

Research

The taxonomic utility of micromorphology in *Lepidaploa* (Vernonieae: Asteraceae)

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Nordic Journal of Botany

2018: e01686

doi: 10.1111/njb.01686

Subject Editor: Bertil Ståhl
Editor-in-Chief: Torbjörn Tyler
Accepted 2 May 2018

Lepidaploa belongs to tribe Vernonieae, one of the most complex tribes of Asteraceae, and the relationships within *Lepidaploa* and among related genera are poorly understood. Microcharacters may be of taxonomic value and may be used in the identification of taxa at different ranks. To evaluate the reliability of microcharacters as taxonomic markers in this group, we analysed the micromorphology of phyllaries, florets and cypselae in detail in 23 species of *Lepidaploa*. The species were studied using stereo, light, and scanning electron microscopy. Eight trichome types (eglandular and glandular) were observed on phyllaries, florets and cypselae, in addition to crystals, idioblasts and other microstructures. The results demonstrates that the occurrence of different combinations of trichome types and crystals, presence of a stylar basal node, idioblasts and glandular apical anther appendages are highly useful to differentiate between related species of *Lepidaploa* and a diagnostic key using these characters is presented. However, these characters are not of much use to distinguish between closely related genera of Vernonieae since most characters appear homeoplastic and are found in representatives of different genera.

Keywords: anthers, crystals, cypselae, idioblasts, phyllaries, style, trichome

Introduction

Microcharacters are structures such as trichomes, crystals and idioblasts and these are considered to be of great taxonomic value in many different angiosperm groups (Franceschi and Horner 1980, Lersten and Horner 2000, Fuller and Hickey 2005, Méric 2009, Santos-Silva et al. 2013). Cassini (1813a, b, c, 1814, 1816a, b, 1817, 1818a, b, 1819a, b) was a pioneer in studies of floral microcharacters in Asteraceae and he used them to circumscribe several tribes. These kinds of characters have since been repeatedly used as a taxonomic tool in Asteraceae (Robinson 1988a, b, 1990, 1999, Isawumi 1999, Méric 2009, Redonda-Martínez et al. 2012, 2016, Wagner et al. 2013, Ângulo and Dematteis 2014, Ângulo et al. 2015, Via do Pico et al. 2016, Redonda-Martínez 2017).

The Vernonieae is one of the major tribes of Asteraceae, including 126 genera and approximately 1500 species, mostly distributed in the tropical regions of the world



(Keeley and Robinson 2009). The tribe has been considered one of the taxonomically most complex tribes of Asteraceae and has been denominated as the “evil tribe” (Keeley and Robinson 2009). In a taxonomic review of American Vernoniae, Robinson (1999) split the genus *Vernonia* Schreb. into several genera based mainly on micromorphological, cytological and palynological characters. Currently, *Vernonia* sensu stricto includes 22 species, the majority distributed in eastern North America (including the type species), and with five in central Mexico and two in South America (Robinson 1999, Keeley and Robinson 2009).

Lepidaploa (Cass.) Cass. is one of the genera resurrected from *Vernonia* sensu lato by Robinson (1990). It comprises 150 species, most of which occur in South America (Angulo et al. 2012). In general, *Lepidaploa* is characterized by sessile heads, nodular style bases, sometimes glandular cypselae, no glands on the anther appendages, elongated raphids on cypselae and pollen of the types C (pollen tricolporate and with polar lacuna), D (pollen triporate and without polar lacuna) and G (pollen tricolporate and without polar lacuna) (Robinson 1990, 1999, Keeley and Robinson 2009). According to molecular studies, this genus is the sister group of *Chrysolaena* H. Rob., *Echinocoryne* H. Rob. and *Lessingianthus* H. Rob. (Keeley et al. 2007, Keeley and Robinson 2009, Loeuille et al. 2015). However, the characters cited by Robinson (1990, 1999) as characteristic of the genus *Lepidaploa* are shared with *Chrysolaena* and *Lessingianthus* (Angulo and Dematteis 2010a, 2014, Via do Pico and Dematteis 2013, Marques and Dematteis 2014, Angulo et al. 2015, Via do Pico et al. 2016). For example, some species of *Lepidaploa* have floral microcharacters that also occur in *Chrysolaena* or *Lessingianthus*, such as the presence of glands on anthers and cypselae, elongated crystals in cypselae walls, and a basal stylar node (Angulo and Dematteis 2014, Via do Pico et al. 2016). However, microcharacters are known only for a few species of *Lepidaploa* and no detailed micromorphological study is available for the genus as a whole.

In this study we describe and compare the micromorphology of phyllaries, florets and cypselae in species of *Lepidaploa* from southern South America (Argentina, southern Brazil, Bolivia and Paraguay), exploring taxonomic differences among the species studied and related genera. A key to species of *Lepidaploa*, based on microcharacters, is presented for the first time.

Material and methods

Mature phyllaries, florets and cypselae of 23 species of *Lepidaploa* were studied using stereo, light and scanning electron microscopy (SEM). At least four different florets and cypselae per specimen were examined. Inner and outer phyllaries of three heads per specimen were studied. In total, three specimens per species were analysed, depending on availability of specimens. We selected samples from different

regions for each species, analysing specimens from different countries within the species distribution range. The specimens for this study were obtained from the herbarium of the Instituto de Botánica del Nordeste (CTES). A complete voucher list is given in Appendix 1.

For the micromorphological analyses, florets were softened in boiling water containing a drop of detergent, dissected under a stereomicroscope and mounted in Hoyer's solution (Anderson 1954, King and Robinson 1970). For observations of phyllaries and cypselae, the phyllaries and fruits were softened in distilled water for 5 min, and then cleared in sodium hypochlorite:water (1:1). Transparent samples were washed in running water, dissected under a stereomicroscope and mounted in Hoyer's solution (Anderson 1954, King and Robinson 1970). All samples were examined and photographed using a Zeiss Axioplan microscope equipped with a digital camera.

For SEM analysis, samples were first dehydrated and then immersed in CO₂ for critical point drying before sputter-coating with gold-palladium. Coated samples were examined and photographed using a JEOL 5800 LV scanning electron microscope.

The terminology of trichome morphology follows Metcalfe and Chalk (1950), Ramayya (1962), Payne (1978), Narayana (1979), Luque et al. (1999) and Werker (2000), and that of the other microcharacters follows Robinson (1988a, b, 1990, 1999), Roque et al. (2009) and Angulo et al. (2015).

For the statistical analysis, a data matrix with 23 operational taxonomic units (OTUs) and 11 qualitative variables was constructed (Supplementary material Appendix 1 Table A1). The crystals of the cypselae, the base of anther thecae and apical appendages were not considered because these showed high intraspecific variation. The remaining characters were coded as binary (Table 1). A cluster analysis UPGMA (unweighted pair group method with arithmetic mean) using the Manhattan distance metric was performed. The program InfoStat ver. 2013 was used for both analyses (Di Rienzo et al. 2013).

Table 1. List of characters and their coding states.

Microcharacter
1. Glandular trichome on corolla: 0: absent; 1: present
2. Arrangement of trichomes on corolla: 1: only inner; 2: inner and marginal
3. Glandular trichome on apical anther appendage: 0: absent; 1: present
4. Basal stylar node: 1: enlarged; 2: narrow
5. Crystals on basal stylar node: 0: absent; 1: present
6. Glandular trichome on style branches: 0: absent; 1: present
7. Glandular trichome on cypselae: 0: absent; 1: present
8. Idioblasts on cypselae: 0: absent; 1: present
9. Glandular trichome on phyllaries: 0: absent; 1: present
10. Crystals on carpodium: 0: absent; 1: present
11. Crystals on phyllaries: 0: absent; 1: present

Data deposition

Data available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.p5q4ph6>> (Marques et al. 2018).

Results

The micromorphological data of florets, cypselae and phyllaries of the 23 species of *Lepidaploa* studied here are summarized in Table 2 and 3. In addition, a key based on these micromorphological features is provided below.

Trichomes

Trichomes observed on florets, cypselae and phyllaries of *Lepidaploa* were variable in shape, number of cells, type (glandular or not), and density. In total, we found eight types of trichomes on corolla lobes, phyllaries and cypselae. Corolla lobes displayed seven types of trichomes, whereas the phyllaries and cypselae exhibited six and two types of trichomes, respectively. Following Metcalfe and Chalk (1950), the trichomes were classified into two categories: non-glandular and glandular. Within these groups, seven trichome types are eglandular (I to VII) and one is glandular (VIII).

Corolla pubescence

All the species analysed showed papillae (Fig. 1a, 3a) on the corolla lobes. In general, papillae are short and composed of rounded cells distributed on the tip of the corolla lobes (Fig. 1a–b). Non-glandular and glandular trichomes were observed on the corolla lobes, as well as on style branches, phyllaries and cypselae. Eight types (Table 2, 3) of trichomes were observed and classified as follows:

Type I: simple conical trichome (Fig. 1b–c, 3a): eglandular, multicellular, apical conical cell with acute apex, foot formed by 1 or 2 cells, the side walls thickened. Present on corolla lobes, style branches and phyllaries.

Type II: simple lageniform trichome (Fig. 1d, 3b): eglandular, multicellular, apical bottle-shaped cell with acute or obtuse apex and narrow base, foot formed by 1 or 2 cells, the side walls thin and the cytoplasm blackish. Present on corolla lobes.

Type III: simple filiform trichome (Fig. 2k): eglandular, thin, multicellular, composed of two or three cells with the terminal cell longer than the other cells, foot formed by 1 or 2 cells, the side walls thin. Present on corolla lobes and phyllaries.

