x	
<i>C. platensis</i> 609	turbinate	2.2–2.5	x	x	x		x	
<i>C. platensis</i> 1478	turbinate	2.0–2.5	x		x	x	x	
<i>C. propinqua</i> 913	turbinate	2.0–2.2	x	x		x	x	
<i>C. propinqua</i> 3038	turbinate	1.7–2.0	x			x	x	
<i>C. propinqua</i> 20	turbinate	2.0–2.1	x		x		x	
<i>C. propinqua</i> 25	turbinate	2.0–2.2	x		x		x	
<i>C. propinqua</i> 35815	turbinate	2.0–2.3	x	x	x		x	

(continued)

Table 2. (Continued)

Species and voucher	Shape	Size (mm)	Trichomes type IIIA	Idioblasts	Crystals			
					Cubic	Estiloid	Prismatic	Druses
<i>C. propinqua</i> D y O	turbinate	2.0–2.2	x	x	x			x
<i>C. sceptrum</i> 76914	turbinate	2.0–2.3	x	x			x	x
<i>C. sceptrum</i> 30442	turbinate	2.7–2.9	x	x	x		x	x
<i>C. sceptrum</i> 3307	turbinate	2.0–2.2	x	x	x			x
<i>C. simplex</i> 547	turbinate	2.9–3.0		x				x
<i>C. simplex</i> 5704	turbinate	2.5–2.7		x	x			x
<i>C. simplex</i> 69630	turbinate	2.2–2.5					x	
<i>C. simplex</i> 23722	cylindrical-turbinate	1.9–2.0		x	x			
<i>C. simplex</i> 6057	cylindrical-turbinate	2.0–2.3		x	x			
<i>C. verbascifolia</i> 12031	cylindrical-turbinate	2.0–2.3	x				x	
<i>C. verbascifolia</i> 34	cylindrical-turbinate	2.0–2.2	x		x			

### Corolla pubescence

All the species have papillae on the adaxial surface of corolla lobes. Most of the species have glandular trichomes type IIIB (Figs 1, 16, 18), usually accompanied by type I (Fig. 20) or twin hairs (Figs 18 and 21). *Chrysolaena simplex* (Less.) Dematt. and *C. guaranitica* Dematt., have only non-glandular trichomes type IA on corolla lobes (Figs 2 and 17). *Chrysolaena nicolackii* H. Rob. and the population of *C. verbascifolia* No. 34 have trichomes type IIIB on the corolla tube and corolla lobes, respectively (Fig. 3). *Chrysolaena simplex* has trichomes type IV on the corolla tube; this feature is unique to this species among all of those examined in this study (Figs 4, 22, 23). No species exhibit entirely glabrous corolla. All trichomes described are in corollas of *Chrysolaena*.

### Style

*Chrysolaena* has style type 'vernonioid': bifid, slender, with sweeping hairs along the branches as well on the upper part of the shaft. It has stigmatic papillae on the inner top of the branches, and is glabrous to sub-glabrous towards the base. Non-glandular (type I) and glandular trichomes (IIIA) occur from the middle part to the apex (Figs 7 and 24). In three species, *C. simplex*, *C. obovata*, and *C. guaranitica*, trichomes type IIIA are absent (Fig. 27).

### Basal stylar node

Most specimens have style without basal nodule (Fig. 6). However, in some entities a conspicuous group of sclerificate cells was observed (Fig. 5) and in others, a little developed nodule formed by a few rows of cells (Fig. 25). This character varied not only between species but also between populations of the same species (see Table 1).

### Base of anther thecae and apical appendages

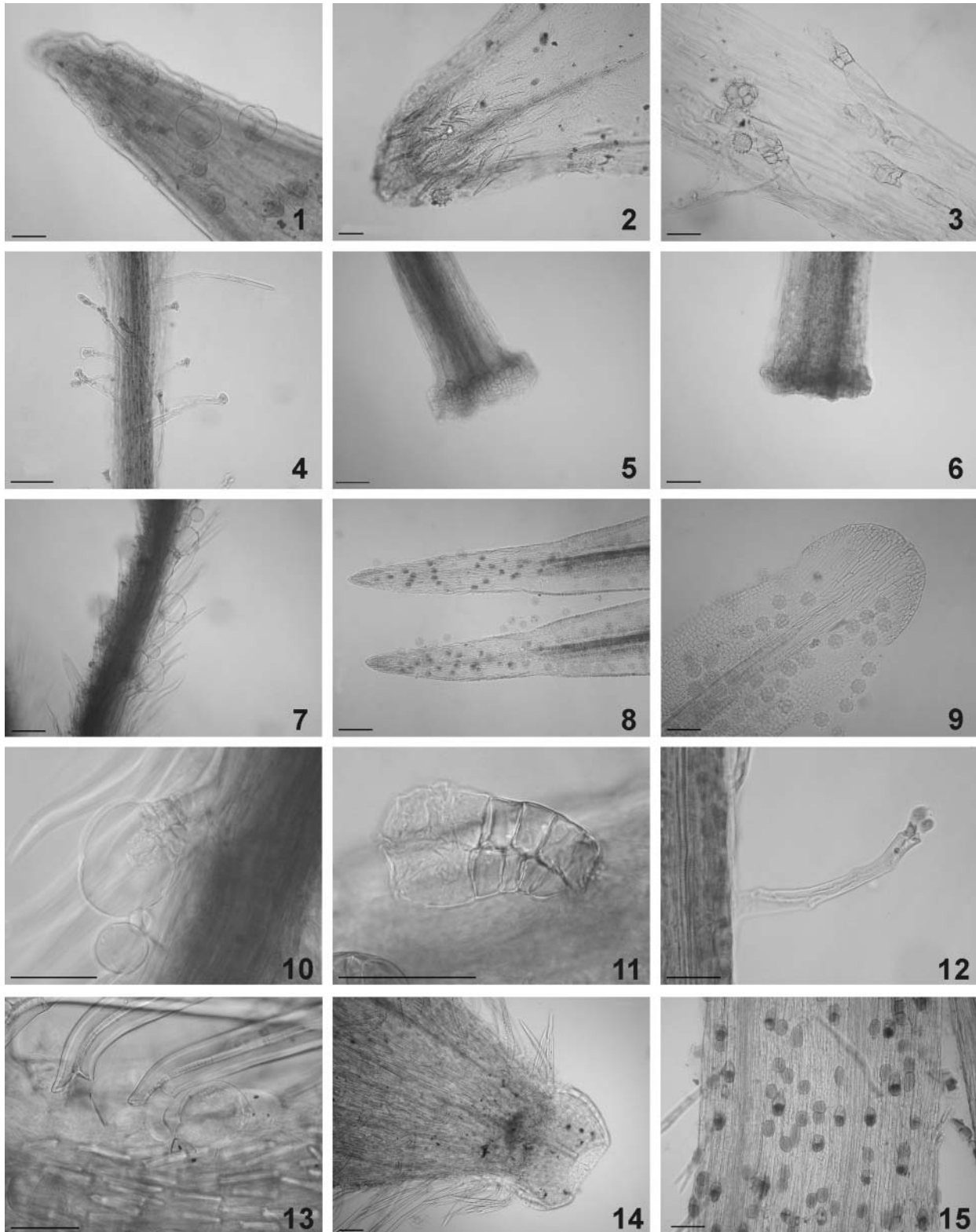
*Chrysolaena* has sagittate anthers. A smaller number of entities have cleft anthers. Regarding to the insertion the anthers are calcarate, as the thecae extend below the point where the filament connects with the anther.

Apical anther appendages are flat and show variable shapes: ovate, ovate-lanceolate, lanceolate, and orbicular (Fig. 8, 9, 26, 27, 45–55). In some species, they are not constricted at the base and are the same width as the thecae. The size varies from 0.4 to 1.1 mm long. In most species the appendages are ovate to ovate-lanceolate with glandular trichomes type IIIA (Fig. 26); except *C. simplex*, *C. obovata*, and *C. guaranitica*, where they are orbicular and glabrous (Figs 9 and 27), and in two populations of *C. obovata* Nos 3475 and 36 where appendages are glabrous. *Chrysolaena campestris* (DC.) Dematt., *C. cordifolia* Dematt. No. 24905, and *C. flexuosa* No. 45 show trichomes type IA in addition to type IIIA.

