

Floral microcharacters in *Lessingianthus* (Vernonieae, Asteraceae) and their taxonomic implications

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Abstract Micromorphological characters of 113 species of the South American genus *Lessingianthus* H.Rob. (Vernonieae, Asteraceae) were analyzed to evaluate their reliability as taxonomic markers. The corolla pubescence was studied in detail for the first time in the genus. Glandular and non-glandular trichomes were studied and described. The trichome types allow differentiation among related species, but do not reflect the relationships among species groups. On the other hand, the basal stylar node only occurs in a distinctive group of species within the genus. The apical appendage of the anthers is non-glandular in all species of the genus. Several character states analyzed in *Lessingianthus* are often widespread in other related genera of Vernonieae. Therefore, *Lessingianthus* can be circumscribed only by a combination of micro- and macromorphological features.

Keywords Floral micromorphology · Lepidaploinae · Taxonomy · Vernonieae

Introduction

Asteraceae currently include 12 subfamilies and 43 tribes (Funk et al. 2009). This classification is based on molecular and mainly macromorphological data. Yet, the micromorphological characters can potentially be an important source of information for classification of the family. For instance, the first classifications of Asteraceae were based on some micromorphological characters (hereafter called

microcharacters) in combination with other traditional macromorphological characters (hereafter called macrocharacters). To circumscribe the tribes, Cassini (1816) used many floral microcharacters, of the stigmatic surface, style appendages, anther base and shape of the anther collar. Many of these characters have been used also by subsequent authors (Lessing 1832; Bentham 1873). Several taxonomically useful microcharacters identified by Bentham (1873) were later used by King and Robinson (1970). Current taxonomic treatments of tribes, genera, and species of Asteraceae use numerous micromorphological features.

The New World tribe Vernonieae is one of the largest tribes of Asteraceae and it is considered biologically and taxonomically a complex group. The tribe was revised taxonomically by Robinson (1999) who used various floral micromorphological characters, such as the style base, ovary wall crystals, carpodium shape, and anther appendages. Based on these microcharacters as well as pollen morphology and chromosome numbers, Robinson (1999) suggested that the genus *Vernonia* Schreb. is restricted to North America, while species from South America would pertain to 16 new genera. Following Robinson's classification, *Lessingianthus* H.Rob. is, with more than 120 species, one of the largest genera of Vernonieae.

Lessingianthus is widely distributed in South America including Venezuela, Colombia, Peru, Brazil, Bolivia, Paraguay, Argentina and Uruguay (Robinson 2007). Almost all species are perennial herbs, with medium- or large-sized capitula and seriate-cymose inflorescence branches (Robinson 1988a). *Lessingianthus* can be distinguished from other American Vernonieae genera based on its “type B” pollen grains, basic chromosome number $x = 16$, non-glandular anther appendages, lack of basal style node, and the presence of cubic crystals in the achene wall (Robinson 1988a, b, 1999; Dematteis 2006).

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The basal node, a disc of thick-walled cells at the style base (Robinson 1988b) of Asteraceae, has been taxonomically useful in some groups of the tribes Eupatorieae, Heliantheae, Senecioneae, and Vernonieae (Robinson 1988a). In Vernonieae, the presence of a basal node is common in numerous neotropical members of the tribe (Robinson 1988b, 1999). For example, *Lepidaploa* (Cass.) DC. is characterized by a well-developed basal node, whereas *Stenocephalum* Sch. Bip., *Vernonanthura* H. Rob., and *Vernonia* sensu stricto have poorly developed basal nodes, and *Lessingianthus* and *Chrysolaena* H. Rob. lack this structure.

The apical anther appendage is another microcharacter used by Robinson (1988a). Robinson noted that such appendage in *Lessingianthus* lacked glands, allowing distinction of this genus from the closely related genus *Chrysolaena* and the other genera of the tribe, all with glandular appendages.

Trichomes also represent a source of taxonomically useful microcharacters. Studies on Asteraceae trichomes have importantly contributed to taxonomic and/or phylogenetic questions (Carlquist 1959; Narayana 1979). Yet, only few identification keys in the Asteraceae actually use trichome morphological characters. The family includes glandular and non-glandular trichomes (Metcalf and Chalk 1957, 1979) that occur in floral and vegetative organs, including underground organs (Appezato-da-Glória et al. 2008, 2012). Non-glandular trichomes are morphologically more diverse than glandular trichomes. The latter are usually formed by a stalk and a head of one or more cells, and usually occur on various parts of the inflorescences, such as receptacles, phyllaries, corollas, and ovaries (Roth 1977).

In Vernonieae, studies have shown the taxonomic value of trichomes to discriminate among genera and similar species (Solereder 1908; Metcalfe and Chalk 1979; Narayana 1979; Appezato-da-Glória et al. 2012). Many of these studies focussed on the morphology and chemical composition of leaf trichomes (Narayana 1979; Milan et al. 2006; Adedeji and Jewoola 2008; Appezato-da-Glória et al. 2012). However, only few studies have comparatively investigated diversity of trichomes on floral organs. Robinson (1999) studied the structure of trichomes on corollas and cypselas in the taxonomic treatment of New World Vernonieae, demonstrating its taxonomic and evolutionary significance. Redonda-Martínez et al. (2012) consider the morphology of corolla trichomes to differentiate species of *Vernonanthura* and *Vernonia* that have similar ecological and/or geographical distribution. Despite the importance of the trichomes in the tribe, their taxonomic value as a source of diagnostic microcharacters remains to be studied in *Lessingianthus*.

Table 1 List of characters and their coding states

Microcharacters

1. Corolla pubescence: glabrous (1) non-glabrous (2)
2. Papillae: absent (0) present (1)
3. Apical anther appendage: lanceolate (1) ovate (2) ovate-lanceolate (3) linear (4)
4. Anther appendage size: 0.3–1 mm (1) > 1mm (2)
5. Anther base: sagittate (1) cuneate (2)
6. Basal stylar node: absent (0) present (1)

Pollen

7. Pollen Type : B (1) B-1 (2) B-2 (3) B-3 (4)

The aim of this study was to examine floral microcharacters and their taxonomic utility in *Lessingianthus*, with almost complete species coverage. These microcharacters are also discussed in relation to other genera of Vernonieae.

Materials and methods

Floral morphology of a total of 113 species was studied with stereo-, light, and scanning electron microscopy (SEM). Samples of florets for each species were collected from herbarium specimens (i.e., voucher specimens) from the herbaria ALCB, BR, CESJ, CTES, ESA, G, K, P, R, RB, S, and UB. Species and specimen information is listed in the Appendix.

For morphological analysis, florets were softened in boiling water including a drop of detergent, dissected under a stereomicroscope, mounted in Hoyer's solution (Anderson 1954; King and Robinson 1970), and then examined with a Zeiss Axioplan microscope with digital camera Canon Power Shot A 640.

For histochemical analyses, material fixed in FAA was hand-sectioned under a stereomicroscope. Cellular content was tested histochemically using Lugol's reagent for starch grains, Sudan IV for lipids, Ruthenium red for pectinaceous materials, and cresyl blue for mucilages. Sections were observed and photographed using the Zeiss Axioplan microscope with digital camera Canon Power Shot A 640.

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.

Terminology of trichome morphology follows Metcalfe and Chalk (1979), while terminology of remaining floral characters follows Bremer (1994), Robinson (1988a, b, 1999), and Roque et al. (2009).