Type IV: simple curly trichome (Fig. 2l, 4k): eglandular, curly, multicellular, composed of two or three cells with the terminal cell longer than the other cells, and with acute apex, foot formed by 1 or 2 cells, the side walls of all cells thin. Present on phyllaries.

Type V: simple digitiform trichome (Fig. 3c): eglandular, cylindrical, multicellular, composed of three cells, with intermediate cells smaller than the other cells, foot formed by

1 or 2 cells. This trichome has an acute apex. The side walls are thin. Present on corolla lobes.

Type VI: branched two-armed trichome (Fig. 1g): eglandular, multicellular, branched, composed of a uniseriate basal part with one or two cells. The apical cell is transversal and enlarged with two arms of different sizes, which are joined at the middle to the basal (s) cell (s), foot formed by 1 or 2 cells. Present on corolla lobes and phyllaries.

Type VII: twin hair, biseriate filiform trichome (Fig. 1e–f, 2e, 3d, 4f–g): eglandular, multicellular, biseriate, composed of four cells of thickened walls, two basal cells and two filiform, parallel apical cells with acute (Fig. 1e) or obtuse (Fig. 1f) apex (biseriate digitiform trichome), foot formed by 1 or 2 cells. The two apical cells can have the same length or vary in length and then separated at the apex. Present on corolla lobes and fruit pericarp.

Type VIII: biseriate vesicular glandular trichome (Fig. 1h–l, 2d, 3e–f, 3l, 4a, d–e, h, l): glandular, multicellular, composed of a biseriate body of four to eight cells in each row. This trichome has a cuticular vesicle in the apex. Present on corolla lobes, style branches, fruit pericarp and phyllaries.

Anther apical appendage

The apical anther appendages presented two variable shapes (lanceolate or ovate) and different apices: obtuse (Fig. 1j, 3i), acute (Fig. 1k, 3h), bilobed and apiculate (Fig. 1l, 3g). The majority of the species displayed apical appendages without glands, the exception being *Lepidaploa pseudomuricata* (Fig. 3l, 4a, Table 2).

Base of anther thecae

All species of *Lepidaploa* have calcarate anthers, with the thecae prolonged below the point of filament insertion. However, this prolongation has two shapes: sagittate (Fig. 3j) and obtuse (Fig. 3k). In some species we observed both shapes (Table 2).

Style branches

Species of *Lepidaploa* have a 'vernonioid' type of style, with thin branches with an acute apex and numerous type I trichomes (Fig. 4c–d), which extend from below the bifurcation to the apex of the branches. The inner surface of the branches has numerous papillae. In addition to type I trichomes, four species had type VIII glandular trichomes (Fig. 2d, 4d–e) on the outer surface of the style branches: *L. chamissonis*, *L. psilostachya*, *L. pseudomuricata* and *L. remotiflora*.

Basal stylar node

The basal stylar node is a ring of sclerified cells located at the style base. Almost all species of *Lepidaploa* have a well-developed ring or enlarged style base (base diameter = 140–350 μm) (Fig. 2b, 4b). However, *L. balansae*

Table 2. Floral microcharacters in *Lepidaploa*. The 'x' indicates the presence of a character, * enlarged base = 140–250 µm diam.; narrow base = 57–70 µm diam.

Species	Trichome type on corolla	Arrangement of trichomes on corolla	Shape of apical appendages	Anthers				Style					
				Glandular trichomes		Basal shape		Glandular trichomes		Basal stylar node* (enlarged or narrow)		Crystals	
				Present	Absent	Absent	Basal shape	Present	Absent	Present	Absent	Present	Absent
<i>L. amambaia</i>	I	only inner	obtuse	x		x	obtuse	x		x	enlarged		x
<i>L. argyrotricha</i>	I, V	only inner	acute or bilobate	x		x	sagittate	x		x	enlarged		x
<i>L. balansae</i>	I, III, VIII	only inner	acute	x		x	sagittate	x		x	narrow		x
<i>L. bakerana</i>	I	only inner	acute or apiculate	x		x	sagittate	x		x	enlarged		x
<i>L. beckii</i>	I	inner and marginal	acute	x		x	sagittate	x		x	enlarged		x
<i>L. buchtienii</i>	I, VII, VIII	only inner	acute	x		x	sagittate	x		x	enlarged		x
<i>L. canescens</i>	I	only inner	acute	x		x	sagittate	x		x	enlarged		x
<i>L. chamissonis</i>	I, VIII	only inner	acute	x		x	sagittate	x		x	enlarged		x
<i>L. cordifolia</i>	I	inner and marginal	acute	x		x	sagittate	x		x	enlarged		x
<i>L. costata</i>	I, VIII	only inner	acute or obtuse	x		x	sagittate or obtuse	x		x	enlarged		x
<i>L. deflexa</i>	V, VIII	only inner	apiculate	x		x	obtuse	x		x	enlarged		x
<i>L. eriolepis</i>	I, II	only inner	obtuse	x		x	obtuse	x		x	enlarged		x
<i>L. fourmetii</i>	I, VIII	only inner	apiculate, acute or obtuse	x		x	obtuse	x		x	enlarged		x
<i>L. myriocephala</i>	I, III, VII	only inner	acute or obtuse	x		x	sagittate or obtuse	x		x	enlarged		x
<i>L. mapirensis</i>	III, VII, VIII	only inner	apiculate, acute or obtuse	x		x	sagittate	x		x	enlarged		x
<i>L. novarae</i>	VII, VIII	only inner	acute	x		x	obtuse	x		x	enlarged		x
<i>L. psilostachya</i>	III, VII	only inner	obtuse, apiculate or bilobate	x		x	sagittate	x		x	enlarged		x
<i>L. pseudomuricata</i>	VIII	only inner	acute or obtuse	x		x	sagittate	x		x	narrow		x
<i>L. remotiflora</i>	VIII	only inner	acute	x		x	sagittate	x		x	enlarged		x
<i>L. salzmännii</i>	III, VI, VIII	only inner	acute	x		x	sagittate	x		x	enlarged		x
<i>L. setososquamosa</i>	I	only inner	acute or obtuse	x		x	obtuse	x		x	enlarged		x
<i>L. sordidopapposa</i>	I	only inner	obtuse	x		x	obtuse	x		x	enlarged		x
<i>L. tarijensis</i>	I	only inner	obtuse or apiculate	x		x	obtuse	x		x	enlarged		x

Table 3. Cypselae and phyllary microcharacters in *Lepidaploa*.

Species	Cypselae						Phyllaries		
	Trichome type	Idioblasts	Crystals			Crystal types on carpodium	Trichome types	Crystal type	
			Cubic	Styloid	Prismatic				Druses
<i>L. amambaia</i>	VII			x			III, VIII		
<i>L. argyrotricha</i>	VII			x	x		III		
<i>L. balansae</i>	VII		x	x	x	druses	III, VIII		
<i>L. bakerana</i>	VII			x	x	druses	IV		
<i>L. beckii</i>	VII	x			x		III		
<i>L. buchtienii</i>	VII	x	x	x	x	druses	III		
<i>L. canescens</i>	VII		x	x	x	x	druses and styloid	IV	
<i>L. chamissonis</i>	VII, VIII		x	x	x	druses	III, VIII		
<i>L. cordiifolia</i>	VII	x			x	druses	I, IV		
<i>L. costata</i>	VII	x			x	druses	I, III, IV, VIII		
<i>L. deflexa</i>	VII		x	x	x	x	druses	I, III	
<i>L. eriolepis</i>	VII		x	x	x	x	druses	I, III	
<i>L. fournetii</i>	VII, VIII				x		I, III		
<i>L. myriocephala</i>	VII		x		x		III		
<i>L. mapirensis</i>	VII		x		x	druses	III		
<i>L. novarae</i>	VII	x		x	x	x	druses	III	styloid
<i>L. psilostachya</i>	VII	x		x	x		III		
<i>L. pseudomuricata</i>	VII, VIII		x	x	x	x	druses	I, III	
<i>L. remotiflora</i>	VII	x		x	x		III		
<i>L. salzmännii</i>	VII, VIII	x			x	x	druses	I, IV, VI, VII, VIII	
<i>L. setososquamosa</i>	VII, VIII	x		x	x		druses, cubic, styloid	IV, VIII	
<i>L. sordidopapposa</i>	VII	x					druses	III, IV	druses
<i>L. tarijensis</i>	VII			x	x		druses	III, VIII	

and *L. pseudomuricata* have a narrow node (base diameter: 57–70 μm) (Fig. 2a). In addition, calcium oxalate (CaOx) crystals (styloid, cubic, and druses) were observed in the style base of nine species (Fig. 2c).

Cypselae pubescence

All species examined showed type VII trichomes (twin hair) on the fruit surface (Fig. 2e, 4f–h). However, *L. chamissonis*, *L. fournetii*, *L. pseudomuricata*, *L. salzmännii* and *L. setososquamosa* also displayed glandular type VIII trichomes (Fig. 4h).

Idioblasts

In addition to twin hairs, idioblasts were observed in nine species. Idioblasts are larger than the other cells of the uniseriate epidermis and are more or less ellipsoid or rounded structures with thickened and lignified walls. These cells are irregularly distributed on the fruit, scattered or longitudinally forming groups of two to many cells. (Fig. 2f–g, 4i–j).

Crystals

Calcium oxalate crystals occur in almost all species, with the exception of *L. sordidopapposa* (Table 3). Crystals are of different size, shape and distribution (Fig. 2h). Prismatic (rectangular or hexagonal in surface view), cubic, styloid,

and druse crystals were observed on the fruit wall. However, the fruits of most species have a combination of one or two crystal types (Table 3).