### Cypselae and pappus

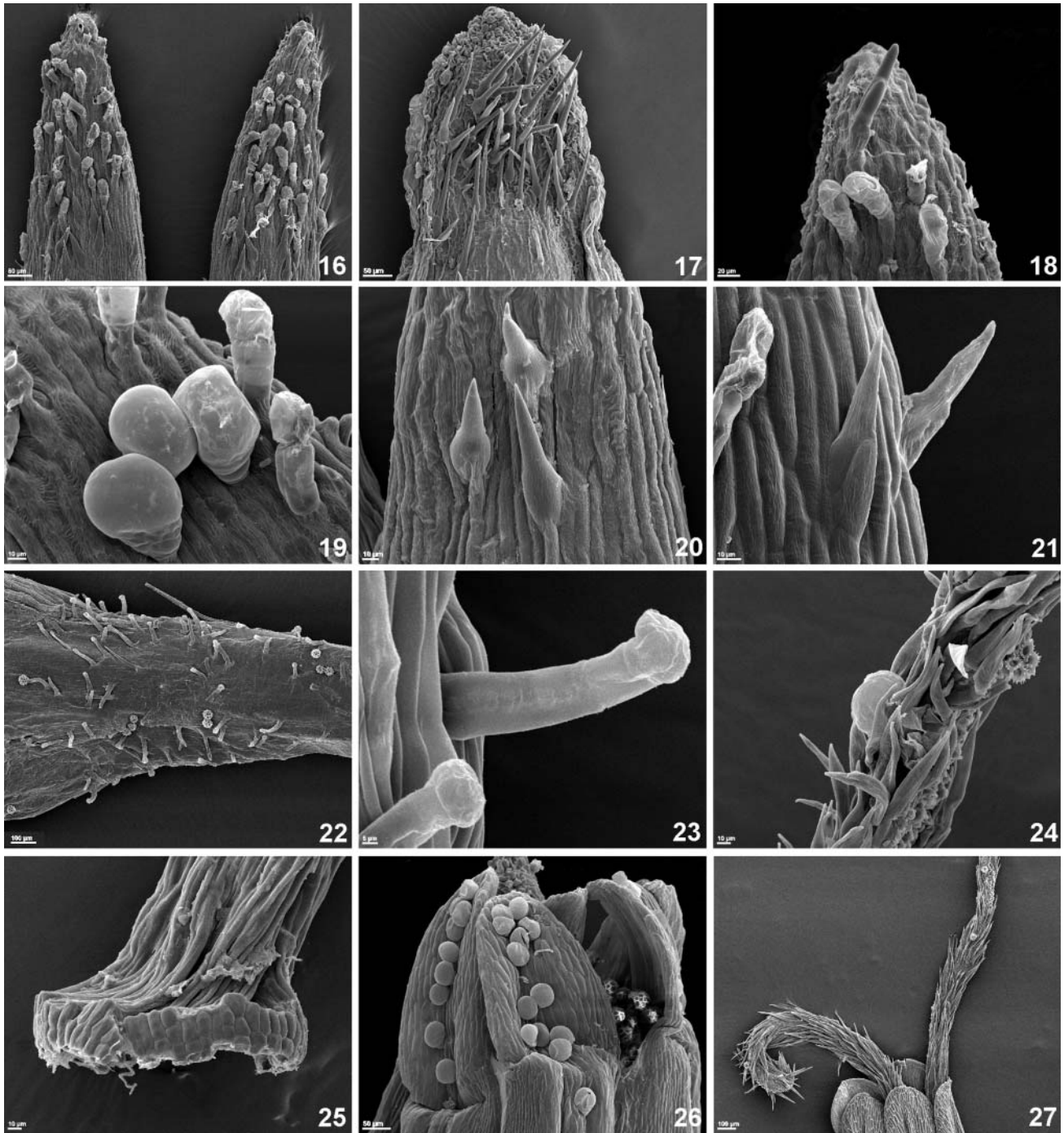
The fruits of *Chrysolaena* species are very similar in shape and indumentum (see Table 2). The cypselae are turbinate (most species) or cylindrical-turbinate (Fig. 56–65). The size varies from 1.3 mm long in *C. cordifolia* No. 24905 to 3.2 mm long in *C. cognata* (Less.) Dematt. No. 46948. Basic colour is brown; however there is variation from dark brown to yellowish brown. In all taxa examined non-glandular trichomes type II (twin hairs) occur throughout the surface of fruit, and most also have glandular trichomes type IIIA (Figs 13, 28–30, 33, 34, 38), located mainly at the base, linearly or scattered. Three species exhibit no glandular cypselae: *C. guaranitica*, *C. simplex*, and *C. obovata* Nos 17361, 36 (Fig. 37).

Besides trichomes, the cypselae usually possess idioblasts. These are more or less rounded and conspicuous