Table 2 Floral microcharacters in *Lessingianthus* species

Species	Corolla pubescence						Anthers								Style base		Species code
							Apical appendage						Base				
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	1	2	
<i>L. adenophyllus</i>		x	x	x			x				x		x		x		ade
<i>L. arachniolepis</i>		x	x	x	x		x				x		x		x		ara
<i>L. arctatus</i>		x	x	x	x			x			x		x		x		arc
<i>L. argenteus</i>		x	x	x	x		x				x		x		x		arg
<i>L. argyrophyllus</i>		x	x	x	x		x				x		x		x		argy
<i>L. bardanoides</i>		x	x	x	x		x				x			x	x		bard
<i>L. barrosoanus</i>		x	x	x	x				x		x		x		x		barr
<i>L. bellulus</i>		x	x	x	x				x			x		x	x		bell
<i>L. bishopi</i>		x	x	x	x		x				x		x		x		bish
<i>L. brevifolius</i>					x		x				x		x			x	brevif
<i>L. brevipetiolatus</i>		x	x	x		x	x				x		x		x		brevip
<i>L. buddleiifolius</i>			x	x	x		x					x	x		x		bud
<i>L. carduoides</i>		x	x	x	x		x				x		x		x		card
<i>L. carvalhoi</i>		x			x		x				x		x			x	carv
<i>L. cataractarum</i>		x	x	x		x	x				x			x	x		cat
<i>L. centauropsideus</i>	x		x	x	x		x				x		x		x		cent
<i>L. cephalotes</i>		x	x	x	x				x		x		x		x		cep
<i>L. cipoensis</i>		x	x	x	x			x			x		x		x		cip
<i>L. clavatus</i>		x	x	x	x		x				x				x		clav
<i>L. compactiflorus</i>		x	x	x	x			x			x		x		x		comp
<i>L. constrictus</i>	x		x	x	x		x				x			x	x		cons
<i>L. cordiger</i>		x	x	x	x				x		x		x		x		cord
<i>L. coriaceus</i>		x	x	x	x		x				x				x		cori
<i>L. coriarius</i>		x	x	x	x		x				x		x		x		coria
<i>L. correntinus</i>	x		x	x	x		x				x		x		x		corr
<i>L. cristalinae</i>			x	x	x				x		x		x		x		cris
<i>L. chamaepueces</i>		x	x	x	x						x		x		x		cha
<i>L. durus</i>		x			x		x				x		x			x	dur
<i>L. eitenii</i>		x	x	x		x			x		x		x		x		eit
<i>L. elegans</i>		x	x	x	x		x				x		x		x		ele
<i>L. exiguus</i>		x	x	x	x		x				x		x		x		exi
<i>L. farinosus</i>		x	x	x	x			x			x		x		x		fari
<i>L. floccosus</i>		x	x	x	x						x			x	x		floc
<i>L. foliosus</i>		x	x	x	x			x			x		x		x		fol
<i>L. fonsecae</i>		x	x	x	x		x				x		x		x		fon
<i>L. glabratus</i>		x	x	x	x		x				x		x		x		glab
<i>L. grandiflorus</i>	x		x	x	x		x				x		x		x		gran
<i>L. grearii</i>			x	x	x		x				x			x	x		gre
<i>L. hasslerianus</i>	x		x	x	x		x				x		x		x		has
<i>L. hovaefolius</i>		x	x	x	x			x			x		x		x		hov
<i>L. hypochaeris</i>		x	x	x	x		x				x		x		x		hyp
<i>L. hystricosus</i>		x	x	x	x			x			x		x		x		hys
<i>L. ibitiipocensis</i>		x	x	x	x			x			x		x		x		ibi
<i>L. intermedius</i>	x		x	x	x		x				x		x		x		inter
<i>L. irwinii</i>		x	x	x	x		x				x			x	x		irwi
<i>L. ixiamensis</i>			x	x	x		x				x		x		x		ixi

Table 2 continued

Species	Corolla pubescence						Anthers								Style base		Species code
							Apical appendage						Base				
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	1	2	
<i>L. kuntzei</i>		x	x	x	x				x		x		x		x		kun
<i>L. laevigatus</i>		x	x	x	x		x				x		x		x		lae
<i>L. lanatus</i>		x	x	x	x		x				x		x		x		lan
<i>L. laniferus</i>		x	x	x	x				x		x		x		x		lani
<i>L. lanuginosus</i>		x	x	x	x			x			x		x		x		lanu
<i>L. laurifolius</i>		x	x	x	x				x		x		x		x		laur
<i>L. ligulifolius</i>		x	x	x	x		x				x			x	x		ligu
<i>L. linearifolius</i>		x	x	x	x		x				x			x	x		linearif
<i>L. linearis</i>	x		x	x	x		x				x		x		x		linear
<i>L. longicuspis</i>		x	x	x	x			x			x		x		x		long
<i>L. lorentzii</i>		x	x	x	x					x	x		x		x		lor
<i>L. macrocephalus</i>		x	x	x	x		x					x	x		x		macroc
<i>L. macrophyllus</i>			x	x	x		x					x	x		x		macrop
<i>L. mansoanus</i>	x		x	x	x			x			x		x		x		man
<i>L. membranifolius</i>			x	x	x				x		x		x		x		mem
<i>L. minimus</i>			x	x	x		x				x		x		x		min
<i>L. mollissimus</i>		x	x	x	x		x				x		x		x		mol
<i>L. morii</i>		x	x	x	x		x				x			x	x		mor
<i>L. myrsinites</i>		x	x	x	x		x				x			x	x		myr
<i>L. niederleinii</i>		x	x	x	x		x				x		x		x		nie
<i>L. obscurus</i>		x	x	x	x		x				x		x		x		obs
<i>L. obtusatus</i>		x	x	x	x		x				x		x		x		obt
<i>L. onopordioides</i>		x	x	x	x		x				x		x		x		ono
<i>L. paraguariensis</i>		x	x	x	x			x			x		x		x		par
<i>L. parvifolius</i>		x	x	x	x				x		x		x		x		parv
<i>L. pentacontus</i>		x	x	x	x		x				x		x		x		pen
<i>L. plantaginoides</i>		x	x	x	x				x		x		x		x		plan
<i>L. platyphyllus</i>	x		x	x	x		x				x		x		x		plat
<i>L. polyphyllus</i>		x	x	x	x		x				x		x		x		pol
<i>L. profusus</i>		x	x	x	x				x		x		x		x		prof
<i>L. pseudoincanus</i>		x	x	x	x		x				x		x		x		pseudoi
<i>L. pseudopiptocarpus</i>		x	x	x	x		x				x		x		x		pseudoip
<i>L. psilophyllus</i>		x	x	x	x		x				x		x		x		psi
<i>L. pumillus</i>		x	x	x	x				x		x		x		x		pum
<i>L. pusillus</i>		x	x	x	x				x		x		x		x		pus
<i>L. pycnostachyus</i>		x	x	x	x				x		x			x	x		pyc
<i>L. regis</i>		x			x				x		x		x			x	reg
<i>L. reitzianus</i>			x	x		x	x				x		x		x		reit
<i>L. rigescens</i>	x		x	x	x		x				x		x		x		rig
<i>L. robustus</i>		x	x	x	x		x				x		x		x		rob
<i>L. roseus</i>		x	x	x	x				x		x		x		x		ros
<i>L. rosmarinifolius</i>	x		x	x	x		x				x		x		x		rosm
<i>L. rubricaulis</i>		x	x	x	x		x				x		x		x		rubri
<i>L. saltensis</i>		x	x	x	x				x		x		x		x		salt
<i>L. sancti-pauli</i>		x	x	x	x		x				x		x		x		sanct

Table 2 continued

Species	Corolla pubescence						Anthers								Style base		Species code
							Apical appendage										
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	1	2	
<i>L. santosii</i>		x			x		x				x		x			x	sant
<i>L. scabrifolius</i>		x	x	x		x			x		x		x			x	sca
<i>L. secundus</i>			x	x	x			x			x		x			x	sec
<i>L. sellowii</i>		x	x	x	x				x		x		x			x	sel
<i>L. soderstroemii</i>		x	x	x	x		x					x	x			x	sod
<i>L. souzae</i>		x	x	x	x		x				x		x			x	sou
<i>L. spicatus</i>		x	x	x	x		x				x		x			x	spi
<i>L. stoechas</i>		x	x	x	x		x				x		x			x	sto
<i>L. subcarduoides</i>		x	x	x	x			x			x		x			x	subc
<i>L. subobscurus</i>		x	x	x	x		x				x		x			x	subo
<i>L. syncephalus</i>		x	x	x	x		x				x		x			x	syn
<i>L. teyucarensis</i>		x	x	x	x		x				x		x			x	tey
<i>L. tomentellus</i>		x	x	x	x		x					x	x			x	tom
<i>L. ulei</i>		x	x	x	x		x				x		x			x	ule
<i>L. varroniifolius</i>		x	x	x	x		x				x		x			x	var
<i>L. venosissimus</i>		x	x	x	x			x				x	x			x	ven
<i>L. vepretorum</i>		x	x	x	x				x		x		x			x	vep
<i>L. vestitus</i>		x	x	x	x				x		x		x			x	vest
<i>L. warmingianus</i>		x	x	x	x		x				x		x			x	war
<i>L. westermanii</i>		x	x	x	x		x				x		x			x	wes
<i>L. xanthophyllus</i>		x	x	x	x				x		x		x			x	xan
<i>L. zuccarinianus</i>		x	x	x	x				x		x		x	x		x	zuc

Corolla pubescence: 1: glabrous, 2: papillae, 3: trichome type I, 4: trichome type II, 5: trichome type III, 6: trichome type IV

Anthers Apical appendage: 1: lanceolate, 2: ovate, 3: ovate-lanceolate, 4: linear. Size of apical appendage: 5: 0.3–1 mm, 6: > 1 mm. Anther base: 1: sagittate, 2: cuneate. *Style base*: 1: without basal node, 2: with basal node

A data matrix of 133 OTUs (operational taxonomic units) \times 7 variables was constructed. All characters were examined here, and the pollen type of each species (taken from Angulo and Dematteis 2010) were added to the data matrix. The different character states for each of them are given in Table 1.

Additionally, to evaluate the micromorphological and palynological variability among species, principal coordinate analyses (PCoA) using Manhattan distance metric were performed based on the data matrix mentioned above.

The analysis was carried out with the Infostat software, version 2009 (Di Rienzo et al. 2009).

The complete data matrix used for statistical analysis is available upon request from the senior author.

Results

Floral microcharacters including corolla pubescence, style base, shape and size of apical anther appendage, and base of the thecae were studied in a total of 113 species of *Lessingianthus* listed in Table 2.

Corolla pubescence

The corolla lobes are papillose in almost all species (only 21 species lack papillae, see Table 2). The papillae are short cells concentrated at the corolla lobe tips (Fig. 1a, b). This character is not consistent within the species, since there are both specimens with and specimens without papillae.