Carpodium

The carpodium is the scar of the abscission zone on the cypselae base formed by one or more rows of cells that are usually morphologically distinct from the other cells of the fruit wall (Roque et al. 2009). In *Lepidaploa*, the carpodium is a complete ring (symmetrical) with more than three rows of oblong cells of thickened walls (Fig. 2i, 4g–h). Most of the species have druses in the carpodium (Table 2, Fig. 2j). However, *L. canescens* also has styloid crystals, whereas *L. setososquamosa* has druses, styloid and cubic crystals in the carpodium.

Phyllaries

Six different trichome types (I, III, IV, VI, VII, VIII) were observed on the phyllaries (Fig. 2k–l, 4k–l). Type I was observed only in *L. costata* and *L. salzmännii*. Most of the species (17 spp.) have type III trichomes (Fig. 2k). Type IV trichomes was observed in *L. bakerana*, *L. canescens*, *L. cordiifolia*, *L. costata*, *L. salzmännii*, *L. setososquamosa* and *L. sordidopapposa* (Fig. 2l, 4k), whereas type VIII was found on the phyllaries of *L. amambaia*, *L. balansae*, *L. chamissonis*, *L. costata*, *L. salzmännii*, *L. setososquamosa* and *L. tarijensis* (Fig. 4l). *Lepidaploa salzmännii* showed the richest flora of

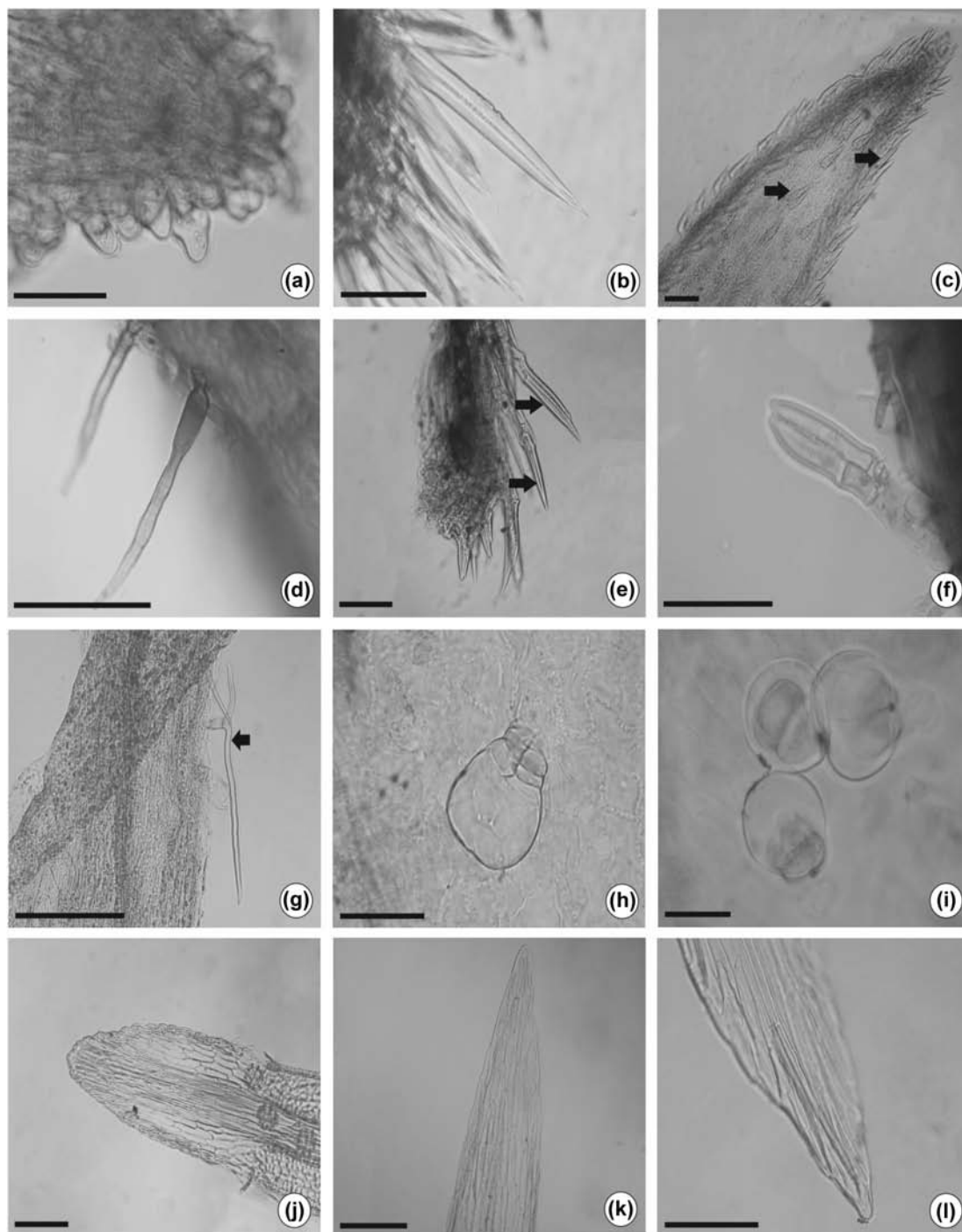


Figure 1. Microcharacters of *Lepidaploa* (LM). (a) *L. balansae*: corolla lobe with papillae; (b) *L. argyrotricha*: corolla lobe with trichome type I; (c) *L. beckii*: corolla lobe with trichome type I on inner surface and margin (black arrow); (d) *L. eriolepis*: corolla lobe with trichome type II; (e) *L. mapiensis*: corolla lobe with trichome type VII. (f) *L. novarae*: corolla lobe with trichome type VII. (g) *L. salzmanni*: corolla lobe with trichome type VI (black arrow). (h–i) *L. balansae*: corolla lobe with trichome type VIII; (j) *L. amambaia*: ovate apical anther appendage with obtuse apex; (k) *L. beckii* lanceolate apical anther appendage with acute apex; (l) *L. fournetii*: lanceolate apical anther appendage with apiculate apex. Scale bar: (b), (d–l) = 50 μm ; (a), (c) = 100 μm .

trichomes, with five different types (I, IV, VI, VII and VIII) (Table 3).

Lepidaploa novarae and *L. sordidopaposa* were the only species with crystals in the phyllaries, styloid and druses, respectively (Table 3).

Cluster analysis

The phenogram (Fig. 5) of the UPGMA shows a main group including 95% of species, characterized by the absence of type VIII glandular trichomes on the apical anther

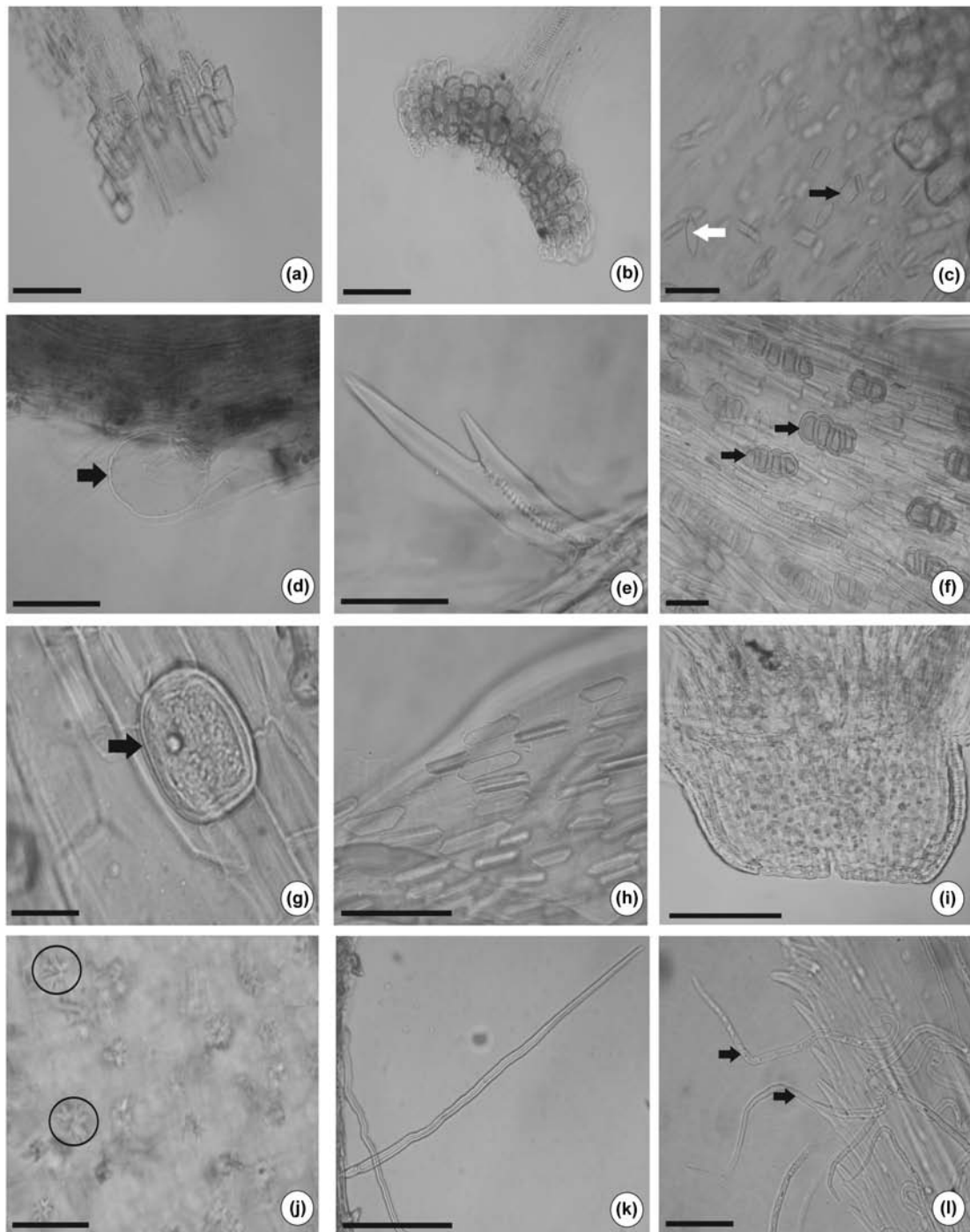


Figure 2. Microcharacters of *Lepidaploa* (LM). (a) *L. balansae*: style with narrow basal node; (b) *L. fournetii*: style with enlarged basal node; (c) *L. tarijensis*: stylar base with styloid (white arrow) and cubic (black arrow) crystals; (d) *L. psilostachya*: stylar branches with trichome type VIII (black arrow); (e) *L. costata*: cypsela with trichome type VII. (f) *L. setosquamosa*: cypsela with idioblasts (black arrow); (g) *L. buchtienii*: cypsela with idioblasts (black arrow); (h) *L. novarae*: cypsela with prismatic crystals; (i) *L. chamissonis*: carpodium with druses (black dots); (j) *L. canescens*: details of druses on carpodium (black circle); (k) *L. salzmannii*: phyllaries with trichome type III; (l) *L. bakerana*: phyllaries with trichome type IV (black arrow). Scale bar: (l) = 20 μm ; (h) = 30 μm ; (a–g), (i), (k) = 50 μm , (j) = 100 μm .