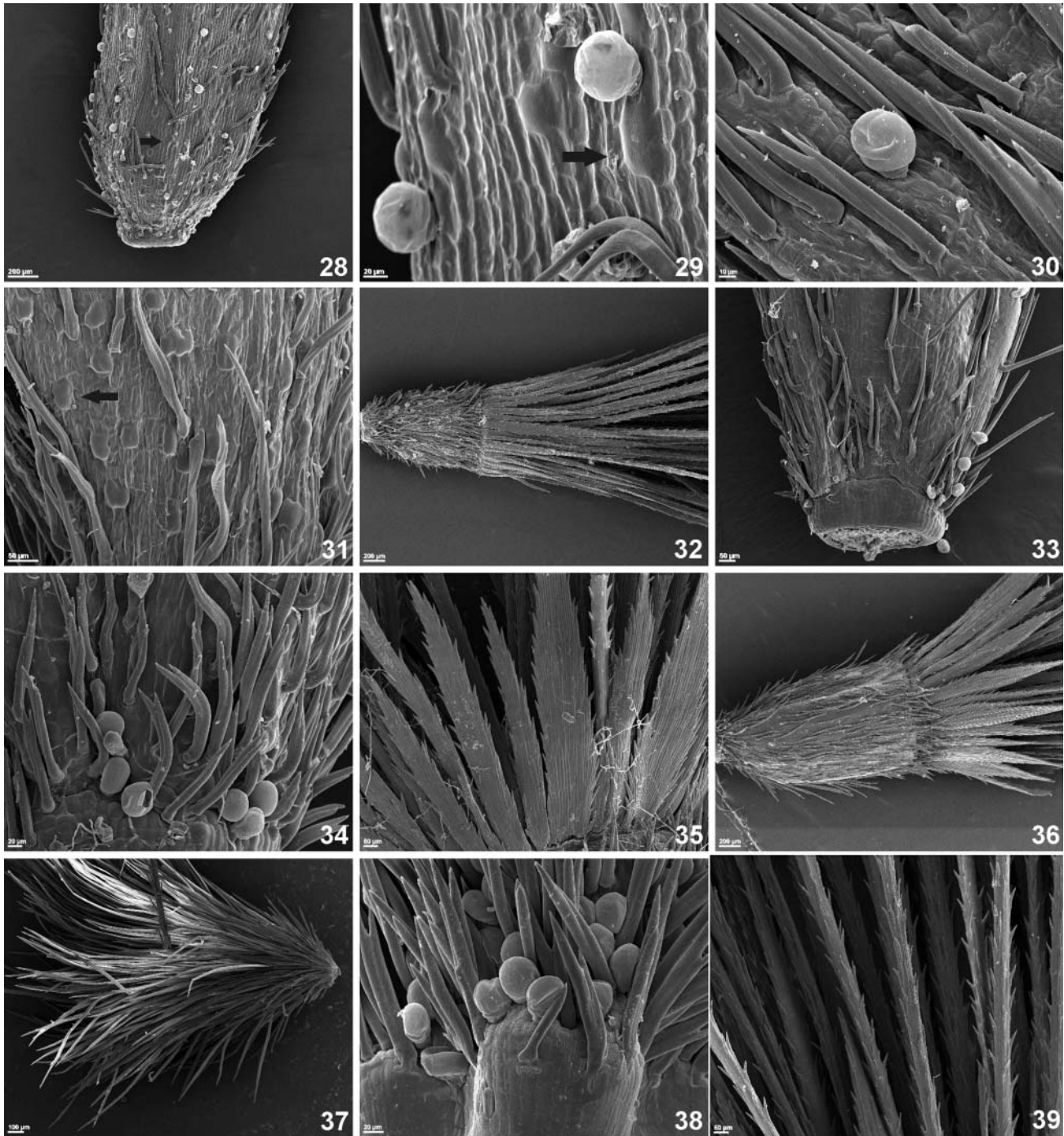


**Fig. 1–15.** Floral microcharacters of *Chrysolea* (LM). (1) *C. cordifolia* No. 24905: corolla lobes with glandular trichomes type IIIA; (2) *C. guaranitica*: corolla lobes with non-glandular trichomes type I; (3) *C. nicolackii* No. 12023: corolla tube with trichomes type IIIB; (4) *C. simplex*: corolla tube with trichomes type IV; (5) *C. campestris* No. 8707: basal stylar node; (6) *C. lithospermifolia* No. 5884: style without basal node; (7) *C. candelabrum* No. 45761: style branch with trichomes type IIIA; (8) *C. platensis* No. 3083: lanceolate apical anther appendage with trichomes type IIIA; (9) *C. simplex* No. 5704: orbicular and glabrous apical anther appendage; (10) *C. candelabrum* No. 45761: glandular trichome type IIIA of style; (11) *C. nicolackii* No. 5533: trichome type IIIB; (12) *C. simplex*: trichome type IV; (13) *C. nicolackii* N° 682: trichomes type II and IIIA, and crystals of the cypsela; (14) *C. lithospermifolia* No. 8: detail of carpopodium; (15) *C. cognata* No. 3053: idioblasts on the cypsela wall. Scale Bars: 50  $\mu\text{m}$  in (1–8, 12, 14); 100  $\mu\text{m}$  in (9–11, 13, 15).

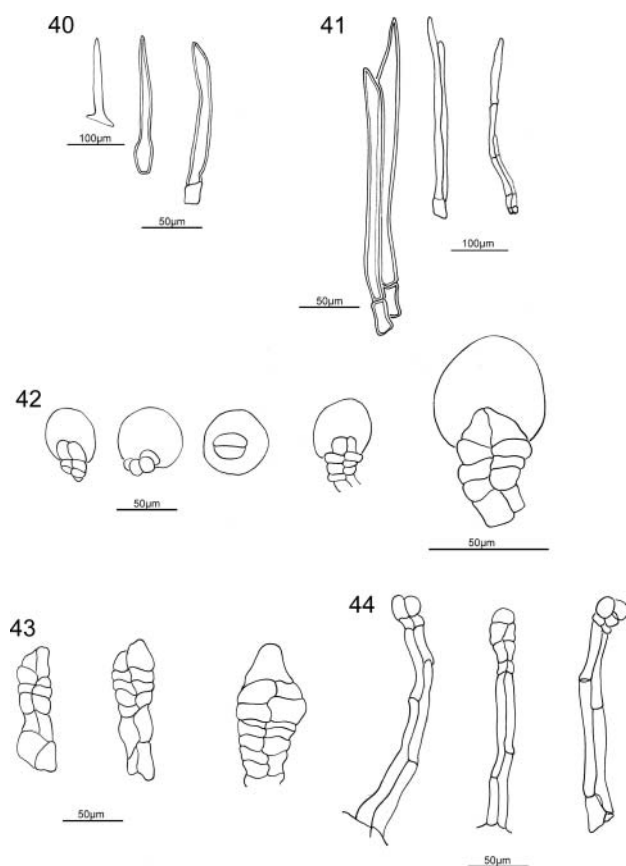




**Fig. 16–27.** Floral microcharacters of *Chrysolaena* (SEM). (16) *C. candelabrum*: corolla lobes with trichomes type I and IIIA; (17) *C. guaranitica*: corolla lobe with trichomes type I; (18) *C. oligophylla* No. 2953: corolla lobe with trichomes type II and IIIA; (19) *C. plattensis* No. 1478: detail of trichomes type IIIA of corolla; (20) *C. sceptrum* No. 3307: detail of trichomes type I of corolla; (21) *C. obovata* No. 3475: trichomes type II (twin hairs) of corolla; (22–23) *C. simplex*: corolla tube and detail of trichome type IV, respectively; (24) *C. cristobaliana*: detail of style branch with trichome type IIIA; (25) *C. verbascifolia* No. 34: basal styler node poorly developed; (26) *C. propinqua*: trichomes type IIIA on the apical anther appendage; (27) *C. simplex*: style branches and anther appendages without glandular trichomes.



**Fig. 28–39.** Floral microcharacters of *Chrysolaena* (SEM). (28–29) *C. cognata* No. 3053: (28) general view of cypselal; (29) cypselal wall with trichomes type II, IIIA and idioblasts; (30) *C. flexuosa* No. 5: trichomes type II and IIIA of the cypselal; (31) *C. obovata* No. 36: detail of cypselal wall; (32) *C. propinqua* No. 25: general view of cypselal; (33–35) *C. sceptrum* No. 3307: (33) detail of carpodium with sub-squares cells; (34) trichomes of the cypselal wall; (35) external series bristle of pappus (flat bristles); (36) *C. sceptrum* No. 76914: general view of cypselal; (37) *C. simplex* No. 6057: general view of cypselal without glands; (38–39) *C. verbascifolia* No. 34: (38) detail of a portion of carpodium, glandular trichomes and twin hairs; (39) detail of the cylindrical inner series bristles of pappus. Black arrows show the idioblasts.



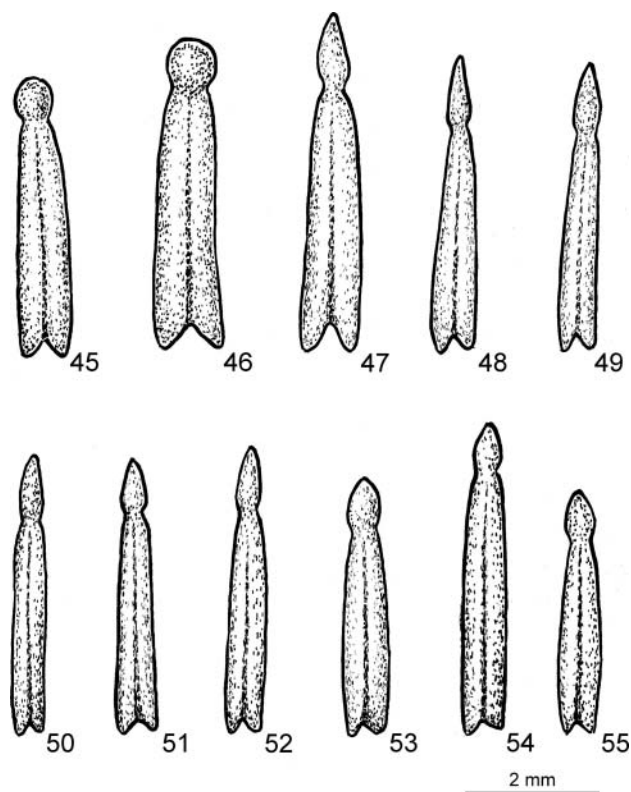
**Fig. 40–44.** Trichome types of florets and cypselae of *Chrysolaena*. (40) Type I: simple filiform trichome; (41) Type II: twin hair, regular and atypical forms; (42) Type IIIA: short-stalked capitate glands. (43) Type IIIB; (44) Type IV: long-stalked glandular trichome.

structures, different from other cells that form the wall of the fruit. With LM are clearly distinguishable by their intense brownish colour. They are distributed irregularly over the fruit wall and are arranged in groups of 2–8 cells (Figs 15, 28, 29, 31). The presence of idioblasts also varies at intraspecific level.