Corolla lobes of most taxa (102 species) have trichomes and only in 11 taxa they are glabrous (Fig. 2a; see Table 2).

The trichomes can be simple, unicellular or multicellular, glandular or non-glandular, and short to long in size. Four different types of trichomes were observed and here classified as follows:

Type I: non-glandular, unicellular, short (Fig. 3d).

Type II: non-glandular, multicellular, long, with a terminal cell longer than the other cells (Figs. 1g, i–l, 2b, d, 3e).

Type III: glandular with a uniseriate stalk and unicellular head (Figs. 1c, d, 2d, 3a, b, f).

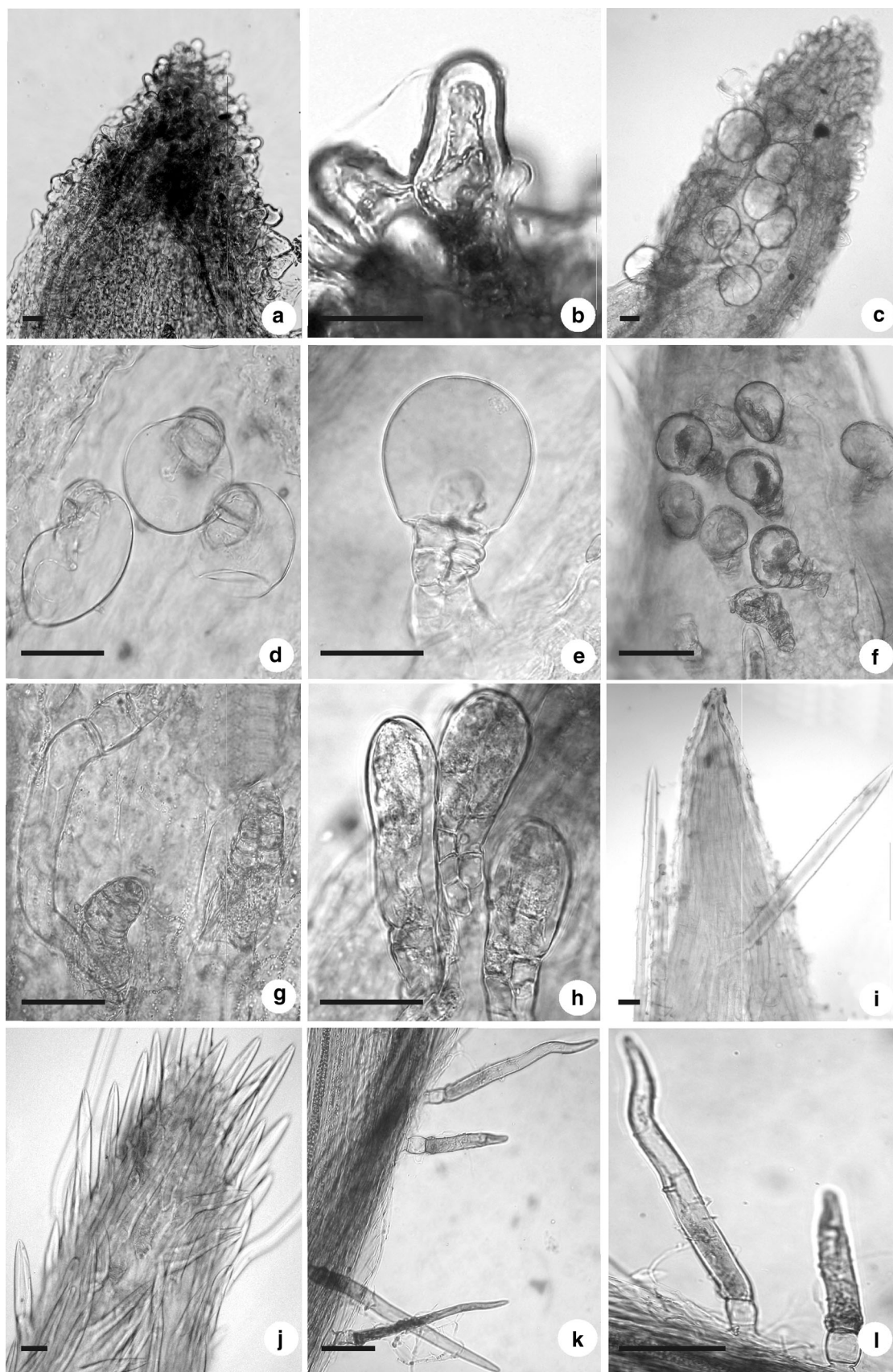


Fig. 1 Floral microcharacters of *Lessingianthus*. **L. arachniolepis**: **a** corolla lobe tip showing papillae, **b** details of papillae. **L. carvalhoi**: **c** corolla lobe tip with glandular trichome type III, **d** details of glandular trichome type III. **e** *L. reitzianus*, glandular trichome type III. **f** *L. psilophyllus*, glandular trichome type III. **g** *L. bellulus*, non-glandular (type II) and glandular (type IV) trichomes. **h** *L. varroniifolius*, details of glandular trichomes type IV. **i** *L. adenophyllus*, non-glandular trichomes type II. **j** *L. clavatus*, corolla lobe tip showing non-glandular trichomes type II. **k**, **l** *L. bardanoides*, non-glandular trichomes type II. Scale bar 10 μ m

Type IV: glandular with a multiseriate stalk and unicellular head (Figs. 1e–h, 2b, c, e, f, 3c).

Almost all species have trichomes at the apex of the corolla lobes, which are always located on the dorsal side of the lobes. However, some species have trichomes on the throats of the limb and scattered all the way to the tubes, such as *L. brevifolius* and *L. glabratus* with only type III trichomes, while *L. reitzianus* has only type II trichomes.

Among the examined species, 11 have exclusively non-glandular trichomes and 12 species have exclusively glandular trichomes (Table 2). Most of the species bear both glandular and non-glandular trichomes on their corollas, and have different combinations of trichome types, with up to three different trichome types (e.g. two glandular and one non-glandular types in *L. morii* with types I–III–IV or one glandular and two non-glandular types in *L. exiguus* with types I–III, Table 2).

Contents of the head cell of the glandular trichomes stain positively with Sudan IV (Fig. 4a, b), indicating the presence of lipids. The histochemical tests with Lugol's reactive, Ruthenium red, and cresyl blue showed negative results, indicating the absence of starch grains, pectinaceous materials, and mucilages, respectively.

Style base

Most species lack a basal node (Fig. 2g), whereas *L. brevifolius*, *L. carvalhoi*, *L. durus*, *L. regis*, and *L. santosii*, have a distinctive basal node in form of a small disc with several rows of thick-walled cells (Fig. 2h, i).

Apical anther appendage

Different shapes of the apical anther appendages were observed: lanceolate, ovate, ovate-lanceolate, and linear. Appendages are 0.3–1.3 μ m long. *Lessingianthus hypochaeris*, *L. exiguus*, and *L. ulei* show the smallest appendages (0.3 μ m), while *L. bellulus* and *L. tomentellus* have the longest ones (1.2 and 1.3 μ m, respectively). Appendages are non-glandular in all taxa.

Base of anther thecae

The base of anther thecae of all the species is calcarate with a pollen-containing portion prolonged below the point of filament insertion. This prolongation in most cases is sagittate (thecae are gradually enlarged at the base into two acute straight lobes, as the tip of an arrow), but in 14 taxa is cuneate (each theca at the base has an inversely triangular form, with straight margins forming an angle of 45°–90°) (see Table 2).

Statistical analysis

The PCoA (Fig. 5) of all 113 OTUs based on Manhattan distance metric was performed. Although the first two principal coordinates represent only 40 % of the total variation, one distinct group is differentiated. This group includes 95 % of the species, which lack node at the style base and have types B and B-3 (only in *L. chamaepeuces*) pollen. Several subgroups containing species with the same distribution (same coordinates) are distinguished within. The same coordinates for the species indicate that they share the same micromorphological characteristics. Subgroup 1 includes 34 species with corollas with papillae and trichomes, anthers with lanceolate apical appendages, 0.3–1 mm long, and sagittate thecae. Taxa of the subgroups 2 (with 20 species) and 3 (with 12 species) have almost all the characteristics of subgroup 1, but differ in the shape of the apical appendages. Subgroup 2 has ovate-lanceolate apical appendages and subgroup 3 has ovate appendages. Subgroup 4 has six species with corollas with trichomes, without papillae, lanceolate apical appendages, 0.3–1 mm long, and sagittate thecae. Species with glabrous corollas are included in subgroup 5. Subgroup 6 includes seven species with the same characteristics of subgroup 1, but the bases of the thecae are cuneate. Several taxa have a combination of microcharacters, which does not correspond to the characteristics of the above subgroups (see Table 2). *Lessingianthus constrictus* is separated (on the top of the negative extreme of coordinate 1) from most of species, because it has glabrous corollas and the bases of the thecae are cuneate. On the other hand, *L. bellulus* (on the lower negative extreme of coordinate 1) is also isolated from the rest of the species because it has a combination of characters such as ovate-lanceolate appendage, 1.2 mm long, and cuneate thecae.