appendages, whereas *L. pseudomuricata* is isolated from the remaining taxa e.g. by the presence of glandular trichomes (type VIII) on the apical anther appendages. The large main group is divided into two groups. **Group 1** includes 14

species lacking crystals in the basal stylar node and phyllaries, and **Group 2** contains eight taxa with crystals in the basal stylar node of the style base. **Group 1** is divided into two subgroups: subgroup **1A**, comprising *L. cordifolia* and

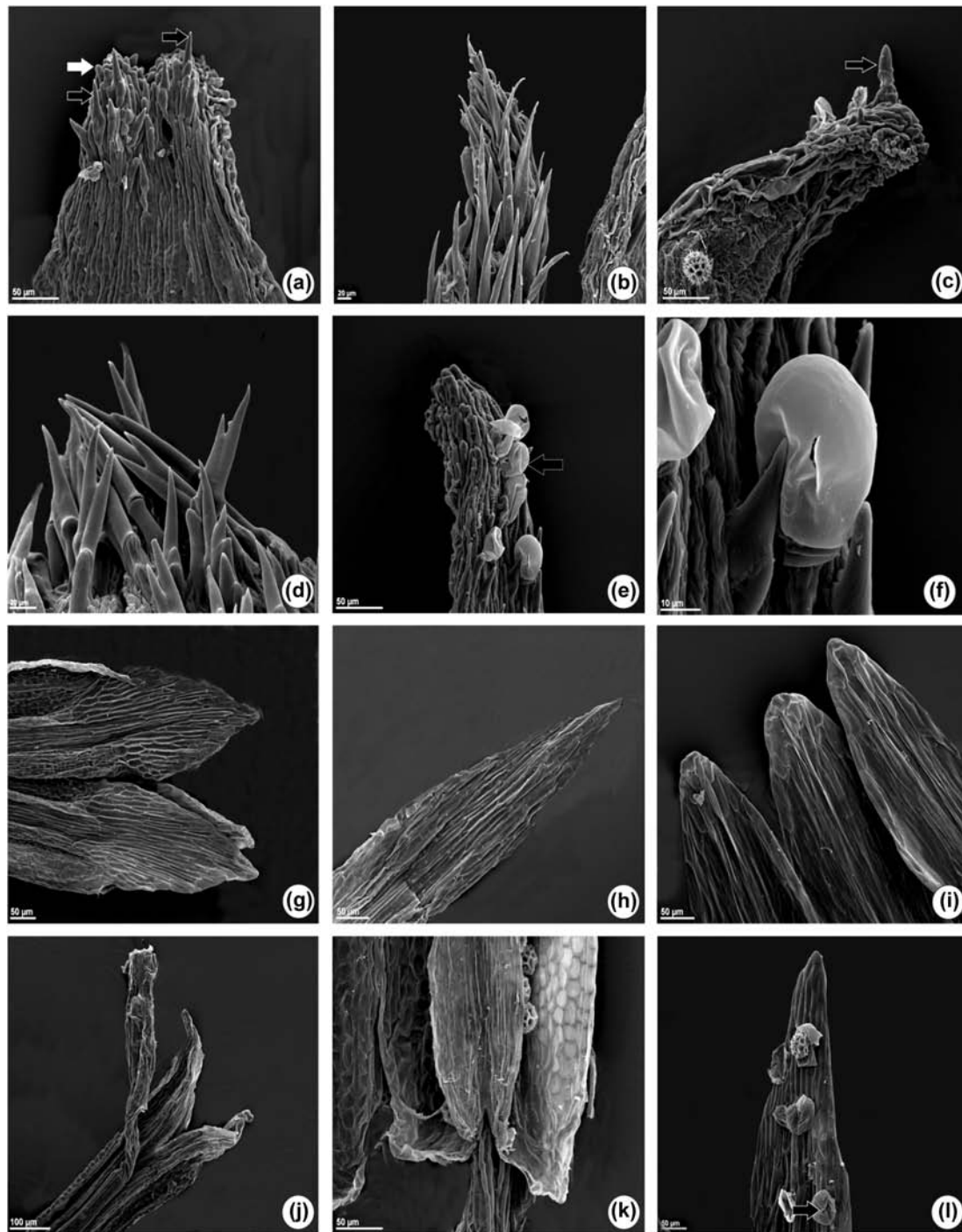


Figure 3. Microcharacters of *Lepidaploa* (SEM). (a) *L. bakerana*: corolla lobe with papillae (white arrow) and trichome type I (black arrow); (b) *L. eriolepis*: corolla lobe with trichome type II; (c) *L. buchtienii*: corolla lobe with trichome type V (black arrow); (d) *L. myriocephala*: corolla lobe with trichome type VII; (e–f) *L. chamissonis*: corolla lobe with trichome type VIII (black arrow); (g) *L. bakerana*: lanceolate apical anther appendage with apiculate apex; (h) *L. beckii*: lanceolate apical anther appendage with acute apex; (i) *L. buchtienii*: lanceolate apical anther appendage with obtuse apex; (j) *L. beckii* sagittate base of anther; (k) *L. costata*: obtuse base of anther; (l) *L. pseudomuricata*: apical anther appendage with trichome type VIII.

L. beckii, which have a marginal arrangement of trichomes on corolla lobes, and subgroup **1B** comprising seven species with an external arrangement of trichomes on this structure. The latter subgroup is further subdivided into two subgroups:

1Ba and **1Bb**. The first subgroup comprises two species with idioblasts on fruit walls (*L. costata* and *L. buchtienii*), and the latter subgroup (**1Bb**) is divided in two subgroups. Subgroup **1Bba** comprises four species with glandular