A biseriate and double pappus is present in the fruits of all species of *Chrysolaena*. The bristles have short teeth. The external series is flat and shortest. The internal series has cylindrical and longer bristles (Fig. 32, 35, 36, 39).

### Carpopodium

The carpopodium is the basal abscission area of the cypselae formed by one or more rows of cells which usually are morphologically distinct from other cells of the wall cypselae. In the taxa investigated the carpopodium is well developed and differentiated; is cylindrical or slightly turbinate. It is ring-like without interruptions covering the cypselae base, and is formed by sub-oblong or square cells with thickened walls (Figs 14, 33, 38). In a few species the carpopodium shows small crystals druses (e.g., *C. nicolackii*).



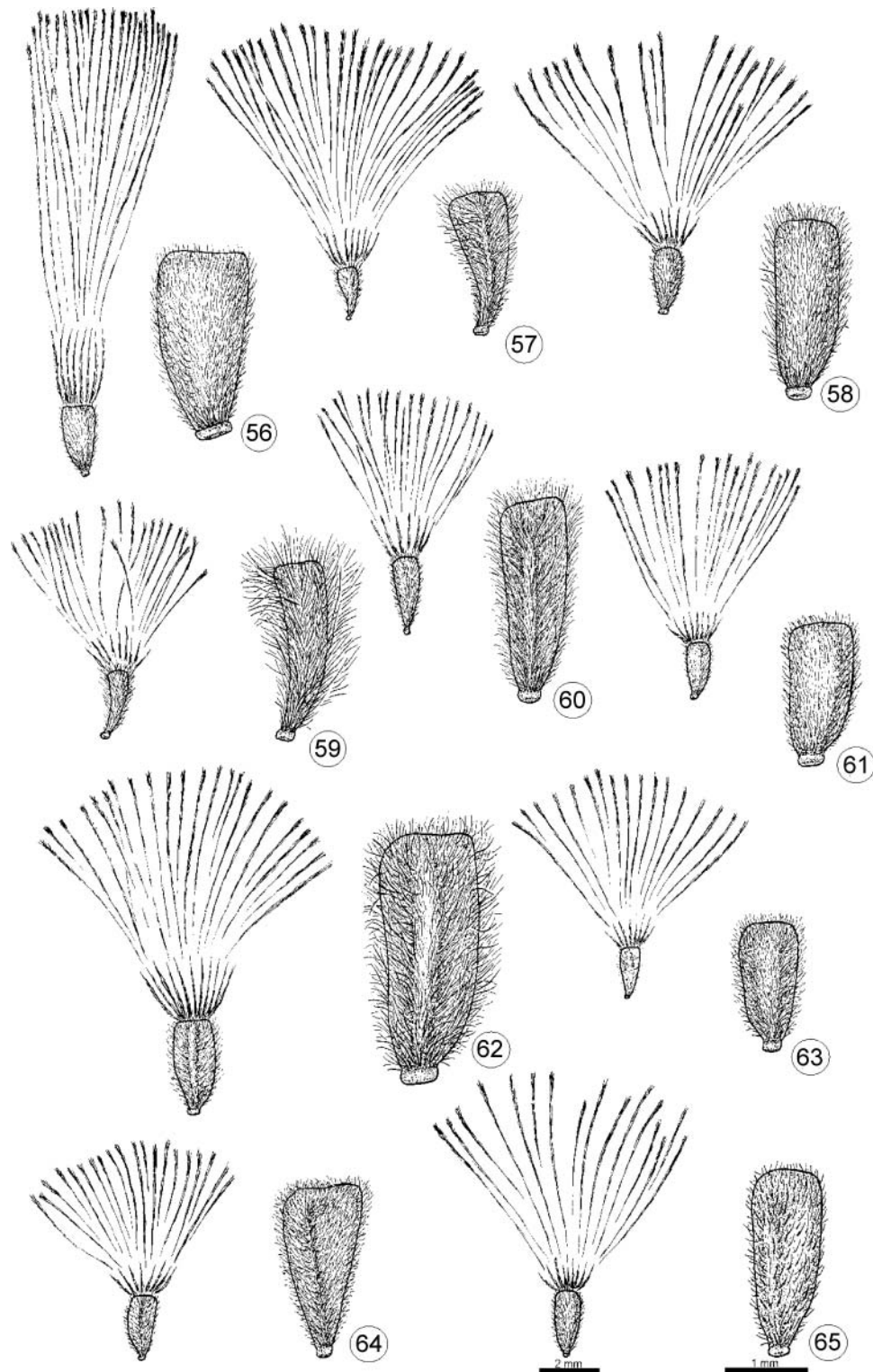
**Fig. 45–55.** Anthers of *Chrysolaena*. (45) *C. guaranitica* No. 12524; (46) *C. simplex* No. 5704; (47) *C. dusenii* No. 1286; (48) *C. candelabrum* No. 45761; (49) *C. platensis* No. 5123; (50) *C. obovata* No. 1736; (51) *C. lithospermifolia* No. 5884; (52) *C. flexuosa* No. 45; (53) *C. desertorum* No. 5840; (54) *C. verbascifolia* No. 12031; (55) *C. flexuosa* No. 44199.

### Ovary wall crystals

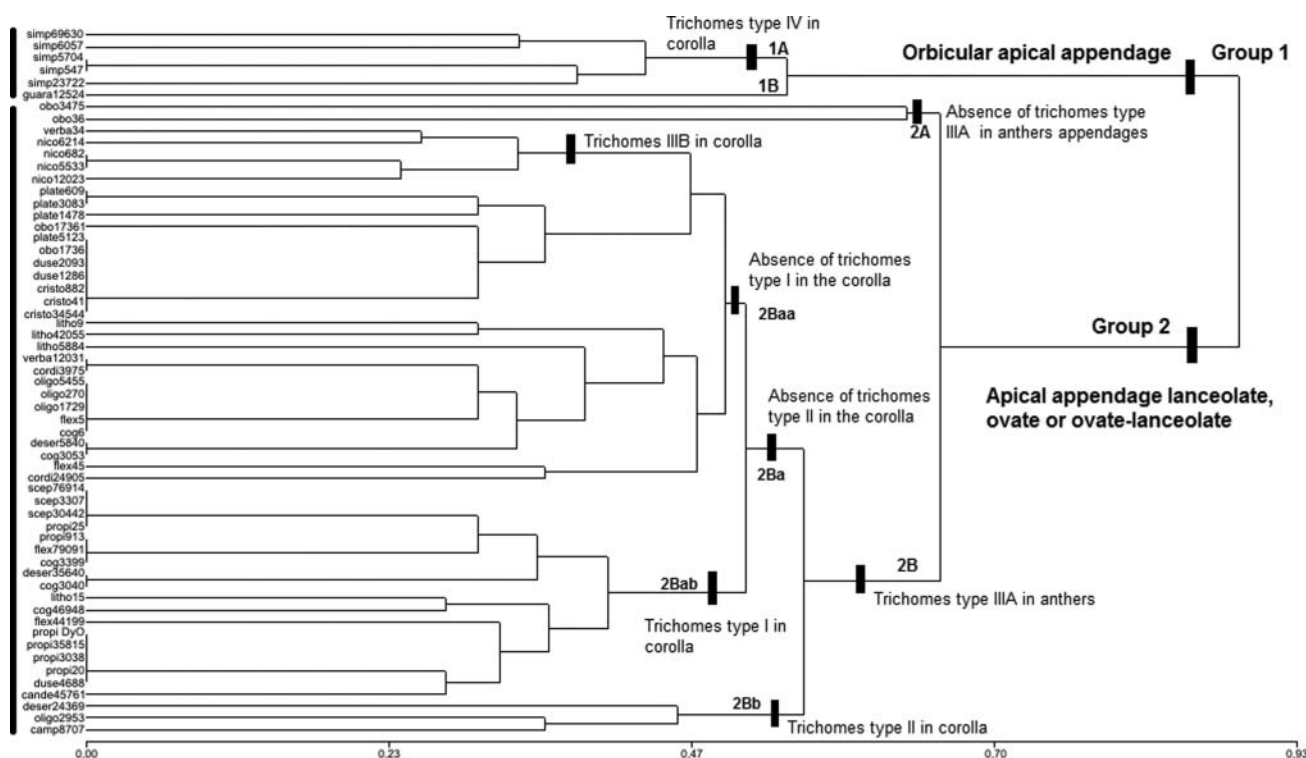
Ovary walls present several forms of calcium oxalate crystals: cubic, prismatic, styloid, and druses. Their sizes and density are variable between the specimens (Fig. 13–15). Most species showed crystals, but not always all the shapes. The most common crystals among species studied are prismatic and cubic. *Chrysolaena cristobaliana* Dematt. Nos 34544, 24369 and *C. desertorum* (Mart. ex DC.) Dematt. No. 5840 do not exhibit crystals on the ovary walls.