Five taxa are isolated from the main group (on the positive extreme of coordinate 1) by having a node at style base and pollen type B-1 (*L. brevifolius*, *L. durus*, *L. regis*, *L. santosii*, and *L. soderstroemii*) and B-2 (*L. santosii*).

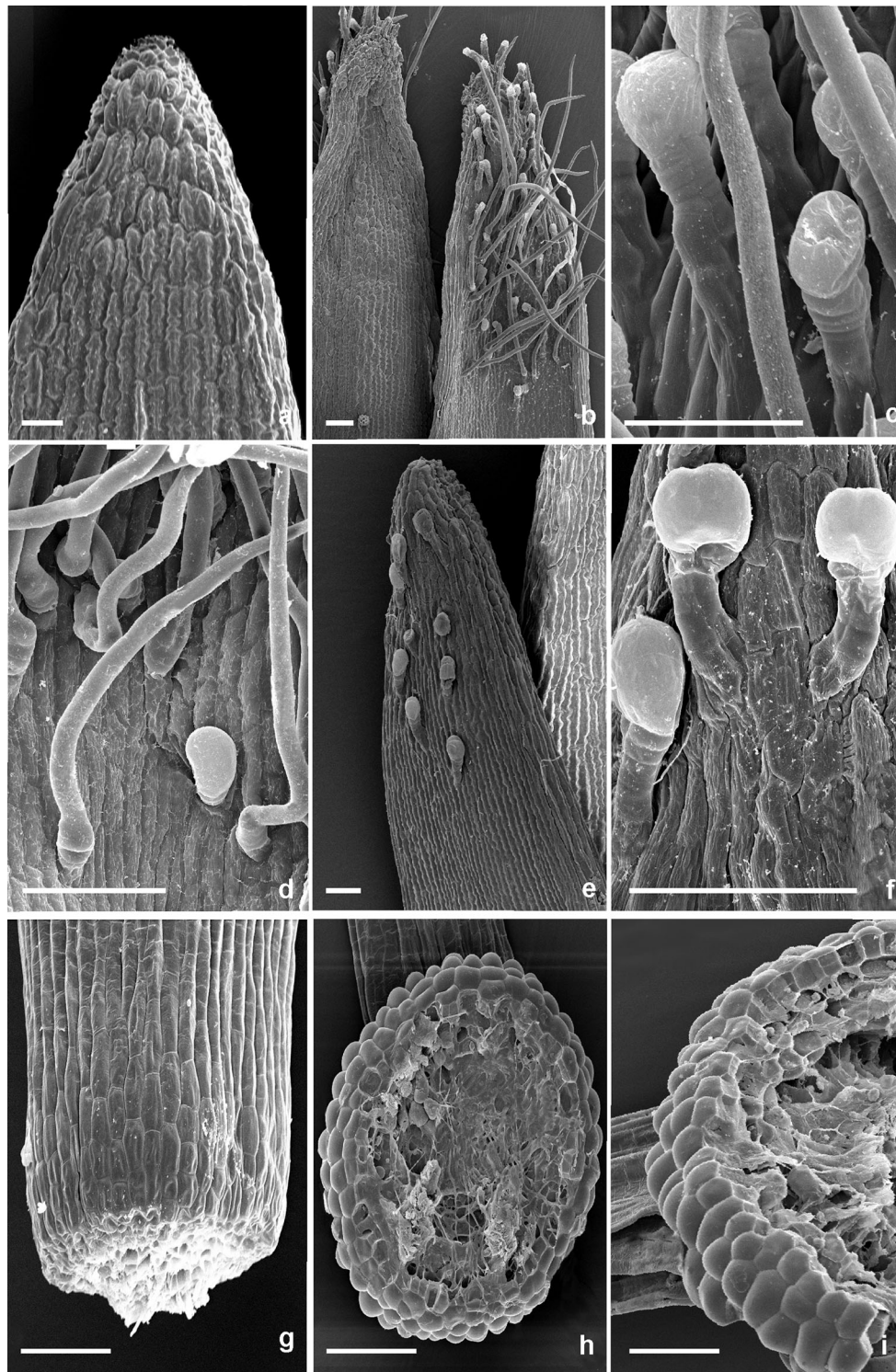


Fig. 2 Floral microcharacters of *Lessingianthus*. **a** *L. centauropsi-deus*, glabrous corolla lobe tip. *L. durus*. **b** ventral (without trichomes) and dorsal surface of corolla lobes (glandular and non-glandular trichomes), **c** details of glandular trichomes type III. **d** *L. pseudoincanus*, non-glandular (type II) and glandular (type III) trichome.

L. scabrifolius. **e** corolla lobe tip showing glandular trichomes, **f** details of glandular trichomes type IV. **g** *L. plantaginoides*, details of style base without basal node. **h**, **i** *L. brevifolius*, style base with basal node. Scale bar 10 μ m

Discussion

This study represents the first comprehensive survey of floral microcharacters of *Lessingianthus*, considering almost all species of the genus. In addition, this is the first

detailed comparative analysis of floral micromorphology in New World species of Vernonieae.

Corolla pubescence

Pubescence of the corolla lobes is a common feature in Vernonieae (Isawumi 1999). The presence of papillae is variable in *Lessingianthus*, since some species have both individuals with and individuals without papillae. This had already been observed in West African species of Vernonieae such as *Pseudoelephantopus spicatus* (Juss. ex Aubl.) C.F. Baker, and *Ethulia conyzoides* L.f. (Isawumi 1999).

Vernonieae show considerable trichome diversity, particularly in the genus *Vernonia* sensu lato, which includes species with several types of non-glandular trichomes and a few glandular ones (Narayana 1979). Most studies have focused on leaves (Cabrera 1944; Hunter and Austin 1967; Narayana 1979; Adedeji and Jewoola 2008; Bonissoni and Duarte 2008), and corolla trichomes had been analyzed in detail only in Old World Vernonieae, such as in species of *Baccharoides* Moench (Isawumi et al. 1996), *Cyanthillium* Blume (Isawumi 1995a), and *Vernonia* (Isawumi 1995b). Recently, Redonda-Martínez et al. (2012) studied the trichomes on the leaves and florets of Mexican species of *Vernonanthura* and *Vernonia*. Therefore, this study is the first to analyze corolla pubescence in South American species of Vernonieae.

In *Lessingianthus*, corolla pubescence is the most variable of all microcharacters examined. The four trichome types identified (types I–IV) vary in structure, distribution, and number of cells within and among species.

The corolla of *Lessingianthus* species bears either glandular or non-glandular trichomes, or a combination of both. Glandular trichomes (i.e., types III, IV) in

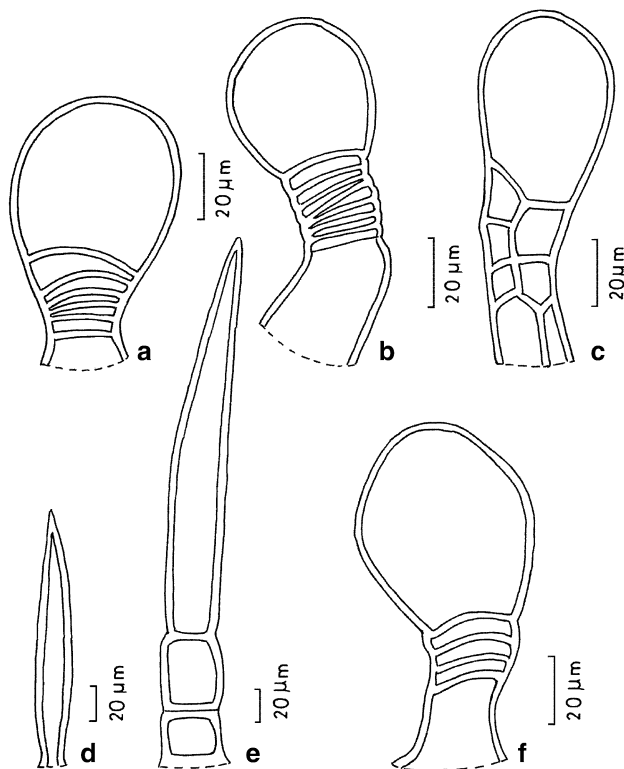


Fig. 3 Trichomes of corolla of *Lessingianthus* species. Glandular trichomes type III, **a** *L. durus*, **b** *L. carvalhoi*. **c** Glandular trichomes type IV of *L. varroniifolius*. **d** Non-glandular trichomes type I of *L. cataractarum*. **e** Non-glandular trichomes type II of *L. polyphyllus*. **f** Glandular trichomes of corolla tube of *L. brevifolius*. Scale bar 20 µm



Fig. 4 Glandular trichomes stained with Sudan IV (arrows). **a** *L. scarifolius*, **b** *L. varroniifolius*. Scale bar 20 µm

Lessingianthus differ morphologically from those in closely related genera. In *Chrysolaena*, glandular trichomes of leaves and underground systems are multicellular and biseriate formed by a pair of basal cells and three pairs of secretory cells (Appezato-da-Glória et al. 2012). In *Vernonanthura*, glandular trichomes are formed by 2–3 short basal cells plus a pair of semiglobose apical cells, and occur on vegetative parts and on the corolla, androecium and cypselae (Redonda-Martínez et al. 2012). Whereas the corollas of *Lessingianthus* also bear non-glandular trichomes, that of *Vernonanthura* and *Vernonia* have only glandular trichomes (Redonda-Martínez et al. 2012). In *Lessingianthus*, trichomes only occur on the dorsal side of the corolla, as in most other Vernonieae, except for the genus *Dasyanthina* H. Rob. bearing short non-glandular trichomes on the ventral side (Keeley and Robinson 2009).