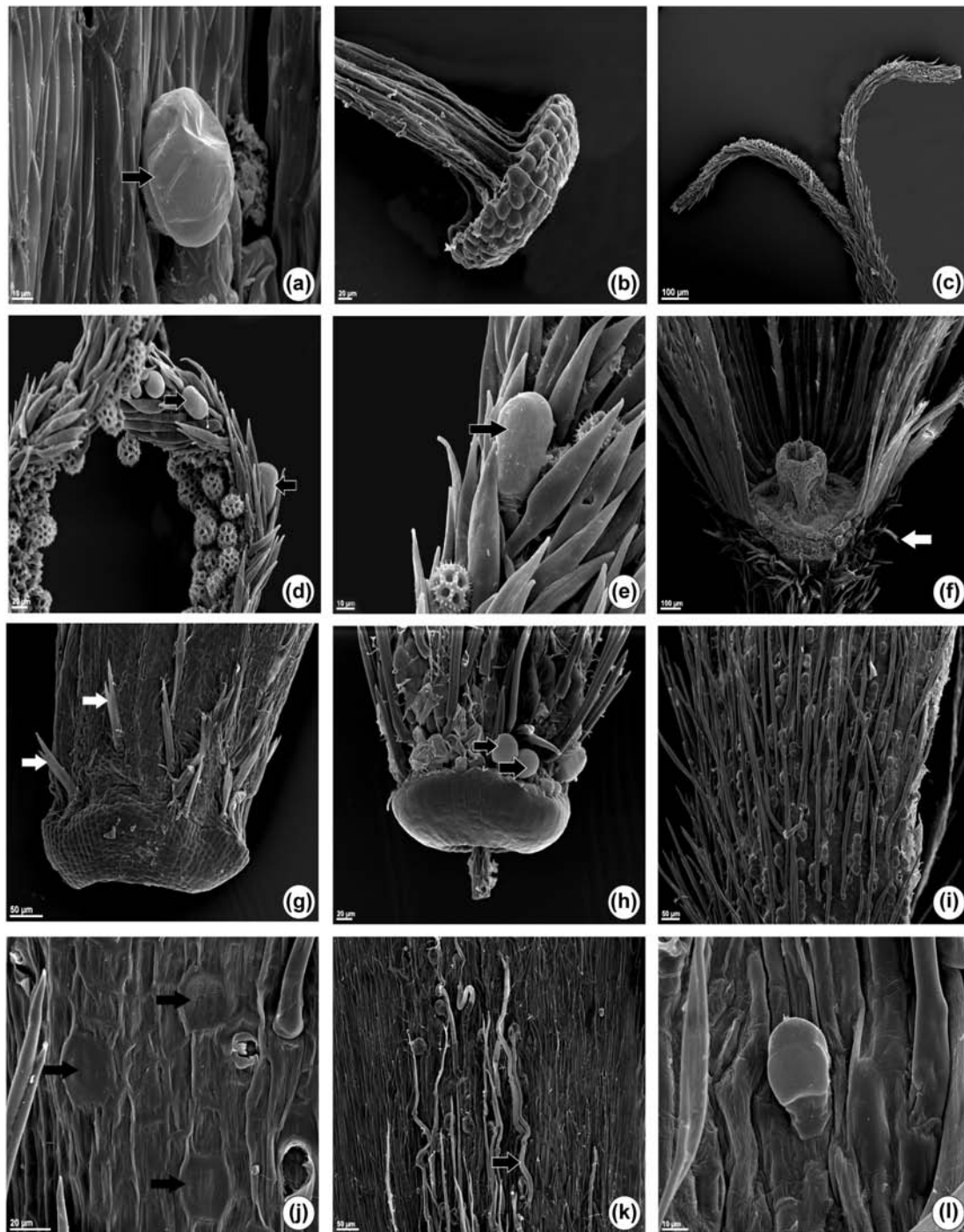


Figure 4. Microcharacters of *Lepidaploa* (SEM). (a) *L. pseudomuricata*: detail of trichome type VIII on apical anther appendage; (b) *L. fourmetii*: style with enlarged basal node; (c) *L. bakerana*: stylar branches; (d–e) *L. chamissonis*: stylar branches with trichome type VIII (black arrow); (f) *L. myriocephala*: apex of cypsel with trichome type VII (white arrow); (g) *L. balansae*: base of cypsel with trichome type VII (white arrow) and carpodium (basal zone); (h) *L. remotiflora*: base of cypsel with trichome type VIII (black arrow) and carpodium (basal zone); (i) *L. psilostachya*: cypselae with idioblasts (spherical structure); (j) *L. buchtienii*: idioblasts (black arrow); (k) *L. costata*: phyllaries with trichome type IV (black arrow); (l) *L. costata*: phyllaries with detail of trichome type VIII.

trichomes on the corolla lobes. Of these, *Lepidaploa balansae* appears isolated by having a poorly developed basal stylar node. Subgroup **1Bbb** comprises five taxa, which forms two subgroups of species with the same microcharacters (Table 1, 2).

Species in **Group 2** also formed two subgroups: **2A** and **2B** based on the presence or absence of glandular trichomes on phyllaries. The former comprises four taxa grouped into two subgroups (**2Aa** and **2Ab**) that differ in the presence (**2Aa**)

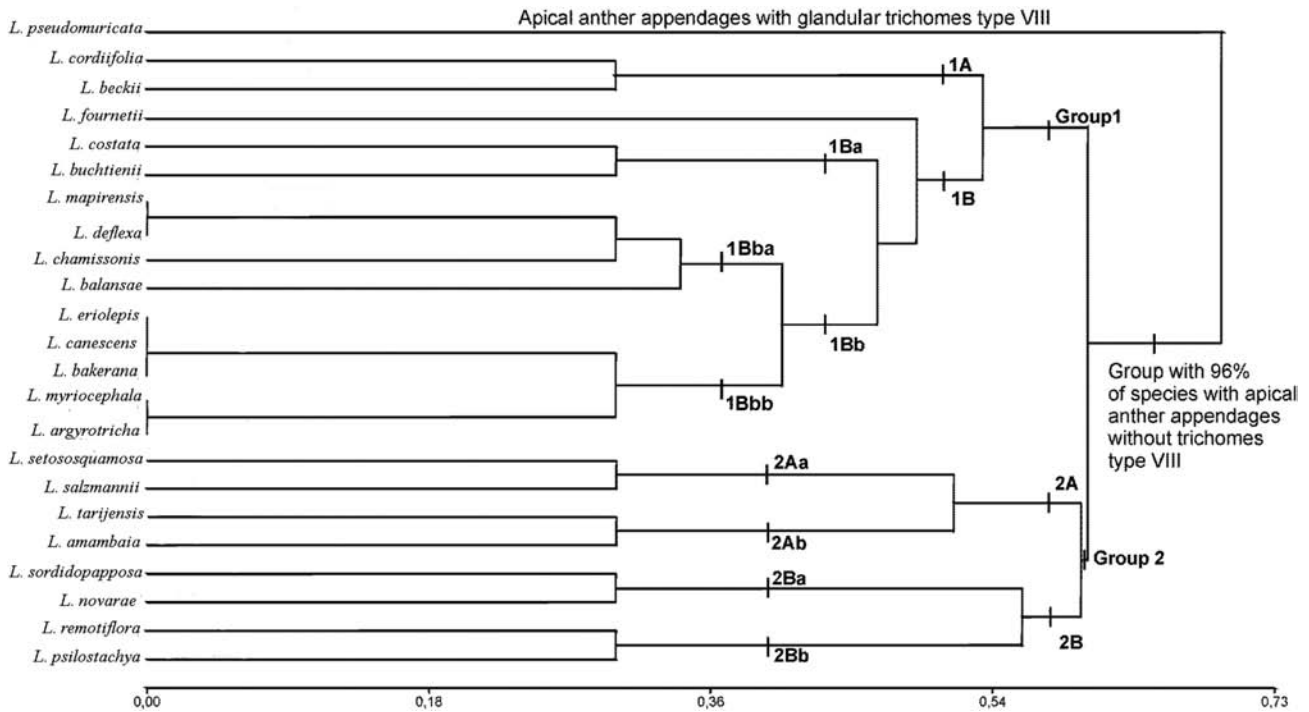


Figure 5. Phenogram of 11 characters and 23 OTUs resulting from UPGMA.

or absence (**2Ab**) of glandular type VIII trichomes on cypsela and idioblasts of the fruit wall. **Group 2B** also comprises four species that form two subgroups: **2Ba** and **2Bb**, which are distinguished by the presence (**2Ba**) or absence (**2Bb**) of crystals in the carpodium and phyllaries and type VIII glandular trichomes on style branches.

Key to species of *Lepidaploa* from southern South America based on floral microcharacters

- | | |
|---|--------------------------|
| 1. Style base with calcium oxalate crystals | 2 |
| – Style base without calcium oxalate crystals | 10 |
| 2. Cypselae without idioblasts | 3 |
| – Cypselae with idioblasts | 5 |
| 3. Type VIII glandular trichomes present on apical anther appendages, style branches and cypselae; basal stylar node not enlarged; prismatic, cubic, and styloid crystals in the fruit wall | <i>L. pseudomuricata</i> |
| – Type VIII glandular trichome absent on apical anther appendages, style branches and cypselae; basal stylar node enlarged; prismatic and styloid crystals in the fruit wall | 4 |
| 4. Cypselae with prismatic and styloid crystals; carpodium with crystals | <i>L. tarijensis</i> |
| – Cypselae only with styloid crystals; carpodium without crystals | <i>L. amambaia</i> |
| 5. Cypselae and phyllaries with type VIII glandular trichomes | 6 |
| – Cypselae and phyllaries without type VIII glandular trichomes | 7 |
| 6. Corolla lobes with types III and VI eglandular trichomes, as well as type VIII glandular trichomes; phyllaries with types I, IV, VI, VII eglandular trichomes | <i>L. salzmannii</i> |
| – Corolla lobes only with type I eglandular trichomes, without type VIII glandular trichome; phyllaries only with type IV eglandular trichomes | <i>L. setososquamosa</i> |
| 7. Phyllaries with styloid and druse crystals; style branches without type VIII glandular trichomes | 8 |
| – Phyllaries without crystals; style branches with type VIII glandular trichomes | 9 |
| 8. Corolla lobes with types VI and VIII non-glandular trichomes; style branches with type VIII glandular trichomes; phyllaries only with type III eglandular trichomes and styloid crystals. | <i>L. novarae</i> |
| – Corolla lobes only with type I eglandular trichomes; style branches without type VIII glandular trichome; phyllaries with types III and IV eglandular trichomes and druse crystals. | <i>L. sordidopapposa</i> |
| 9. Corolla with eglandular trichomes | <i>L. psilostachya</i> |
| – Corolla with glandular trichomes | <i>L. remotiflora</i> |
| 10. Margin of corollas with trichomes | 11 |
| – Margin of corollas without trichomes | 12 |
| 11. Carpodium without druses. | <i>L. beckii</i> |
| – Carpodium with druses. | <i>L. cordifolia</i> |
| 12. Corolla lobes without glandular trichomes | 13 |
| – Corolla lobes with glandular trichomes | 17 |
| 13. Corolla lobes with only type I eglandular trichomes; phyllaries with type IV eglandular trichomes | 14 |
| – Corolla lobes with other eglandular trichomes in addition to type I; phyllaries with other trichome types | 15 |

14. Cypselae with styloid and prismatic crystals; carpodium with druse crystals *L. bakerana*
– Cypselae with prismatic, cubic, styloid and druse crystals; carpodium with druse and styloid crystals *L. canescens*
15. Corolla lobes with type II eglandular trichomes; carpodium with druses. *L. eriolepis*
– Corolla lobes without type II eglandular trichomes; carpodium without druses 16
16. Corolla lobes with type I and V eglandular trichomes; cypselae with styloid and prismatic crystals
..... *L. argyrotricha*
– Corolla lobes with type I, III and VII eglandular trichomes; cypselae with cubic crystals *L. myriocephala*
17. Fruit wall with idioblasts 18
– Fruit wall without idioblasts 19
18. Corolla lobes with type VII eglandular trichomes; phyllaries with type III eglandular trichomes; cypselae with cubic, prismatic and styloid crystals
..... *L. buchtienii*
– Corolla lobes without type VII eglandular trichomes; phyllaries with type I, III, IV and VIII trichomes; cypselae with prismatic crystals *L. costata*
19. Phyllaries with type I eglandular trichomes 20
– Phyllaries without type I eglandular trichomes 21
20. Corolla lobes with type V eglandular trichomes; cypselae without type VIII glandular trichomes *L. deflexa*
– Corolla lobes with type I eglandular trichomes; cypselae with type VIII glandular trichomes *L. fournetii*
21. Corolla lobes with type VII eglandular trichomes; cypselae without styloid crystals and without type VIII glandular trichomes; phyllaries without type VIII glandular trichome *L. mapirensis*
– Corolla lobes with other trichome types; cypselae with styloid crystals; phyllaries with type VIII glandular trichomes 22
22. Style branches and cypselae with type VIII glandular trichomes; style base enlarged *L. chamissonis*
– Style branches and cypselae without type VIII glandular trichomes; style base narrow *L. balansae*

Discussion

Corolla pubescence

Over the last few years, detailed studies have shown the species level taxonomic value of the corolla pubescence in *Lessingianthus*, with four trichome types (Angulo and Dematteis 2014), and *Chrysolaena* with five types (Via do Pico et al. 2016). However, *Lepidaploa* has seven trichome types on the corolla lobes, which allowed us to differentiate closely related species, indicating the relevance of these trichomes from a taxonomic viewpoint.