### Cluster analysis (UPGMA) and principal coordinates analysis (PCoA)

Figure 66 shows the phenogram of the UPGMA. Cluster analysis divided the 59 OTUs into two main groups based on the shape of the apical anther appendage. Group 1 contains species with orbicular apical appendages (Fig. 45 and 46), while in Group 2 are all other entities that may present apical appendages ovate, lanceolate or ovate-lanceolate. Group 1 is subdivided in two: group 1A, that contains taxa with trichomes type IV on the corolla tube (*C. simplex*), and



**Fig. 56–65.** Cypselae of *Chrysolea*. (56) *C. verbascifolia* No. 34; (57) *C. sceptrum* No. 30442; (58) *C. cognata* No. 3053; (59) *C. platensis* No. 5123; (60) *C. obovata* No. 36; (61) *C. propinqua* No. D y O s/n; (62) *C. nicolackii* No. 12023; (63) *C. flexuosa* No. 45; (64) *C. flexuosa* No. 44199; (65) *C. cognata* 30422. Turbinate cypselae in (56, 57, 64); Cylindrical-turbinate cypselae in (58–63, 65).

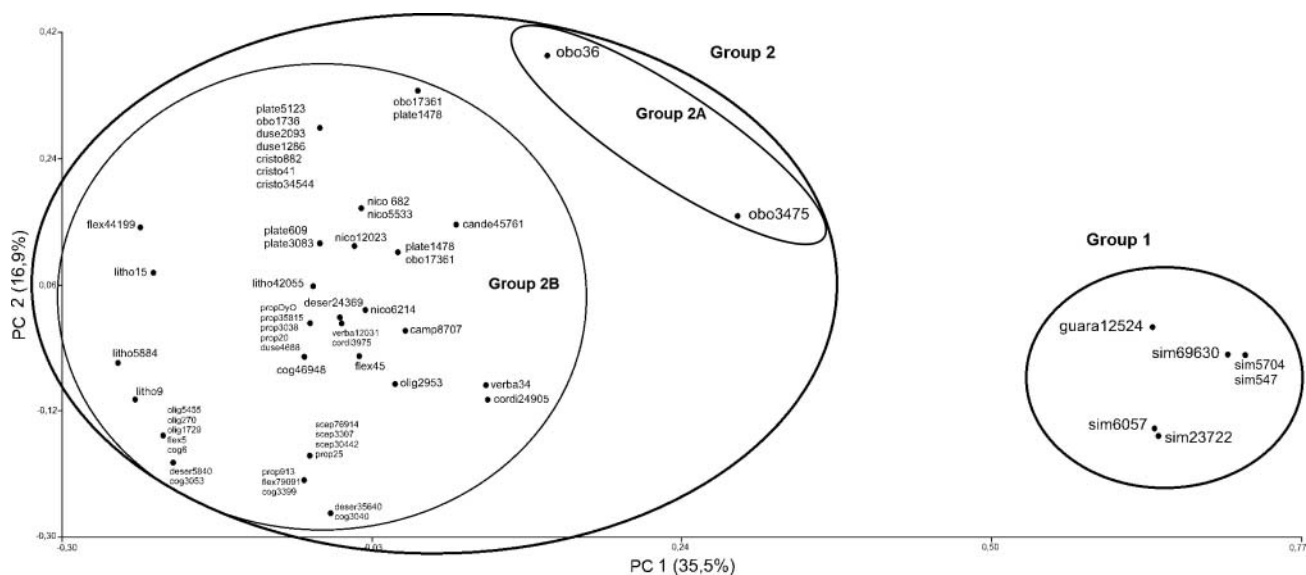


**Fig. 66.** Phenogram of 13 characters and 59 OTUs resulting from UPGMA based on the Dice coefficient.

group 1B, taxa with glabrous corolla tube (*C. guaranitica*). Subgroup 1A is divided, again, into two groups based on the presence or absence of type II trichomes on the corolla, and these groups are subdivided according to the fruit shape (turbinate or cylindrical-turbinate). Group 2 includes the largest number of taxa and is subdivided several times based

mainly on the presence of the different types of trichomes on apical anther appendages and corollas.

The PCoA shows that 52.4% of the variation can be explained by the first two principal coordinates (PC), PC 1 and 2 (Fig. 67). PC 1 explains 35.5% of the total variation, while PC 2 explains 16.9%. The PCoA diagram



**Fig. 67.** Principal Coordinates Analysis (PCoA) of *Chrysolaena* species based on floral microcharacters. Abbreviations: PC – principal coordinate; *camp* – *Chrysolaena campestris*; *cande* – *C. candelabrum*; *cog* – *C. cognata*; *cordi* – *C. cordifolia*; *cristo* – *C. critobaliana*; *deser* – *C. desertorum*; *duse* – *C. dusenii*; *flex* – *C. flexuosa*; *guara* – *C. guaranitica*; *litho* – *C. lithospermifolia*; *nico* – *C. nicolackii*; *obo* – *C. obovata*; *olig* – *C. oligophylla*; *plat* – *C. platensis*; *pro* – *C. propinqua*; *scep* – *C. scepstrum*; *sim* – *C. simplex*; *verb* – *C. verbascifolia*. Circles show the taxon groups which correspond to the clustering phenogram of Fig. 66.

shows distinctive groups of taxa. These groups correspond to the grouping of phenogram of cluster analysis.

## Discussion

The species of *Chrysolaena* are completely uniform in the presence of papillae on the style, the insertion of the anther in the filament (calcarate), the pappus (biseriate with external bristles flattened and short, and the inner cylindrical and long) and carpodium (forming a cylindrical or slightly turbinate ring, with oblong or sub-square cells). In addition to the microcharacters used by Robinson (1988b) for circumscription of the genus, other characters of taxonomic significance were analysed for the first time. Below we discuss separately the microcharacters examined.

## Trichomes

The Asteraceae family is characterized by glandular and non-glandular trichomes (Metcalf & Chalk, 1950, 1979) in floral and vegetative organs, and even in the underground organs (Apezzato-da-Glória, Hayashi, Cury, Soares, & Rochao, 2008; Apezzato-da-Glória *et al.*, 2012). Type and proportion can vary throughout the plant and between different taxonomic levels (Werker, 2000). Based on its glandular and non-glandular nature and the number and organization of their cells, a total of five morphological types of trichomes were described for *Chrysolaena*. The morphologies observed are consistent with those described for other species of Asteraceae and Vernoniaeae. According to these previous studies, the morphologies of the trichomes here described as types I, II, and IIIA are the most common among species (Angulo & Dematteis, 2014; Apezzato-da-Glória *et al.*, 2012; Freire, Katinas, & Sancho, 2002; Isawumi, 1999, Isawumi *et al.*, 1996; Metcalke & Chalk, 1950; Narayana, 1979; Ramayya, 1962; Redonda-Martínez, Villaseñor, & Terrazas, 2012; Robinson, 2009; Sancho & Katinas, 2002). The morphology of trichomes type I agree with those described as types I and II on the corollas of most *Lessingianthus* species (Angulo & Dematteis, 2014). The type II trichomes are of the same morphology as the twin hairs, or also called double hairs or Zwillingshaare (Kraus, 1866), typical of the fruits of most genera of Asteraceae (Hess, 1938; Ramayya, 1962; Robinson, 2009). However, variations of basic type were observed, mainly on corollas and anthers. Previous studies have shown that twin hairs may vary morphologically in the size, length, wall thickness, or the degree of divergence, the degree of development and reduction of hair cells and/or basal cells (Freire & Katinas, 1995; Hess, 1938; Ml'khi R.ike & Nordensstam, 2012). Atypical morphologies seen in *Chrysolaena* showed variations in the length, the number of cells that

form the hair and the degree of divergence of the hair cells at the apex.