Trichome type is consistent within *Lessingianthus* species and the presence or absence of certain trichome types allows differentiation among related species that are morphologically similar. For example, *L. plantaginoides* (Kuntze) H. Rob. and *L. ulei* (Hieron.) H. Rob. can be distinguished by the presence of type I and III trichomes on corolla lobes in the former and type II trichomes in the latter. Both species are also related to *L. intermedius* (DC.) Dematt., which has glabrous corollas. Therefore, the presence or absence of trichome types can be used to identify some of the species within the genus.

The taxonomic value of trichomes increases when they are considered together with other characters. For example, three species of the *L. saltensis* complex can be distinguished from each other by morphological features, such as texture and shape of leaves, phyllary apex, cypselae pubescence, as well as by the chromosome number (Angulo and Dematteis 2012a). All three species bear glandular trichomes on the corolla lobes, but *L. saltensis* (Hieron.) H. Rob. bears type IV trichomes, while both *L. coriarius* M. B. Angulo and *L. membranifolius* M. B. Angulo bear type III trichomes. Although these two species have the same trichome type, they differ in other morphological traits, such as leaf texture and cypselae pubescence (membranaceous and glabrous in *L. membranifolius*, and coriaceous and pilose in *L. coriarius*).

In *Lessingianthus*, the glandular trichomes secrete only lipids. In other species of Asteraceae, glandular trichomes secrete lipids, polysaccharides, and proteins as well (Werker and Fahn 1981; Werker et al. 1994; Monteiro et al. 2001). Secondary metabolites, such as flavonoids, are common in Asteraceae; however, in Vernonieae they are barely documented, and have been reported only in *Chrysolaena obovata* and *C. platensis* (Spreng.) H. Rob. (Appezato-da-Glória et al. 2012).

Secretions produced by the trichomes in plants are considered to function as protection against herbivores and

pathogens, for attraction of pollinators or fruit dispersal (Ascensão et al. 1998; Monteiro et al. 2001). Also, flavonoids inside the trichomes may prevent oxidation of other compounds (Appezato-da-Glória et al. 2012). Detailed chemical studies of the trichome secretion are necessary to infer possible functions of such trichomes in *Lessingianthus*.

Style base

The basal stylar node was used in the classification of Vernonieae of both hemispheres. Most Vernonieae of the Eastern Hemisphere lack nodes; however, the African genus *Distephanus* Cass. has a highly developed node. In the Western Hemisphere, the stylar node is common and its absence appears to be more significant (Robinson 1988a). *Lessingianthus* species lack the node (Robinson 1988a). However, 5 species: *L. brevifolius* (Less.) H. Rob., *L. carvalhoi* (H. Rob.) H. Rob., *L. durus* (Mart. ex DC.) H. Rob., and *L. regis* (H. Rob.) H. Rob. showed a basal stylar node. The results obtained in *L. regis* and *L. brevifolius* agree with a previous study carried out by Robinson (1990). These five species also share palynological characteristics. Angulo and Dematteis (2010) found pollen type “B-1” in these species. The type “B-1” differs from the typical “B” pollen of the genus. Whereas the first is triporate, echinolophate without equatorial lacunae; the second is tricolporate, echinolophate with equatorial lacunae (Keeley and Jones 1979; Dematteis and Pire 2008; Angulo and Dematteis 2010). Only *L. soderstroemii* (H. Rob.) H. Rob., which also has type B-1 pollen, lacks a node at the stylar base.

The principal coordinate analysis (PCoA) ordination of the *Lessingianthus* species demonstrates a grouping by correspondence to the presence or absence of basal stylar node and pollen type. Both characters allow to separate the species without basal stylar node and with pollen “type B” from taxa with node at the stylar base and pollen “type B-1”. Also, several subgroups have been differentiated showing that many species of *Lessingianthus* share the same micromorphological characteristics.

Apical anther appendage

The apical anther appendages of some Vernonieae have small glandular punctations (Robinson 1990). However, *Lessingianthus* species lack them. Thus, its presence or absence allows the distinction between some genera of Vernonieae. For example, in contrast to *Lessingianthus*, *Chrysolaena* species have glandular apical appendages (Dematteis 2007, 2009). Dematteis (2007) used this character in combination with other features to transfer the two species *L. simplex* (Less.) H. Rob. and *L. desertorum* (Mart.

PCA plot showing the first two principal components (Coordinate 1: 24,3%; Coordinate 2: 15,8%) for 24 species. The plot is divided into six subgroups (Subg 1 to Subg 6) based on morphological characters. Species are labeled with their names, and a large circle encompasses the main cluster of species.

Species and Subgroups:

- Subg 1 (Orange):** urg, ara, gra, pen, mol, fon, bish, pol, exi, sub, card, tey, lae, brevip, sanct, card, tey, lae, psi, syn, de, pseudop, coria, ven, pseudop, spi, subo, var, reb, det, val, no, ele, sou, pseudop, ade, obs.
- Subg 2 (Green):** lor, lani, sel, plan, laur, prof, cep, can, salt, ros, pum, zuc, west, sca, pus, ad, kun, cord, ved, parv, bam.
- Subg 3 (Blue):** comp, tan, long, par, fol, sado, laru, cip, hys, havi, lbi, arc.
- Subg 4 (Red):** hyp, eno, lan, min, ret, oi.
- Subg 5 (Purple):** gran, has, roem, inter, cris, men, ru, salt, plat, sec, barc, chav, foc, nvi, cat, nve, con.
- Subg 6 (Brown):** macrop, tom, macrop, pyr, nian, ligu.

Species outside the circle: constrictus, brevifolius, santosii, carvalhoi, soderstroemii, regis.

The shape of the anther apical appendage is widely used in Asteraceae classification. Liu and Yang (2011) demonstrated that this feature along with other floral microcharacters combined with cytological and molecular evidence strongly supports the polyphyletic nature of the genus *Sinosenecio* B. Nord. (Senecioneae). In *Lessingianthus* the shape of the anther apical appendage is variable, being a useful tool to differentiate among morphologically related species within the group.

Lessingianthus was segregated from *Vernonia* on the base of a combination of micro- and macromorphological characters (Robinson 1988a). As mentioned above, *Lessingianthus* differs from the remaining members of Vernoniaeae by a set of cytological, palynological, and morphological features (Robinson 1999; Angulo and Dematteis 2010, 2012b). Molecular and morphological data suggest that *Lessingianthus* is closely related to the genera *Chrysolaena* and *Lepidaploa* (Robinson 1999; Dematteis 2007, 2009; Keeley et al. 2007). Some micromorphological features are useful to distinguish these genera from one another, whereas some others are shared by the three genera (see Table 3). For example, glandular hairs on the corolla lobes are present in the three genera, but only *Lessingianthus* shows non-glandular trichomes. With regard to the style base, *Lepidaploa* often has a node

Microcharacters	<i>Lessingianthus</i>	<i>Lepidaploa</i>	<i>Chrysolaena</i>
Trichomes of corolla lobes	Glandular and non-glandular	Glandular	Glandular
Apical anther appendage	Without glands	Rarely glandular	Glandular
Basal stylar node	Rarely present	Present	Absent
Ovary wall crystals	Cubic	Prismatic	Prismatic
Trichomes of cypselas	Non-glandular	Non-glandular, rarely glandular	Non-glandular and glandular

In conclusion, *Lessingianthus* shares several micro-morphological features with overlaps with *Lepidaploa* and *Chrysolaena*; some characters exhibit various states, some of which occur across these genera. Therefore, the investigated microcharacters here are difficult to be used as good

taxonomic markers at the genus level, but they are valuable within genera to differentiate related species from one another.

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Appendix

Specimens analyzed

Lessingianthus adenophyllus (Mart. ex DC.) H.Rob.

BRAZIL, Minas Gerais: Menezes et al. 11878 (CTES).

L. arachniolepis (Ekman & Dusen) H.Rob.

BRAZIL, Paraná: Barbosa and Costa 1025 (CTES).

L. arctatus Dematt.

BRAZIL, Goiás: Magenta et al. 266 (CTES).

L. argenteus (Less.) H.Rob.

BRAZIL Paraná: Hatschbach 1989 (CTES). PARAGUAY, Amambay: Dematteis et al. 3396 (CTES).

L. argyrophyllus (Less.) H.Rob.

BRAZIL, Brasília: Krapovickas et al. 31158 (CTES). Minas Gerais: Krapovickas et al. 33353 (CTES).

L. bardanoides (Less.) H.Rob.

BRAZIL, Mato Grosso do Sul: Hatschbach et al. 58736 (CTES). PARAGUAY, Amambay: Dematteis et al. 3393 (CTES).