The presence of papillae on the corolla is constant in all species of *Lepidaploa*, as in *Chrysolaena* (Via do Pico et al.

2016). However, in other genera, such as *Leiboldia* Schtdl. ex Gleason, *Lepidonia* S. F. Blake (Redonda-Martínez et al. 2016), *Lessingianthus* (Angulo and Dematteis 2014) and *Baccharoides* Moench (Isawumi 1999), papillae can be present or absent on the corolla. Our present results show that trichomes in *Lepidaploa* are highly variable and of diagnostic value for some species.

Type I trichomes agrees morphologically with type I reported from *Lessingianthus* (Angulo and Dematteis 2014) and *Chrysolaena* (Via do Pico et al. 2016). This trichome type was also found on the style branches and phyllaries of some species of *Lepidaploa* (Table 2, 3). In other Vernoneae, such as *Vernonia peninsularis* C.B. Clarke, type I trichomes have also been observed on styles and bracts (Narayana 1979), as well as on the corolla and phyllaries of species of *Leiboldiinae* (Redonda-Martínez et al. 2016, Redonda-Martínez 2017).

Lageniform trichomes (type II) was only found on the corolla lobes of *Lepidaploa eriolepis*. This trichome type differs from those found by Redonda-Martínez et al. (2012) on the leaves of *Vernonia* and *Vernonanthura*, because type II trichomes has a dense cytoplasm and a narrow base. Moreover, it differs from type I (conical trichome) by their dark content, tapered base and larger size.

Type III trichomes, observed in some species of *Lepidaploa* on the corolla lobes and phyllaries, is common in Vernoneae and have previously been found on the corolla and bracts of the inflorescence of some species of *Vernonia* studied by Narayana (1979), on the corolla of *Lessingianthus* (Angulo and Dematteis 2014) and *Chrysolaena* (Via do Pico et al. 2016), and on the leaves of species of *Chronopappus* DC., *Eremanthus* Less., *Lychnophora* Mart., *Lychnophoriopsis* Sch. Bip., *Paralychnophora* MacLeish and *Piptolepis* Sch. Bip. (Wagner et al. 2013).

The simple digitiform trichome (type V) is similar to the trichomes found on the leaves of *Vernonia monosis* Sch. Bip., *V. shevaroyensis* Gamble (Narayana 1979) and *Chronopappus bifrons* (DC. ex Pers.) DC. (Wagner et al. 2013). However, the terminal cell of type V trichome observed in *Lepidaploa* is digitiform, whereas in both species of *Vernonia* (Narayana 1979) and in *C. bifrons* (Wagner et al. 2013) the terminal cell is filiform. Simple digitiform trichomes were observed for the first time on the corolla of Vernoneae in this study.

Trichome type VI was found exclusively in *Lepidaploa salzmannii*, and is the first record of this trichome type on the corolla in this genus. In the study of Narayana (1979), type VI trichomes were observed on the leaves of *Vernonia elaeagnifolia* DC. and *Cyanthillium cinereum* (L.) H. Rob.. Robinson (1992) also observed it on the corolla of some species of *Vernonia* s. str. Subsequently, Redonda-Martínez et al. (2012) also found type VI trichomes on the leaf surfaces of species of *Vernonia* and *Vernonanthura*. In addition, other related genera, such as *Anteremanthus* H. Rob., *Lychnophora* Mart. and *Vinicia* Dematt., also present type VI trichomes on the leaves (Wagner et al. 2013).

Type VII trichomes in species of *Lepidaploa* is morphologically similar to type II trichome observed on the corolla

lobes of *Chrysolaena* (Via do Pico et al. 2016). These authors considered that type II trichome is equivalent to twin hairs (biseriate eglandular trichome) found on the cypsela. In *Lepidaploa*, we also consider that the biseriate eglandular trichomes found on the corolla of some species is equivalent to the twin hairs observed on the cypsela. However, regarding the corolla of *Lepidaploa*, the apex of type VII trichome is usually variable, with some trichomes having an acute apex (biseriate filiform trichome) and others an obtuse apex (biseriate digitiform trichome). Type VII trichomes with an obtuse apex is morphologically similar to the trichomes found by Narayana (1979) on the cypsela wall of *Vernonia monosis*, and type VII trichome with acute apex is similar to the twin hairs on the cypsela of *Chrysolaena* (Via do Pico et al. 2016) and *Lessingianthus* (Angulo et al. 2015). According to Sancho and Katinas (2002), twin hairs on the corolla may be produced as a response to environmental characteristics.

Type VIII glandular trichomes are common in different reproductive and vegetative organs of Asteraceae, and is present in several species of Vernonieae. It was described by Robinson (1988a, b, 1990) as glands and can be found on the anther apex, corolla lobes and cypselae of *Lepidaploa*, as well as in *Chrysolaena* and *Lessingianthus*. However, our morphological study indicates that they are in fact trichomes and should not be considered glands because glandular trichomes are epidermal appendices formed by a few cells, while glands are complex structures consisting of many cells (Evert 2006, Angulo et al. 2015, Via do Pico et al. 2016). These trichomes were also found on the vegetative parts of some species of *Chrysolaena* (Apezato-da-Glória et al. 2012). In *Vernonia* and *Vernonanthura* this trichome type was also observed on leaves, corollas, anthers and fruits (Redonda-Martínez et al. 2012), and on the phyllaries of *Leiboldia guerreroana* (S.B. Jones) H. Rob. (Redonda-Martínez et al. 2016).

Style branches

Type I trichomes found on the style branches of *Lepidaploa* agrees with the morphology of trichome types found on the style branches of *Chrysolaena* and *Lessingianthus* (Angulo and Dematteis 2014, Via do Pico et al. 2016), and so cannot be used for distinguishing these genera. However, presence of type VIII glandular trichome allowed to differentiate four species of *Lepidaploa*. These glandular trichomes are not common in Asteraceae but they have been found in some taxa of Heliantheae (King and Robinson 1987) and Eupatorieae (King and Robinson 1987, Barreto et al. 2016). In Vernonieae, they have previously been observed on the style branches of some species of *Chrysolaena* (Via do Pico et al. 2016).

Basal stylar node

The presence or absence of a basal stylar node is a useful character in several tribes, such as Eupatorieae, Heliantheae and Vernonieae (Robinson 1988a). In Vernonieae, the basal stylar node is lacking or poorly differentiated in *Chrysolaena*,

Lessingianthus and *Stenocephalum* (Robinson 1999). However, in *Lepidaploa* most species have a well-developed basal node, and from a taxonomic point of view, this microcharacter is useful at species level in *Lepidaploa*, because it can be used to separate species with a well-developed basal node from *L. balansae* and *L. pseudomuricata*, which shares a poorly developed basal node.

Anther apical appendage

The different shapes of the apical anther appendages observed in *Lepidaploa* also occur in other genera of tribe Vernonieae and in other tribes of Asteraceae (Hattori and Nakajima 2011, Moreira and Teles 2014, Marques and Nakajima 2015). These shapes are not a diagnostic character at genus and species levels because the apical appendage is variable at the population level.

However, the presence of glandular trichomes on apical anther appendages is of taxonomic value as it distinguishes *Lepidaploa pseudomuricata*, the only species that exhibits type VIII trichomes on the anther appendages. Although the presence of glandular trichomes on apical appendages is not common in *Lepidaploa*, Robinson (1990) observed this structure in *L. helophila*. The presence of this trichome type on the anthers of *Chrysolaena* is common (Via do Pico et al. 2016).

Base of anther thecae

The two shapes (obtuse or sagittate) observed at the base of the anther thecae of *Lepidaploa* have previously been observed in other genera of Vernonieae and other tribes of Asteraceae (Hattori and Nakajima 2011, Moreira and Teles 2014, Marques and Nakajima 2015). These shapes are not of taxonomic value due to their variability at the population level.

Cypselae pubescence

Species of *Lepidaploa* exhibit two trichome types on the cypselae: type VII and VIII. Type VII trichomes are morphologically similar to type II and to the twin hairs of *Chrysolaena* (Via do Pico et al. 2016) and *Lessingianthus* (Angulo et al. 2015), respectively. Type VIII glandular trichomes are morphologically similar to type III trichomes found on the corolla of *Chrysolaena* (Via do Pico et al. 2016) and cypselae of *Lessingianthus* (Angulo et al. 2015).