The trichome type IIIA correspond morphologically with the typical vesicular glands, usually observed as glandular dots in different organs of the Asteraceae. They usually contain sesquiterpene lactones (Ramayya, 1962; Robinson, 2009). This type of trichome is very common in Vernoniaeae and is the structure that Robinson (1988a, 1988b, 1990) referred to as 'glands' and noted in apical anther appendages, the corolla lobes and the cypselae in his treatment of *Lepidaploa* complex. Previous studies revealed the occurrence of this type of glandular trichomes on leaves, stems, and underground organs (rhizophores) of *Chrysolaena obovata*, *C. platensis*, *C. cognata*, *C. flexuosa*, *C. lithospermifolia*, *C. propinqua*, and *C. verbascifolia* (Apezzato-da-Glória *et al.*, 2012; Sosa, Via do Pico, & Dematteis, 2014).

Similar morphology of trichome type IV was observed in petioles of *Echinops echinatus* Roxb. (Ramayya, 1962), in leaves of *Madia sativa* Mol. (Asteraceae) (Carlquist, 1958) and members of the subtribe Espeletiinae in Heliantheae tribe (Robinson, 2009). These trichomes usually contain monoterpenes (Robinson, 2009).

The use of trichomes is well established in comparative systematic studies of several groups of angiosperms because of their variety, wide occurrence, ease of preparation for study and close relation of their variation patterns to the taxonomic system (Carlquist, 1961). Within Vernoniaeae, numerous studies have supported the utility of trichomes for systematic studies (Faust, 1972; Hunter & Austin, 1967; Urbatsch, 1972; Wagner *et al.*, 2014).

The trichomes morphology in *Chrysolaena* is variable and some types presented diagnostic value at species level. The presence or absence of glandular trichomes is the main character that allowed distinguishing some species, because some morphotypes are restricted to certain structures or to particular species. For example, *C. simplex* and *C. nicolackii* can be separated from the other species by the presence of glandular trichomes on corolla tube. Besides, *C. simplex* differs by the exclusive presence of glandular trichomes type IV. Moreover, *C. guaranitica* and *C. simplex* have only non-glandular trichomes type I on corolla lobes, and lack glandular trichomes type IIIA, while the remaining species possess them. The lack of glandular trichomes on corolla lobes and cypselae are typical of most species of *Lessingianthus* (Robinson, 1988a).

## Corolla pubescence

Pubescence of corolla is a feature widely used in Vernoniaeae systematics (Isawumi, 1999). However, it has only been analysed in detail in Old World genera such as *Baccharoides* Moench (Isawumi *et al.*, 1996), *Cyanthillium* Blume (Isawumi, 1995b), and some African species of

*Vernonia s.l.* (Isawumi, 1995a). For American Vernonieae the only studies were carried out in some entities of *Lepidaploa* complex (Robinson, 1987a, 1987b, 1988a, 1988b, 1990) and the most recently, in the genus *Lessingianthus*, which also lists and describes the morphology of the trichomes (Angulo & Dematteis, 2014).

The results of this study show that all species of *Chrysolaena* are homogeneous in the presence of stigmatic papillae in corolla lobes. In *Baccharoides* and *Lessingianthus* this feature varies with species with papillae and species lacking these (Angulo & Dematteis, 2014; Isawumi, 1999). Excluding the presence of papillae, the corolla pubescence was the most variable character and provided greater taxonomic information. In the florets of the species analysed the five morphological types of trichomes described (I, II, IIIA, IIIB, and IV) are present in different combinations. However, some entities lack glands in corolla lobes, such as *C. simplex*, *C. guaranitica*, and two populations of *C. obovata*, which exhibit only non-glandular trichomes type I. This distinctive feature distinguishes them from other species. The exclusive presence of glandular trichomes on corolla lobes was observed in some species of the genus *Lessingianthus* (Angulo & Dematteis, 2014). Additionally, *C. nicolackii* and *C. simplex* differ from other species of the genus by trichomes type IIIB and IV, respectively, on the corolla tube. The presence of trichomes on the corolla tube was also reported in some species of *Lessingianthus* (Angulo & Dematteis, 2014) and *Baccharoides* (Isawumi et al., 1996), but they differ morphologically from trichomes of *Chrysolaena*.

This study noted for the first time the presence of twin hairs on the corolla of *Chrysolaena* (Vernonieae). As previously mentioned, traditionally such trichomes are considered typical and unique to Asteraceae cypselae (Hess, 1938; Ramayya, 1962; Robinson, 2009; Roth, 1977). However, it has been observed that the morphology and ontogeny of trichomes of the corollas of some *Onoseris* Willd., *Trichocline* Cass. and *Uechtrizia* Freyn (Mutisieae) species are the same as the twin hairs of cypselae. Despite having a great morphological variation, all show the same ontogeny (Sancho & Katinas, 2002). Based upon this background in other Asteraceae groups, the trichomes type II observed in this study in corollas of *Chrysolaena* correspond to the morphology of a 'twin hair'. Nevertheless, ontogenetic studies should be performed to confirm their origin.

## Style

The general morphology of style coincides with most Vernonieae and it was not a useful taxonomic character, since all species had the same characteristics.

In this study the trichome types of the style were analysed for the first time. In the Asteraceae this feature, along with the morphology of style, has been very important taxonomically (Keeley & Robinson, 2009). However there are no studies using this character at species or genus level among American Vernonieae. In *Chrysolaena*, the presence or absence of glandular trichomes type IIIA on branches style varies between species hence was useful to distinguish some taxa. Most species have this type of trichome in branching style, except *C. guaranitica*, *C. obovata*, and *C. simplex*.

## Basal stylar node

The presence of basal stylar node varies greatly among taxa of the same species and among populations. This variation was also observed in *Lessingianthus*. Species such as *L. brevifolius* (Less.) H. Rob., *L. carvalhoi* (H. Rob.) H. Rob., *L. durus* (Mart. ex DC.) H. Rob., *L. regis* (H. Rob.) H. Rob., and *L. santosii* H. Rob. present a slight development of the stylar node with several rows of thick-walled cells (Angulo & Dematteis, 2014) as noted in some of the entities of *Chrysolaena*.

The basal stylar node was a character used for the classification of Vernonieae of both hemispheres. It is considered typical in many members of Neotropical Vernonieae and characteristic of some genera of the *Lepidaploa* complex (Robinson, 1988a). The genus *Lepidaploa* presents a well-developed basal node. However, in three genera of the complex (*Chrysolaena*, *Stenocephalum* Sch. Bip., and *Lessingianthus*) the node seems lost or poorly differentiated (Robinson, 1988a, 1988b, 1990). It has been suggested that the lack of node is due to a loss. In Vernonieae, apparently the basal node does not play a significant role, so there is no variability between groups and, therefore, the observed changes in complex *Lepidaploa* members should be considered rare and random (Robinson, 1990). From the taxonomic point of view, although its absence is considered typical of *Chrysolaena*, apparently, this character is not well defined in the genus (also in *Lessingianthus*) and, therefore, does not have much systematic value.

## Base of anther thecae and apical appendage

*Chrysolaena* has calcarate base of anther thecae, as well as *Lessingianthus* and *Lepidaploa* (Angulo & Dematteis, 2014). The shape can be sagittate or cleft.