L. barrosoanus Dematt.

BRAZIL, Bahía: Guédès et al. s. n. (SPF).

L. bellulus Dematt.

BRAZIL, Mato grosso do Sul: Hatschbach et al. 58687 (CTES).

L. bishopii (H.Rob.) H.Rob.

BRAZIL, Goiás: Saint Hilaire 487 (P).

L. brevifolius (Less.) H.Rob.

ARGENTINA, Corrientes: Angulo 11 (CTES). BRAZIL, Paraná: Silva et al. 5152 (CTES). PARAGUAY, Alto Paraná: Schinini et al. 28196 (CTES). URUGUAY, Rivera: Bonifacino and Wilson 2140 (CTES).

L. brevipetiolatus (Sch. Bip. ex Baker) H.Rob.

BRAZIL, Minas Gerais: Roth 1615 (CESJ).

L. buddleiifolius (Mart. ex DC.) H.Rob.

BRAZIL, Goiás: Krapovickas et al. 33270 (CTES).

L. carduoides (Baker) H.Rob.

BRAZIL, Goiás: Gardner 4192 (P).

L. carvalhoi (H.Rob.) H.Rob.

BRAZIL, Bahía: Conceição785 (ALCB); Harley et al. 50404 (CTES).

L. cataractarum (Hieron.) H.Rob.

ARGENTINA, Misiones: Gatti 28 (CTES). BRAZIL, Parana: Ribas et al. 6258 (CTES).

L. centauropsideus (Hieron.) Dematt.

ARGENTINA, Salta: Arbo et al. 9107 (CTES).

L. cephalotes (DC) H.Rob.

BRAZIL, Goiás: Glaziou 21577 (G). Minas Gerais: Mello Silva et al. CfCR 5768 SPF 35568 (CTES).

L. cipoensis Dematt.

BRAZIL, Minas Gerais: Hatschbach 30041 (MBM).

L. clavatus (Gardner) Dematt.

BRAZIL, Minas Gerais: Mexia 5536 (P).

L. compactiflorus (Mart. ex Baker) H.Rob.

BRAZIL, Goiás: Hatschbach 43705 (CTES).

L. constrictus (Matzenb. & Mafioleti) Dematt.

BRAZIL, Río Grande do Sul: Krapovickas and Cristóbal 44772 (CTES). Irgang 9865 (CTES)

L. cordiger (Mart. ex DC.) H.Rob.

BRAZIL, Minas Gerais: Menezes et al. CFCR 11843 (CTES); Semir et al. SPF 42759 CFCR 9510 (CTES); Hatschbach et al. 72053 (CTES).

L. coriaceus (Less.) H.Rob.

BRAZIL, Minas Gerais: Krapovickas et al. 33375 (CTES). BOLIVIA, Santa Cruz: Dematteis et al. 2066 (CTES).

L. coriarius M.B. Angulo

BOLIVIA, Chuquisaca: Beck 6252 (CTES). Santa Cruz: Dematteis et al. 3660 (CTES); Fuentes 2372 (CTES).

L. correntinus (Cabrera & Cristóbal) Dematt.

ARGENTINA, Corrientes: Schinini and Ahumada 13895 (CTES).

L. cristalinae (H.Rob.) H.Rob.

BRAZIL, Goiás: Barroso 522 (UB).

L. chamaepeuces (Sch. Bip. ex Baker) H.Rob.

BRAZIL, Mato Grosso: Malme 3442 (S).

L. durus (Mart. ex DC.) H.Rob.

BOLIVIA, Santa Cruz: Dematteis et al. 2102 (CTES). BRAZIL, Goiás: Hatschbach et al. 70856 (CTES).

L. eitenii (H.Rob.) H.Rob.

BRAZIL, Distrito Federal: Fonseca and Alvarenga 2220 (RB).

L. elegans (Gardner) H.Rob.

BRAZIL, Minas Gerais: Souza et al. 10107 B (CTES).

L. exiguus (Cabrera) H.Rob.

BRAZIL, Paraná: Krapovickas and Cristobal 40842 (CTES).

L. farinosus (Baker) H.Rob.

BRAZIL, Bahia: Giulietti et al. CFCR 1293 (CTES).

L. floccosus (Gardner) H.Rob.

BRAZIL, Minas Gerais: Gardner 4786 (G).

L. foliosus Dematt.

BRAZIL, San Pablo: Glaziou 8133 (P).

L. fonsecae (H.Rob.) H.Rob.

- BRAZIL, Goiás: Pirani 1835 (K).
L. glabratus (Less.) H.Rob.
 ARGENTINA, Corrientes: Vanni et al. 1476 (CTES).
L. grandiflorus (Less.) H.Rob.
 BRAZIL, Goiás: Hatschbach et al. 70744 (CTES).
 PARAGUAY, Amambay: Dematteis and Schinini 859 (CTES).
L. grearii (H.Rob.) H.Rob.
 BRAZIL, Goiás: Paula-Souza et al. 4563 (CTES).
L. hasslerianus (Chodat) Dematt.
 PARAGUAY, Itapúa: Montes 7161 (CTES).
L. hovaefolius (Gardn.) H.Rob.
 BRAZIL, Goiás: Hatschbach et al. 70576 (CTES).
L. hypochaeris (DC) H.Rob.
 BRAZIL, Paraná: Silva and Ribas 3491 (CTES).
L. hystricosus (Cabrera & Dematt.) Dematt.
 PARAGUAY, Amambay: Dematteis et al. 905 (CTES).
 Cordillera: Schinini 10908 (CTES).
L. ibitipocensis Borges & Dematt.
 BRAZIL, Minas Gerais: Saint Hilaire 200 (P).
L. intermedius (DC.) Dematt.
 URUGUAY, Colonia: Solis Neffa et al. 2089 (CTES).
 Montevideo: Sellow s. n. (BR).
L. irwinii (G.M. Barroso) H.Rob.
 BRAZIL, Goiás: Glaziou s. n. (G).
L. ixiamensis (Rusby) H.Rob.
 BOLIVIA, Beni: Krapovickas and Schinini 34882 (CTES). BRAZIL, Mato Grosso: Pedersen 12213 (CTES).
L. kuntzei (Hieron.) Dematt.
 BOLIVIA, Santa Cruz: Saravia Toledo 12309 (CTES); Vargas 880 (CTES).
L. laevigatus (Mar. ex DC.) H.Rob.
 BOLIVIA, Beni: Solomon 7881 (CTES). BRAZIL, Minas Gerais: Hatschbach et al. 64730 (CTES).
L. lanatus (Cabrera) Dematt.
 PARAGUAY, Caaguazú: Schinini et al. 36145 (CTES).
 Cordillera: Schinini 2211 (CTES)
L. laniferus (Cristobal & Dematt.) M. B. Angulo
 ARGENTINA, Corrientes: Krapovickas et al. 18056b (CTES). Misiones: Maruñak 119 (CTES). BRAZIL, Santa Catarina: Ribas and Hatschbach 7513 (CTES). URUGUAY, Rivera: Dematteis and Schinini 1448 (CTES).
L. lanuginosus Dematt.
 BRAZIL, Brasília: Krapovickas et al. 33175 (CTES).
 Goiás: Serra dos Pireneus, Hatschbach et al. 70059 (CTES).
L. laurifolius (H. B. K.) H.Rob.
 BOLIVIA, La Paz: Beck 19805 (CTES).
L. ligulifolius (Mart. ex DC.) H.Rob.
 BRAZIL, Distrito Federal: Krapovickas and Cristóbal 33190 (CTES). Goiás: Hatschbach and Silva 59996 (CTES).
L. linearifolius (Less.) H.Rob.
 BRAZIL, Minas Gerais: Saavedra et al. 270 (CTES).
 Serra de Ibitipoca: Sucre and Krieger 6850 (CTES).
L. linearis (Spreng.) H.Rob.
 BRAZIL, Bahia: Ganev 186 (CTES). Minas Gerais: Vidal I-828 (CTES).
L. longicuspis Dematt.
 BOLIVIA, Santa Cruz: Dematteis et al. 2186 (CTES).
L. lorentzii (Hieron.) H.Rob.
 ARGENTINA, Corrientes: Carnevalli 4413 (CTES).
 Entre Rios: Martínez Crovetto 4818 (CTES).
L. macrocephalus (Less.) H.Rob.
 BRAZIL, Rio Grande do Sul: Deble et al. s.n. (CTES).
 URUGUAY, Rivera: Pedersen 11648 (CTES).
L. macrophyllus (Less.) H.Rob.
 BRAZIL, Paraná: Hatschbach et al. 66520 (CTES)
L. mansoanus (Baker) H.Rob.
 BRAZIL, Mato Grosso do Sul: Hatschbach et al. 66643 (CTES). Mato Grosso: Hatschbach et al. 66727 (CTES).
L. membranifolius M.B. Angulo
 BOLIVIA, Santa Cruz: Dematteis et al. 2383 (CTES); Dematteis et al. 2362 (CTES).
L. minimus Dematt.
 BRAZIL, Goiás: Hatschbach and Kummrow 37253 (CTES).
L. mollissimus (D. Don ex Hook & Arn.) H.Rob.
 ARGENTINA, Misiones: Krapovickas and Cristóbal 28841 (CTES). BRAZIL, Rio Grande do Sul: Hagelund 7851 (CTES). PARAGUAY, Alto Paraná: Caballero Mar-mori s.n. (CTES).
L. morii (H.Rob.) H.Rob.
 BRAZIL, Bahia: Hatschbach et al. 67666 (CTES).
L. myrsinites H.Rob.
 BRAZIL, Goiás: Hatschbach et al. 60128 (CTES).
L. niederleinii (Hieron.) H.Rob.
 ARGENTINA, Misiones: Montes 192 (CTES). BRAZIL, Mato Grosso do Sul: Hatschbach et al. 74473 (CTES).
 PARAGUAY, Caaguazú: Lopez et al. 196 (CTES).
L. obscurus (Less.) H.Rob.
 BRAZIL, Goiás: Gardner 3791 (G).
L. obtusatus (Less.) H.Rob.
 BOLIVIA, Santa Cruz: Killeen et al. 6499 (CTES).
 BRAZIL, Goiás: Hatschbach et al. 60116 (CTES).
L. onopordioides (Baker) H.Rob.
 BRAZIL, Mato Grosso do Sul: Souza et al. 16972 (CTES).
L. paraguariensis Dematt.
 PARAGUAY, Amambay: Schinini et al. 5897 (CTES).
L. parvifolius (Chodat) H.Rob.
 PARAGUAY, Cordillera: Bordas 1257 (CTES). Par-aguari: Mereles et al. 8453 (CTES).
L. pentacontus (DC) H.Rob.
 BRAZIL, Minas Gerais: Hatschbach et al. 64852 (CTES); Hatschbach et al. 64644 (CTES).
L. plantaginoides (Kuntze) H.Rob.