Type VII trichomes have the same morphology as that of the twin hairs observed on the pericarp of the fruits of most Asteraceae (Hess 1938, Ramayya 1962, Bremer 1994, Robinson 2009). In *Lepidaploa*, it was found in all cypselae examined, as well as in the fruits of all species of *Chrysolaena* (Via do Pico et al. 2016). The glabrous fruits observed in *Lessingianthus* (Angulo et al. 2015) were not observed in any species of *Lepidaploa* analysed here.

Presence of Type VIII glandular trichomes found in the cypselae of *Lepidaploa* are valuable for distinguishing *L. fournetii*, *L. salzmännii* and *L. setos squamosa*. Notably,

L. setosquamosa and *L. remotiflora* are closely related species, but *L. remotiflora* does not have glandular trichomes on the cypsela.

Idioblasts

Idioblasts are often found on the cypsela wall of several Vernoniae (Robinson 2009). In *Chrysolaena* (Via do Pico et al. 2016) the presence of idioblasts varied within and between species, whereas in *Lessingianthus* the presence of idioblasts varied only between species (Angulo et al. 2015). In *Lepidaploa*, the presence of idioblasts did not vary within species and therefore allowed us to distinguish species groups with and without idioblasts.

Crystals

The fruits of *Lepidaploa* display a large diversity of crystals, and these structures are also found on the styles and phyllaries. According to Meric (2009), crystals on the ovary wall in tribe Inuleae act as protection against predation by herbivores and as supporting tissue during seed maturation. The function of crystals in floral organs (anthers, style, etc.) would be to provide the tissue with mechanical resistance (Meric 2009). Likewise, the presence of crystals in the different organs of *Lepidaploa* is likely to be involved in tissue resistance of the style and protection against herbivores in the phyllaries. Robinson (1990, 1999) described only prismatic crystals in *Lepidaploa*. However, in this study we found three additional crystal types (cubic, styloid, and druses) on the cypsela, styles and phyllaries, occurring in different tissues and organs. This variation was also observed by Meric (2009) in tribe Inuleae, who described three types of crystals: druses (found in the stem, style and filament), styloid (anther), and prismatic (ovary epidermis). In two species of Heliantheae, styloid and/or prismatic crystals were observed on the corolla and style (Meric and Dane 2004), whereas styloid crystals were found on the tapetum cells and anther endothecium (Horner 1977, Meric and Dane 2004), and druses in the corolla and style of two species in tribe Astereae (Meric 2008).

In Vernoniae, styloid and prismatic crystals were also found in the cypsela wall of *Lessingianthus* (Angulo et al. 2015), whereas *Chrysolaena* (Via do Pico et al. 2016) presented four crystal types in the fruit wall, as observed in *Lepidaploa* in our study. In addition, in *Lepidaploa* species the style base and phyllaries presented druses and styloid crystals. The different crystal types and their location in *Lepidaploa* are of taxonomic value at species level.

Carpopodium

The carpopodium is defined as the abscission region of the fruit that connects the ovary to the inflorescence or head (Haque and Godward 1984, Roque and Bautista 2008). This structure consists of one or more rows of cells (Haque and Godward 1984, Roque et al. 2009). The carpopodium can be

present or absent in the basal zone of the fruits of Asteraceae (Haque and Godward 1984).

In Vernoniae a carpopodium is present in most genera, except in *Camchaya* Gagnep. (Robinson 2009). In *Heterocoma* DC., for example, the carpopodium has taxonomic value because species exhibit differences in its size and shape (Freitas et al. 2015). In *Chrysolaena* (Via do Pico et al. 2016), *Lessingianthus* (Angulo et al. 2015) and *Lepidaploa*, the shape and size of the carpopodium are uniform both among the three genera and within species and therefore are not taxonomically useful. However, the presence and shape of crystals in the carpopodium is of taxonomic value, distinguishing some species of *Lepidaploa*.

Phyllaries

Our results showed that microcharacters of the phyllaries may be used for the recognition of some species. Simple curly trichomes, or type IV, is morphologically similar to the flagellate trichomes found on the leaves of *Vernonia gossypina* Gamble, *V. indica* C. B. Clarke, and *V. comorinensis* W. W. Smith (Narayana 1979), and similar to the flagelliform trichome found on the phyllaries and leaves of species of Leiboldiinae (Redonda-Martínez et al. 2016, Redonda-Martínez 2017). The same trichome type, called A2, was also observed on the leaves of some species of *Chronopappus* and *Lychnophora*, of subtribe Lychnophorineae (Wagner et al. 2013).

Some species of *Lepidaploa* display a peculiar combination of different trichomes, enabling identification of these taxa. Additionally, the phyllaries of *L. novariae* and *L. sordidopapposa* presented crystals, making it possible to distinguish these two species.

Cluster analysis

The UPGMA cluster analysis highlights the importance of certain micromorphological structures for grouping species. The presence or absence of crystals on the basal stylar node and carpopodium, arrangement of trichomes on the corolla, idioblasts on the fruit walls and glandular trichomes on the corolla lobes, style branches, cypselae and phyllaries are the most useful characters for grouping taxa. As such, the cluster analysis clearly corroborates the importance of microcharacters to separate *Lepidaploa* species groups. The taxonomic importance of microcharacters for *Lessingianthus* was shown by Angulo and Dematteis (2014) and Angulo et al. (2015). Although there are some fused terminals in the phenogram (UPGMA), the species present in these terminals are distinguished in the key by means of trichome types on the phyllaries and corolla, and crystal types on the cypsela and carpopodium. However, according to Via do Pico et al. (2016), the microcharacters in different species and populations of *Chrysolaena* overlap, and separation of species is therefore problematic.

Taxonomic implications

Chrysolaena, *Echinocoryne*, *Lepidaploa* and *Lessingianthus* are very close phylogenetically (Keeley et al. 2007, Loeuille et al. 2015), with *Lepidaploa* more closely related to *Chrysolaena*, and *Lessingianthus* possibly being the sister group to both genera (Keeley et al. 2007). Palynological studies (Robinson 1988a, b, 1990, 1999, Angulo and Dematteis 2010a, Via do Pico and Dematteis 2013) and previous studies also show that *Chrysolaena*, *Lepidaploa* and *Lessingianthus* are closely related and share microcharacters (Table 4). Species of *Chrysolaena* and *Lepidaploa* shared all microcharacters analysed here, whereas some species of *Lessingianthus* exhibited microcharacters common to both genera, a result that agrees with the phylogenetic relationships (Keeley et al. 2007).

One of the most distinctive features among the three genera is the presence of glandular trichomes on the anthers and style branches, which is unique to species of *Chrysolaena* within subtribe Lepidaploinae, and absent in *Lepidaploa* and *Lessingianthus* (Robinson 1999). However, *L. pseudomuricata* also has glandular anthers and style branches, characters shared with *Chrysolaena*. The taxonomic position of this species should therefore be reconsidered.

The well-developed stylar basal node has been considered as the main microcharacter that distinguish *Lepidaploa* from *Chrysolaena* and *Lessingianthus* (Robinson 1999), but it is quite variable and cannot be used as a diagnostic microcharacter for the genus. Angulo and Dematteis (2014) and Via do Pico et al. (2016) showed that some species of *Lessingianthus* and *Chrysolaena* have a basal stylar node, although it is poorly developed. In *Lepidaploa* most species have a well-developed basal stylar node; however, in *L. balansae* and *L. pseudomuricata*, a poorly developed node was observed, made up from several rows of thick-walled cells such as those found in *Chrysolaena* and *Lessingianthus*. The anther base also does not contribute to distinguish *Chrysolaena* (Via do Pico et al. 2016), *Lessingianthus* (Angulo and Dematteis 2014) and *Lepidaploa*. These three genera lack a caudate anther (Angulo and Dematteis 2014, Via do Pico et al. 2016). However, the caudate shape is characteristic of other genera in the tribe, such as in some species of *Vernonanthura* (Robinson 1992),

Piptocarpha R. Br. (Smith 1982), *Distephanus* Cass. (Robinson and Kahn 1986) and *Critoniopsis* Sch. Bip. (Robinson 1993). *Lepidaploa* and *Vernonanthura* share morphological features (e.g. paniculate inflorescence and campanulate heads) and the anther base is important to differentiate *Lepidaploa* from *Vernonanthura*. Robinson (1988a, b, 1990) used the presence of type VIII trichomes on the cypsela to distinguish *Lepidaploa* and *Chrysolaena* from *Lessingianthus*. However, this trichome type has been observed in some taxa of all three genera. In *Lessingianthus*, 19 species have these glandular trichomes on the fruits (Angulo et al. 2015). Finally, the presence of crystals and their shapes are highly variable in species of *Lepidaploa*, and therefore could not be used to separate *Lepidaploa* from *Chrysolaena* and *Lessingianthus* (Robinson 1999).

The present study reveals the taxonomic value and diversity of microcharacters among species of *Lepidaploa*, showing that related species can be distinguished by different combinations of microcharacters. For example, microcharacters can be useful to differentiate *L. remotiflora* and *L. setosquamosa*, which have previously been considered varieties of the same species (Cabrera 1944) or synonyms of a single species (Robinson 1999). These taxa can be separated by the size of the heads, shape of phyllaries, pollen morphology, and chromosome number (Angulo and Dematteis 2010b). This study shows that they also differ considerably with respect to corolla trichomes, presence of cypsela crystals, anther base and phyllary trichome types. *Lepidaploa amambaia* shares macromorphological affinities with both species mentioned above. However, *L. amambaia* can be distinguished by the presence of idioblasts on the cypsela wall, presence of crystals on the carpodium and phyllary trichome types.