As in most of the Asteraceae, all *Chrysolaena* species have an apical anther appendage. This structure can be seen as an adaptation to protect the pollen from humidity and insect predators, until the stigma and style push it up for the presentation of pollen (Stuessy, Sang, & Devore,

1996). The apical appendages are important among the New World Vernoniaeae, since variations occur in the presence of glands or thickening. However, the Old World taxa are very uniform (Keeley & Robinson, 2009). Most entities of *Chrysolaena* have an ovate apical appendage. However, other forms (ovate-lanceolate, lanceolate, and orbicular) were also observed. This character allowed separation *C. simplex* and *C. guaranitica* from the other species, since they were the only taxa with orbicular apical appendages.

Regarding pubescence, Robinson (1988b, 1990) states that numerous small glands are present in apical anther appendages of Vernoniaeae and this characteristic is typical of *Chrysolaena*. Dematteis (2007) used this character, in combination with pollen morphology (type 'C') and base chromosome number ( $x = 10$ ), for transferring to two species, *Lessingianthus simplex* (Less.) H. Rob. and *L. desertorum* (Mart. ex DC.) to *Chrysolaena* (previously located in *Lessingianthus* subgen. *Oligocephalus*), because they have glandular apical appendages and cypselae. However, this study demonstrates that *C. simplex* can present an apical appendage without glands, a typical characteristic of *Lessingianthus* and most species of *Lepidaploa*. Despite being closely related genera, a distinguishing feature between *C. simplex* and *Lessingianthus* is the shape of the apical appendages. In *Lessingianthus* the appendages are ovate, ovate-lanceolate, lanceolate, or linear (Angulo & Dematteis, 2014), and this study has shown that *C. simplex* has orbicular apical appendages. The absence of glands in anthers was also denoted in previous studies as uncommon in *C. obovata* (sub nom. = *Chrysolaena herbacea*) (Robinson, 1988b) and in *C. guaranitica* (Dematteis, 2007).

### Cypselae, pappus, and carpopodium

Wagenitz (1976) suggested that morphoanatomical studies of the fruits of the Asteraceae might aid in elucidating its systematics. Characteristics of the indumentum (Ritter & Miotto, 2006), pappus, carpopodium (Haque & Godward, 1984), and the anatomy of the pericarp (Bruhl & Quinn, 1990), or a combination of all of the characters cited above have been used to delimit tribes, genera, and even species of this family. According to Bremer (1987), cypselae and pappus provide a wealth of characters at the lower taxonomic levels because of their considerable variation.

The results of this study show that the shape and pubescence of cypselae, as well as pappus features are fairly uniform among *Chrysolaena* species. All entities analysed have cypselae with pappus, non-glandular trichomes type II (twin hairs), and most also glandular trichomes type IIIA. Both types of trichomes are biseriate and occur in most genera of the Asteraceae family (Robinson, 2009).

However, *C. guaranitica*, *C. simplex*, and two populations of *C. obovata* do not have glandular trichomes, a typical characteristic of the fruits of *Lessingianthus* and most *Lepidaploa* species (Dematteis 2007, 2009; Robinson 1988a, 1988b, 1990, 1999).

The structure of pappus of *Chrysolaena* species is common among Lepidaploinae taxa (Vernoniaeae), and also in other tribes such as Eupatorieae (*Ageratina* Spach) and Astereae (*Symphotrichum* Nees) (Robinson, 2009).

King & Robinson (1966) have shown that special features of the carpopodium can be useful taxonomic characters. Many genera of Asteraceae could be differentiated on the basis of shape and cellular structure of the carpopodium (King & Robinson, 1966, 1987). Haque and Godward (1984) examined the carpopodium and its usefulness in the taxonomy of the Asteraceae. According to Robinson (2009), in general, the Vernoniaeae has a carpopodium of uniform characteristics, except *Camchaya* Gagnep. that lack a carpopodium. Previous studies in some species of *Chrysolaena* have described a turbinate carpopodium formed by oblong cells (Robinson, 1988b). The results of this study show that all species of *Chrysolaena* have a carpopodium with uniform characteristics: differentiated in the cypselae base, cylindrical-turbinate and formed by sub-square or slightly oblong cells.

### Idioblasts

In plant histology, idioblasts are isolated cells that differ in shape, size, content, and structure of the wall elements with respect to surrounding tissue (Foster, 1955). It is a term with more than a specific morphological or physiological conventional connotation use, because it applies to a variety of cell types (Foster, 1955).

Isawumi (1996, 1999) and Robinson (2009) refer to idioblasts as the structure commonly found on the cypselae walls of numerous Vernoniaeae. These are arranged in groups of three or more cells and scattered or in longitudinal rows. There is no background on the structure or function of these elements within the group, but the presence or absence of idioblasts has been used as a taxonomic character (Isawumi et al., 1996; Robinson, 2009). These structures were observed in the fruits of some species of *Chrysolaena* analysed in this study. However, their presence was highly variable among species and populations of the same species, so it was not a useful basis for systematics of the group.

### Ovary wall crystals

Calcium oxalate (CaOx) crystals are found in over 215 plant families and distributed in organs such as stems, roots, leaves, floral structures, and seeds (Franceschi &



Horner, 1980; Lersten & Horner, 2000; Prychid & Rudall, 1999). Although their functional significance in plant development remains unclear, various functions have been attributed to them, including calcium regulation in plant cells (Franceschi, 1989), protection against herbivory (Molano-Flores, 2001), detoxification of heavy metals or oxalic acid (Franceschi & Nakata, 2005), tissue strength, light gathering, and reflection (Franceschi & Horner, 1980; Kuo-Huang, Ku, & Franceschi, 2007). Even though the size, location, and other properties of the crystals in plants may be affected by physical, chemical and biological conditions, it is thought that crystal formation within the cell is under genetic control (Ilarslan, Palmer, & Horner, 2001). Thus the type, presence or absence of crystals may be represented as a taxonomic character (Lersten & Horner, 2000; Prychid & Rudall, 1999).

Ovary wall crystals have been successfully studied for the taxonomic evaluation of several tribes in the family Asteraceae (Dormer, 1961; Isawumi, 1995a, 1995b; Isawumi et al., 1996; Nordenstam & El-Ghazaly, 1977). Studies by Robinson (1988a, 1988b, 1999) suggest that the elongated crystals (the correct term would be prisms, because it is a structure with volume) of the fruit wall are typical of the sorts *Chrysolaena* and *Lepidaploa*; opposed to *Lessingianthus*, which is generally square crystals sub squares (cubic or nearly cubic). However, in this study four types of crystals were found on the cypselae of *Chrysolaena*: druses, styloid, prismatic, and cubic. Other studies have also observed druses, styloid, and prismatic crystals in the ovary walls and carpelodium of *C. platensis* [sub nom. *Vernonia platensis*] (Galastri & Oliveira, 2010) and *C. obovata* [sub nom. *V. herbacea*] (Martins & Oliveira, 2007). Besides presenting various forms, the presence of different types of crystals in *Chrysolaena* varied to interspecific and intraspecific level.

From the taxonomic point of view, the results of this analysis suggest that while the prismatic crystals (elongated in terms of Robinson, 1988b) are among the most common forms observed in *Chrysolaena* species, they do not constitute a distinctive character of the genus because various shape crystals were observed in most entities. Besides, the morphology and the presence of crystals are not constant between *Chrysolaena* species, so this would not be a useful character to recognize species taxonomically.

### Statistical analyses: UPGMA and PCoA

The results of the statistical analyses performed (UPGMA and PCoA) correspond to each other. The grouping of species according to the analyses of clusters is consistent with the clustering seen in the diagram PCoA. Both graphs

identify the same groups of taxa with common characteristics (Figs 66 and 67). For both analyses it can be deduced that the shape of apical anther appendages and the presence/absence of certain types of trichomes on certain structures are the determining variables in taxa grouping.

The statistical analyses clearly showed that *C. simplex* and *C. guaranitica* (Group 1) are very similar to each other by the presence of orbicular apical appendage and the absence of glands in the lobes of the corolla, apical appendages, and cypselae. In turn, these characteristics are very different from other species *Chrysolaena*, and grouped separately from them. With respect to the remaining species (Group 2), one can identify remarkably consistent subgroups based on common features that are mainly the presence/absence of certain types of trichomes on florets structures. However, in a significant number of taxa there is overlap of characters and separation of species in the innermost clusters becomes difficult. Populations of different species had shared characters, which is reflected in the polyphyletic terminals in the phenogram (UPGMA) consisting of taxa from different species and the separation of populations of the same species in different clusters. This is seen equivalently in the scatterplot (PCoA) and taxa of different species with the same coordinates and populations of the same species with different coordinates.