ARGENTINA, Corrientes: Krapovickas and Cristobal 29013 (CTES). Entre Rios: Krapovickas and Cristobal 46566 (CTES). BRAZIL, Porto Alegre: Bueno 2148 (CTES). URUGUAY, Soriano: Castellanos 17612 (CTES).

L. platyphyllus (Chodat) H.Rob.

BRAZIL, Mato Grosso do Sul: Hatschbach et al. 74558 (CTES). PARAGUAY, Amambay: Dematteis and Schinini 865 (CTES).

L. polyphyllus (Sch. Bip. ex Baker) H.Rob.

ARGENTINA, Misiones: Dematteis 2752 (CTES). BRAZIL, Paraná: Hatschbach 79600 (CTES). PARAGUAY, Amambay: Schinini et al. 36102 (CTES).

L. profusus (Dematt. & Cabrera) M. B. Angulo

BRAZIL, San Pablo: Panizza SPF 17789 (CTES). PARAGUAY, Amambay: Schinini et al. 30440 (CTES).

L. pseudoincanus (Hieron.) Dematt.

ARGENTINA, Buenos Aires: Mazzucconi 967 (CTES). Corrientes: Angulo 9 (CTES). Salta: Tolaba 2039 (CTES). Santiago del Estero: Elisetch 328 (CTES).

L. pseudoptocarphus (H.Rob.) H.Rob.

BRAZIL, Goiás: Machado Teles et al. 1906 (RB).

L. psilophyllus (Sch. Bip. ex Baker) H.Rob.

BRAZIL, Minas Gerais: Hatschbach et al. 51076 (CTES); Hatschbach et al. 64716 (CTES).

L. pumillus (Vell.) H.Rob.

BRAZIL, Paraná: Hatschbach 25932 (CTES)

L. pusillus (Dematt.) M.B. Angulo.

ARGENTINA, Chaco: Dematteis 605 (CTES). Corrientes: Dematteis et al. 2769 (CTES). Formosa: Morel 5107 (CTES). PARAGUAY, Boquerón: Vanni 2628 (CTES).

L. pycnostachys (DC) H.Rob.

BRAZIL, Minas Gerais: Motta 1243 (CTES); Arbo et al. 4949 (CTES).

L. regis (H.Rob.) H.Rob.

BRAZIL, Minas Gerais: Zappi et al. 10301 (CTES).

L. reitzianus (Cabrera) H.Rob.

BRAZIL, Santa Catarina: Silva and Andrade 5462 (CTES). Paraná: Ferrucci et al. 236 (CTES); Hatschbach 39206 (CTES).

L. rigescens (Malme) Dematt.

BRAZIL, Mato Grosso: Malme 1462 (S).

L. robustus (Rusby) H.Rob.

BOLIVIA, La Paz: Krapovickas 46754 (CTES). Santa Cruz: Fuentes and Navarro 2183 (CTES). BRAZIL, Mato Grosso: Riedel 1474 (P).

L. roseus (Mart. ex DC.) H.Rob.

BRAZIL, Minas Gerais: Krapovickas and Cristobal 33517 (CTES); Abudd et al. CFSC 10752 (CTES); Damazio 1465 (RB).

L. rosmarinifolius (Less.) H.Rob.

BRAZIL, Minas Gerais: Arbo et al. 3913 (CTES); Arbo et al. 4170 (CTES).

L. rubricaulis (Humb. & Bonpl.) H.Rob.

ARGENTINA, Corrientes: Dematteis et al. 2756 (CTES). Misiones: Dematteis and Surenciski 2452 (CTES). BOLIVIA, Santa Cruz: Dematteis et al. 3567 (CTES). BRAZIL, Mato Grosso do Sul: Hatschbach et al. 74696 (CTES). PARAGUAY, Amambay: Dematteis et al. 3375 (CTES).

L. saltensis (Hieron.) H.Rob.

ARGENTINA, Jujuy: Dematteis et al. 2952 (CTES). Salta: Pozner and Belgrano 454 (CTES). BOLIVIA, Chiquitos: Dematteis et al. 2334 (CTES, SI).

L. sancti-pauli (Hieron.) Dematt.

BRAZIL, Paraná: Von Lisingen and Sonehara 115 (CTES).

L. santosii (H.Rob.) H.Rob.

BRAZIL, Bahía: Giulietti 1275 (CTES). Minas Gerais: Arbo et al. 5133 (CTES).

L. scabrifolius (Hieron.) H.Rob.

BOLIVIA, Santa Cruz: Seijo 3215 (CTES).

L. secundus (Sch. Bip. ex Baker) H.Rob.

BRAZIL, Goiás: Glaziov 21634 (R).

L. sellowii (Less.) H.Rob.

ARGENTINA, Misiones: Dematteis 588 (CTES). BRAZIL, Rio Grande do Sul: Deble and Oliveira, s.n. (CTES).

L. soderstroemii (H.Rob.) H.Rob.

BRAZIL, Minas Gerais: Souza et al. 25168 (ESA); Hatschbach et al. 54521 (CTES); Hatschbach et al. 58357 (CTES).

L. souzae (H.Rob.) H.Rob.

BRAZIL, Goiás: Glaziov 21632 (BR).

L. spicatus (Cabrera) Dematt.

ARGENTINA, Misiones: Schwatz 5491 (CTES); Schulz 7185 (CTES).

L. stoechas (Mart. ex DC.) H.Rob.

BRAZIL, Minas Gerais: Hatschbach et al. 72051 (CTES).

L. subcarduoides (H.Rob.) H.Rob.

BRAZIL, Minas Gerais: Irwin et al. 28237 (RB).

L. subobscurus (Malme) H.Rob.

BOLIVIA, Santa Cruz: Seijo and Solis Neffa 3248 (CTES).

L. syncephalus (Sch. Bip.) H.Rob.

BRAZIL, San Pablo: Hohene 2102 (CTES). PARAGUAY, Alto Paraná: Schinini et al. 28185 (CTES).

L. teyucuaensis (Cabrera) Dematt.

ARGENTINA, Misiones: Dematteis et al. 515 (CTES).

L. tomentellus (Mart. ex DC.) H.Rob.

BRAZIL, Minas Gerais: Hatschbach et al. 64579 (CTES).

L. ulei (Hieron.) H.Rob.

BRAZIL, Paraná: Barbosa and Cordeiro 1195 (CTES); Ribas et al. 5060 (CTES).

L. varroniifolius (DC.) H.Rob.

BOLIVIA, Santa Cruz: Dematteis et al. 2076 (CTES); Beck 25852 (CTES).

L. venonissimus (Sch. Bip. Ex Baker)

BRAZIL, Mato Grosso: Hatschbach et al. 66618 (CTES). Rondonia: Krapovickas et al. 40142 (CTES).

L. vepretorum (Mart. ex DC.) H.Rob.

BRAZIL, Minas Gerais: Weddell 1073 (G); Pirani CFSC 12815 (CTES).

L. vestitus (Baker) H.Rob.

BRAZIL, Minas Gerais: Claussen s. n. (P).

L. warmingianus (Baker) H.Rob.

BRAZIL, Minas Gerais: Glaziou 20383 (R); Krapovickas and Cristobal 33496 (CTES); Souza et al. 29576 (CTES).

L. westermanii (Ekman & Dusén) H.Rob.

BRAZIL, Paraná: Dusén 16400 (G).