In conclusion, this is the first detailed study of the floral micromorphological characters in *Lepidaploa*. The results indicate that several character states analysed in *Lepidaploa* are useful for species-level identification, but they are often widespread in other related genera of tribe Vernonieae, which could be explained by homoplasy. However, for a more precise evaluation of diagnostic characters, including microcharacters, in relation to the relationship of these genera, more

Table 4. Microcharacters in *Chrysolaena*, *Lessingianthus* and *Lepidaploa* as known from previous studies (Angulo and Dematteis 2014, Angulo et al. 2015, Dematteis 2009, Robinson 1988a, b, 1990, 1999, Via do Pico et al. 2016) and the results from this study.

Microcharacter	<i>Chrysolaena</i>	<i>Lepidaploa</i> (this study)	<i>Lessingianthus</i>
Trichomes on corolla lobe	glandular and/or eglandular	glandular and eglandular	glandular and/or eglandular
Apical anther appendages	glandular, eglandular in populations of <i>C. obovata</i> and in <i>C. guaranitica</i> and <i>C. simplex</i>	glabrous, except in <i>L. pseudomuricata</i>	glabrous
Basal stylar node	present, underdeveloped or absent	present, underdeveloped in <i>L. balansae</i> and <i>L. pseudomuricata</i>	present, underdeveloped in <i>L. brevifolius</i> , <i>L. carvalhoi</i> , <i>L. durus</i> , <i>L. regis</i> and <i>L. santosii</i>
Presence of trichomes on cypsela	glandular, absent only in populations of <i>C. obovata</i> and in <i>C. guaranitica</i> and <i>C. simplex</i>	eglandular, rarely glandular	eglandular, rarely glandular
Cypsela wall crystals	cubic, prismatic, styloid, druses	cubic, prismatic, styloid, druses	prismatic and styloid

through and comprehensive phylogenetic studies will be needed.

Acknowledgements – Funding – We thank the financial support provided by the Secretaría General de Ciencia y Técnica de la Universidad Nacional del Nordeste, the Consejo Nacional de Investigaciones Científicas y Técnicas and the Agencia Nacional de Promoción Científica y Tecnológica (PI no. 15-P002, PIP no. 11220150100125, PICT no. 2016-2686). DM and JNN are grateful to CNPq, CAPES, FAFs (REFLORA proc. 563541 / 2010-5, PROTAX proc. 562290 / 2010-9), for the funding to study Asteraceae in Brazil.

Conflict of interest – The authors declare that they have no conflict of interest.

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Supplementary material (online as Appendix njb-01686 at <www.nordicbotany.org/appendix/njb-01686>). Appendix 1.

Appendix 1

Specimens analyzed

Lepidaploa amambaia H. Rob.

PARAGUAY, Amambay: Dematteis et al. 867 (CTES). PARAGUAY, Caagazú: *A. Schinini s.n.* (CTES 22903). PARAGUAY, Central: E. Bordas 3674 (CTES). PARAGUAY, Concepción: M. Dematteis et al. 3336 (CTES).

Lepidaploa argyrotricha (Sch. Bip. ex Baker) H. Rob.

BRAZIL, Paraná: E. Barbosa et al. 1012 (CTES).

Lepidaploa bakerana (Britton) H. Rob.

BOLIVIA, La Paz: A. Fuentes et al. 7042 (CTES). BOLIVIA, La Paz: St. G. Beck 8317 (CTES).

Lepidaploa balansae (Hieron.) H. Rob.

ARGENTINA, Corrientes: A. Schinini 6799 (CTES). ARGENTINA, Misiones: H. A. Keller 8792 (CTES). BRAZIL, Paraná: A.C. Cervi (MBM). BRAZIL, Santa Catarina: R.M. Klein 5726 (HBR); BRAZIL, Santa Catarina: G. Hatschbach & O. S. Ribas 79945. Brazil, Rio Grande do Sul: A. Sehnem, 2866. PARAGUAY, Alto Paraná: F. González 256 (FCQ). PARAGUAY, Canindeyú: B. Jiménez 1408 (CTES). PARAGUAY, Concepción: A. Krapovickas et al. 45131 (CTES). PARAGUAY, Paraguari: E. Zardini 9801 (FCQ).

Lepidaploa beckii H. Rob.

BOLIVIA, La Paz: St. G. Beck 4678 (CTES). BOLIVIA, La Paz: St. G. Beck 14931 (CTES)

Lepidaploa buchtienii (Gleason) H. Rob.

BOLIVIA, La Paz: St. G. Beck 29463 (CTES)

Lepidaploa canescens (Kunth) H. Rob.

BOLIVIA, La Paz: St. G. Beck 12108 (CTES)

Lepidaploa chamissonis (Less.) H. Rob.

ARGENTINA, Corrientes: A. G. Schinini 15251 (CTES). A. G. Schinini s. n. (CTES 18119). ARGENTINA, Chaco: A. G. Schinini s. n. (CTES 18119). ARGENTINA, Formosa: A. G. Schinini 16621 (CTES). BRAZIL, Santa Catarina: A. Krapovickas et al. 38426 (CTES). BRAZIL, Paraná: G. Hatschbach, 7528 (MBM). PARAGUAY, PARAGUARÍ: G. Schemeda 55. (CTES). PARAGUAY, Presidente Hayes: A. G. Schinini 16585 (CTES).

Lepidaploa cordifolia (Kunth) H. Rob.

BOLIVIA, La Paz: St. G. Beck 29172 (CTES).

Lepidaploa costata (Rusby) H. Rob.

BOLIVIA, La Paz: Beck St. G. 29455 (CTES). BOLIVIA, La Paz: St. G. Beck 28282 (CTES). BOLIVIA, La Paz: Wood et al. 13906 (CTES)

Lepidaploa deflexa (Rusby) H. Rob.

BOLIVIA, La Paz: St. G. Beck 29174 (CTES). BOLIVIA, La Paz: St. G. Beck 24903 (CTES). BOLIVIA, La Paz: St. G. Beck 22758 (CTES)

Lepidaploa eriolepis (Gardner) H. Rob.

BRAZIL, Paraná: J. M. Silva et al. 5007 (CTES); BRAZIL, Paraná: G. Hatschbach et al. 49535 (MBM). BRAZIL, Santa Catarina:

D. B. Falkenberg 4364 (MBM). Brazil, Santa Catarina: A. BreSolín 313 (HBR).

Lepidaploa fournetii (H. Rob. & B. Kahn) H. Rob.

BOLIVIA, La Paz: St. G. Beck. 13578 (CTES). BOLIVIA, La Paz: R. Michel et al. (CTES).

Lepidaploa mapirensis (Gleason) H. Rob.

BOLIVIA, La Paz: J. C. Salomon 9656 (CTES). BOLIVIA, La Paz: J.C. Solomon 15318 (CTES).

Lepidaploa myriocephala (DC.) H. Rob.

BOLIVIA, La Paz: M. Dematteis 1221 (CTES). BOLIVIA, La Paz: A. Fuentes et al. 13068.

Lepidaploa novaruae (Cabrera) A. J. Vega & M. Dematteis.

ARGENTINA, Salta: O. Ahumada et al. 8365. ARGENTINA, Salta: Vervoorst 4581.

Lepidaploa pseudomuricata H. Rob.

BRAZIL, Paraná: Município de São Mateus do Sul, Usina de Xisto da Petrobrás. O. S. Ribas et al. 6518 (CTES). BRAZIL, Santa Catarina: G. Hatschbach et al. 78921 (CTES). BRAZIL, Santa Catarina: A. L. de Gasper 1574 (FURB). BRAZIL, Santa Catarina: A. L. de Gasper 1606 (FURB). BRAZIL, Rio Grande do Sul: G. Hatschbach 79077 (CTES).

Lepidaploa psilostachya (DC.) H. Rob.

ARGENTINA, Misiones: M. Dematteis et al. 3047 (CTES). ARGENTINA, Misiones: M. Dematteis et al. 4139 (CTES). BRAZIL, Santa Catarina: L. P. Deble et al. 12020 (CTES)

Lepidaploa remotiflora (Rich.) H. Rob.

ARGENTINA, Corrientes: A. Krapovickas et al. 29153 (CTES). ARGENTINA, Formosa: A. Krapovickas et al. 13086 (CTES). ARGENTINA, Misiones: A. Schinini 31553 (CTES). BOLIVIA, Santa Cruz: M. Dematteis 3673 (CTES). PARAGUAY, Asunción: A. Schinini 8006 (CTES). PARAGUAY, Central: A. Krapovickas 12125 (CTES). PARAGUAY, Cordillera: A. Krapovickas 41886 (CTES). PARAGUAY, San Pedro: A. Krapovickas 13886 (CTES). PARAGUAY, Trinidad: A. Barbero 313 (SCQ).

Lepidaploa salzmännii (DC.) H. Rob.

BOLIVIA, Tarija: M. Dematteis 3438 (CTES). PARAGUAY, Amambay: A. Schinini et al. 30441 (CTES)

Lepidaploa setos squamosa (Hieron.) M.B. Angulo & Dematt.

ARGENTINA, Salta: L.J. Novara 10877 (CTES). BOLIVIA, Santa Cruz: V.S. Neffa et al. 1305 (CTES). PARAGUAY, Alto Paraguay: R. Degen 3298 (FCQ). PARAGUAY, Boquerón: A. Krapovickas et al. 44197 (CTES).

Lepidaploa sordidopapposa (Hieron.) H. Rob.

BOLIVIA, La Paz: St. G. Beck 22672 (CTES). BOLIVIA, La Paz: St. G. Beck 17717 (CTES)

Lepidaploa tarijensis (Griseb.) H. Rob.

ARGENTINA, Salta: A. Krapovickas et al. 19425 (CTES). ARGENTINA, Salta: A. Krapovickas et al. 47264 (CTES). ARGENTINA, Salta: M. Dematteis et al. 839 (CTES).