### Taxonomic implications

Table 3 shows and compares the microcharacters of *Chrysolaena*, *Lepidaploa*, and *Lessingianthus* based on the results of this study and previous information from the literature.

As previous studies indicated, *Chrysolaena*, *Lepidaploa*, and *Lessingianthus* share some characters (Robinson, 1988a, 1988b). The results of this study agree with this background. Although most *Chrysolaena* species are homogeneous in the typical microcharacters of the genus, some entities may have typical characteristics of *Lessingianthus* (such as apical anther appendages and cypselae without glands) or *Lepidaploa* (style with basal node).

*Chrysolaena simplex*, *C. guaranitica* and some populations of *C. obovata* show systematic inconsistencies since they have microcharacters that show a departure from *Chrysolaena* and are closer to *Lessingianthus*. However, previous studies have reported base chromosome number  $x = 10$  in *C. simplex* and *C. obovata* (Ruas, Ruas, Vieira, Matzenbacher, & Martins, 1991; Via do Pico & Dematteis, 2012), presence of glands on corolla lobes, anther appendages and cypselae in *C. guaranitica* and *C. simplex* (Dematteis, 2007, 2009), and pollen grains type 'C' in all these species (Via do Pico & Dematteis, 2013a), which supports the taxonomic position of these entities within *Chrysolaena*. *Lessingianthus* always has basic chromosome number  $x = 16$  and type 'B' pollen, while *Lepidaploa*

**Table 3.** Microcharacters of *Chrysolaena*, *Lessingianthus* and *Lepidaploa* based on previous studies and the results of this study (Angulo & Dematteis, 2014; Dematteis 2007, 2009; Robinson 1988a, 1988b, 1990, 1999).

Microcharacters	Chrysolaena	Lepidaploa	Lessingianthus
Trichomes of corolla lobes	Glandular and non-glandular, rarely only non-glandular	Glandular	Glandular and non-glandular
Apical anther appendages	Glandular, rarely non-glandular	Rarely glandular	Non-glandular
Basal stylar node	Present or absent	Present	Rarely present
Cypselae	Glandular, rarely non-glandular	Non-glandular, rarely glandular	Non-glandular
Ovary wall crystals	Cubic, prismatic, estiloid, druses	Prismatic	Cubic

presents  $x = 14, 15$  or  $16$  and pollen grains 'C', 'D' or 'G' (Angulo & Dematteis, 2010; Dematteis, 2009).

According to Robinson (1988b), although *Chrysolaena* violates the generic boundaries between *Lessingianthus* and *Lepidaploa* in some secondary characters, the presence of glands on the apical anther appendage is the only microcharacter that allows separation of the genus from the other members of the complex *Lepidaploa*. Although some species lack glands, their presence is too consistent in the genus to be ignored. In this sense, the results obtained here support the points made by the author. The presence of apical anther appendage and glandular cypselae are common characteristics among species *Chrysolaena*. In addition to these, this study shows that presence of glands on the style and corollas is another consistent characteristic in the genus. However, the absence of basal stylar node would not be a diagnostic character since this varies widely among species.

Regarding ovary wall crystals, contrary to what is stated in previous studies (Robinson, 1988b), *Chrysolaena* may have more than one type of crystal (cubic, prismatic, styloid, and druses), and this allows differentiation from *Lessingianthus* and *Lepidaploa* which have one type of crystal (Dematteis, 2009).

At species level, the results show that *Chrysolaena* species differ from each other by having different combinations of microcharacters. The genus presents some species closely similar morphologically and these micro-morphological differences are useful to differentiate them. *Chrysolaena cristobaliana*, *C. cognata*, *C. sceptrum*, *C. platensis*, and *C. cordifolia*, for example, are a closely related group. The results of this study shows that they can be differentiated by the shape of the apical anther appendages, the trichome types on corolla lobes, the presence of basal node and the cypselae shape (see Tables 1 and 2).

*Chrysolaena dusenii* and *C. nicolackii* are very similar in morphology and geographical distribution. Micro-morphologically they can only be differentiated by the florets number of capitula and the leaves being either discolorous (*C. dusenii*) or concolorous (*C. nicolackii*) (Dematteis, 2009). Micro-morphologically they differ from each other by the fruit shape and because *C. nicolackii* has trichomes

type IIIB on the corolla tube and *C. dusenii* may have trichomes type I on corolla lobes.

Another group of related species is *C. guaranitica*, *C. desertorum*, and *C. simplex*. *Chrysolaena guaranitica* and *C. desertorum* differ from *C. simplex* by monocephalic inflorescence; and *C. desertorum* differs from *C. guaranitica* by glabrous corolla lobes (Dematteis, 2009). However, in this study we observed specimens of *C. desertorum* with glabrous corolla and glandular and/or non-glandular trichomes (type I or II). Besides, *C. guaranitica* and *C. simplex* are distinguished from *C. desertorum* by the absence of glands on corolla lobes (it has non-glandular trichomes type I and occasionally type II), on style, cypselae, and the orbicular shape of apical anther appendage. The trichomes type IV on the corolla tube distinguishes *C. simplex* from *C. guaranitica*.

By the arrangement of leaves in a basal rosette, *C. oligophylla*, *C. propinqua*, and *C. flexuosa* are related species. However, *C. oligophylla* differs from these two entities by the pedunculate heads and the umbeliform or pseudo-umbeliform inflorescence; while *C. propinqua* differs from *C. flexuosa* by cylindrical or narrowly campanulate involucre (Dematteis, 2009). The different combinations of trichomes on floral structures allow differentiation.

*Chrysolaena campestris* and *C. lithospermifolia* are similar. Both entities are mainly differentiated by the bracts size of the inflorescence and the leaf length (Dematteis, 2009). *Chrysolaena campestris* is distinguishable by the presence of non-glandular trichomes on corolla lobes and apical anther appendages in addition to glands. Based on their morphological similarity, *C. candelabrum* and *C. verbascifolia* have been considered as synonyms (Zuloaga, Morrone, Belgrano, Marticorena, & Marchesi, 2008). However, they differ by the indumentum type and phyllaries shape (Dematteis, 2009). Micro-morphologically, *C. verbascifolia* can also be distinguished from *C. candelabrum* by the type IIIB trichomes on corolla lobes and turbinate cypselae. *Chrysolaena candelabrum* is distinguished by the four types of calcium oxalate crystals on the ovary walls.

*Chrysolaena obovata* has some resemblance to *C. cristobaliana* and *C. sceptrum*, from which it differs by ovate

leaves and the florets number per capitula (Dematteis, 2009). From the point of view of microcharacters it differs from these two entities because its style, anther appendages and cypselae are non-glandular and its cypselae are cylindrical-turbinate.

## Conclusions

This study analysed for the first time the floral microcharacters of genus *Chrysolaena*. Microcharacters previously studied were considered, and also new characters were revealed, which contributed taxonomically. The presence/absence and types of trichomes on different floral and fruit structures (corolla, style and anthers), and also the shape of the apical anther appendage were the most informative. Besides, the types of trichomes of the floral structures and cypselae were described and typified for the first time.

The results indicate that most of the floral microcharacters of *Chrysolaena* analysed are quite consistent in the genus. However, until now, the pollen morphology and the chromosome base number are most useful for separating *Chrysolaena* from the related genera *Lessingianthus* and *Lepidaploa*.

At species level, the results show that related species can be discriminated by the different combinations of microcharacters. The value of floral microcharacters as taxonomic criterion could be increased if combined with other lines of evidence such as pollen morphology and chromosome number.

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No potential conflict of interest was reported by the authors.

## Supplemental data

Supplemental material for this article can be accessed here: <http://dx.doi.org/10.1080/14772000.2015.1134699>

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