L. xanthophyllus (Mart. ex DC.) H.Rob.

BRAZIL, Tocantins: Paula-Souza et al. 4763 (CTES).

L. zuccarinianus (Mart. ex DC.) H.Rob.

BRAZIL, Goiás: Weddell 2060 (P). Mato Grosso: Hatschbach et al. 62350 (CTES).

References

- Adedeji O, Jewoola OA (2008) Importance of leaf epidermal characters in the Asteraceae family. *Not Bot Hort Agrobot* 36:7–16
- Anderson LE (1954) Hoyer's solution as a rapid mounting medium for bryophytes. *Bryologist* 57:242–247
- Angulo MB, Dematteis M (2010) Pollen morphology of the South American genus *Lessingianthus* (Vernonieae, Asteraceae) and its taxonomic implications. *Grana* 49:12–25
- Angulo MB, Dematteis M (2012a) Taxonomy of the *Lessingianthus saltensis* (Vernonieae, Asteraceae) species complex. *Ann Bot Fenn* 49:239–247
- Angulo MB, Dematteis M (2012b) Cytotaxonomy of some species of the South American genus *Lessingianthus* (Asteraceae, Vernonieae). *Plant Syst Evol* 298:277–285
- Appenzato-da-Glória B, Hayashi AH, Cury G, Soares MKM, Rochao R (2008) Occurrence of secretory structures in underground systems of seven Asteraceae species. *Bot J Linn Soc* 157:789–796
- Appenzato-da-Glória B, Batista Da Costa F, Da Silva VC, Gobbo-Neto L, Garcia Rehder VL, Hissae Hayashi A (2012) Glandular trichomes on aerial and underground organs in *Chrysolaena* species (Vernonieae-Asteraceae): structure, ultrastructure and chemical composition. *Flora* 207:878–887
- Ascensão L, Pais MSS (1987) Glandular trichomes in *Artemisia campestris* (ssp. *maritima*): ontogeny and histochemistry of the secretory product. *Bot Gaz* 148:221–227
- Ascensão L, Figueiredo AC, Barroso JG, Pedro LG, Schripsema J, Deans SG, Scheffer JC (1998) *Plectranthus madagascariensis*: morphology of the glandular trichomes, essential oil composition, and its biology activity. *Int J Plant Sci* 159:31–38
- Bentham G (1873) Vernonieae. In: Bentham G, Hooker JD (eds) *Genera plantarum*, vol 2 (1), pp 227–231
- Bonissoni EC, Duarte MR (2008) Estudo anatômico de folha e caule de *Elephantopus mollis* Kunth (Asteraceae). *Rev Bras Farmacogn* 18:108–116
- Bremer K (1994) Asteraceae: Cladistics and classification. Timber Press, Portland
- Cabrera AL (1944) Vernonieas Argentinas (Compositae). *Darwiniana* 6:265–379
- Carlquist S (1959) Vegetative anatomy of *Dubautia*, *Argyroxiphium* and *Wilkesia* (Compositae). *Pac Sci* 13:195–210
- Cassini H (1816) Troisième mémoire sur les Synanthérées. *J Phys Chim Hist Nat Arts* 82:116–146
- Dematteis M (2006) Two new species of *Lessingianthus* (Vernonieae, Asteraceae) from the Brazilian highlands. *Bot J Linn Soc* 150:487–493
- Dematteis M (2007) Taxonomic notes on the genus *Chrysolaena* (Vernonieae, Asteraceae), including a new species endemic of Paraguay. *Ann Bot Fenn* 44:56–64
- Dematteis M (2009) Revisión taxonómica del género sudamericano *Chrysolaena* (Vernonieae, Asteraceae). *Bol Soc Argent Bot* 44:3–7
- Dematteis M, Pire SM (2008) Pollen morphology of some species of *Vernonia* s. l. (Vernonieae, Asteraceae) from Argentina and Paraguay. *Grana* 47:117–129
- Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo CW (2009) InfoStat version 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. <http://www.infostat.com.ar>
- Funk VA, Susanna A, Stuessy T, Robinson H (2009) Classification of Compositae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ (eds) *Systematics, evolution, and biogeography of Compositae*. International Association for Plant Taxonomy, Vienna, pp 171–189
- Hunter GE, Austin DE (1967) Evidence from trichome morphology of interspecific hybridization in *Vernonia*: Compositae. *Brittonia* 19:38–41
- Isawumi MA (1995a) Floral microcharacters and taxonomy of the *Cyanthillium cinereum* complex (Asteraceae: Vernonieae). *Comp Newsl* 26:11–25
- Isawumi MA (1995b) Notes on *Vernonia* (Vernonieae: Compositae) in West Africa. In: Hind DJN, Jeffrey C, Pope GV (eds) *Advances in Compositae systematics*. Royal Botanical Gardens, Kew, pp 51–106
- Isawumi MA (1999) Floral microcharacters and taxonomy of the tribe Vernonieae: Asteraceae (excluding *Vernonia* Scherb.) in West Africa. *Feddes Repertorium* 110:359–374
- Isawumi MA, El-Ghazaly G, Nordenstam B (1996) Pollen morphology, floral microcharacters and taxonomy of the genus *Baccharoides* Moench (Vernonieae: Asteraceae). *Grana* 35:205–230
- Keeley SC, Jones SB (1979) Distribution of the pollen types in *Vernonia* (Vernonieae: Asteraceae). *Syst Bot* 4:195–202
- Keeley SC, Robinson H (2009) Vernonieae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ (eds) *Systematics, evolution and biogeography of Compositae*. International Association for Plant Taxonomy, Vienna, pp 439–469
- Keeley SC, Forsman ZH, Chan R (2007) A phylogeny of the “evil tribe” (Vernonieae: Compositae) reveals Old/New World long distance dispersal: Support from separate and combined congruent datasets (*trnL-F*, *ndhF*, ITS). *Mol Phylogenet Evol* 44:89–103
- King RM, Robinson H (1970) The new synanthology. *Taxon* 19:6–11
- Lessing CF (1832) Synopsis generum Compositarum. Duncker & Humblot, Berlin
- Liu Y, Yang QE (2011) Floral micromorphology and its systematic implications in the genus *Sinosenecio* (Senecioneae-Asteraceae). *Plant Syst Evol* 291:243–256
- Metcalfe CR, Chalk L (1957) *Anatomy of the dicotyledons*. Clarendon Press, Oxford, pp 783–804
- Metcalfe CR, Chalk L (1979) *Anatomy of the dicotyledons* I. Clarendon Press, Oxford

- Milan P, Hayashi AH, Appezzato-da-Glória B (2006) Comparative leaf morphology and anatomy of three Asteraceae species. *Braz Arch Biol Techn* 49:135–144
- Monteiro WR, Castro MM, Mazzoniviveiros SC, Mahlberg PG (2001) Development, and some histochemical aspects of foliar glandular trichomes of *Stevia rebaudiana* (Bert.) Bert. *Asteraceae*. *Rev Bras Bot* 24:349–357
- Narayana BM (1979) Taxonomic value of trichomes in *Vernonia* Schreb. (Asteraceae). *Proc Ind Acad Sci* 88:347–357
- Redonda-Martínez R, Villaseñor JL, Terrazas T (2012) Trichome diversity in the Vernonieae (Asteraceae) of Mexico I: *Vernonanthur* and *Vernonia* (Vernoniinae). *J Torrey Bot Soc* 139:235–247
- Robinson H (1988a) Studies in the *Lepidaploa* complex (Vernonieae: Asteraceae). IV. The new genus *Lessingianthus*. *Proc Biol Soc Wash* 100:929–951
- Robinson H (1988b) Studies in the *Lepidaploa* complex (Vernonieae: Asteraceae). V. The new genus *Chrysolaena*. *Proc Biol Soc Wash* 101:952–958
- Robinson H (1990) Studies in the *Lepidaploa* complex (Vernonieae: Asteraceae). VII. The new genus *Lepidaploa*. *Proc Biol Soc Wash* 103:464–498
- Robinson H (1999) Generic and subtribal classification of American Vernonieae. *Smithson Contrib Bot* 89:1–116
- Robinson H (2007) Tribe Vernonieae. In: Kadereit J, Jeffrey C (eds) *The families and genera of vascular plants*, vol 8., *Asterales*. Springer, Berlin, pp 165–192
- Roque N, Keil DJ, Susanna A (2009) Illustrated glossary of Compositae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ (eds) *Systematics, evolution, and biogeography of Compositae*. International Association for Plant Taxonomy, Vienna, pp 781–806
- Roth I (1977) Fruits of angiosperms. *Encyclopedia of plant anatomy*. Gebrüder Borntraeger, Berlin
- Solereder H (1908) *Systematic anatomy of dicotyledons*, vol 2. Clarendon Press, Oxford
- Werker E, Fahn A (1981) Secretory hairs of *Inula viscosa* (L.) Ait. development, ultrastructure and secretion. *Bot Gaz* 142:447–461
- Werker E, Putievsky E, Ravid U, Dudai N, Katzir I (1994) Glandular hairs, secretory cavities, and the essential oil in leaves of tarragon (*Artemisia dracunculus* L.). *J Herbs Spices Med Plants* 2:19–32