

Cytotaxonomy of some species of the South American genus *Lessingianthus* (Asteraceae, Vernoniaeae)

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Abstract Mitotic chromosome numbers of 32 populations belonging to 23 species of the genus *Lessingianthus* H. Rob. (Vernoniaeae, Asteraceae) were determined. The chromosome number of all examined plants was found to be based on $x = 16$. The numbers observed varied from $2n = 32$ to $2n = 176$. The results include the first report of the chromosome number for 11 species: *L. lanatus* ($2n = 32$), *L. varroniifolius* ($2n = 32$), *L. cataractarum* ($2n = 64$), *L. intermedius* ($2n = 64$), *L. argenteus* ($2n = 96$), *L. centaurosideus* ($2n = 96$), *L. profusus* ($2n = 96$), *Lessingianthus* sp. nov. 1 ($2n = 96$), *Lessingianthus* sp. nov. 2 ($2n = 128$), *L. robustus* ($2n = 160$), and *L. macrocephalus* ($2n = 176$). New chromosome numbers were found in the four other species: *L. rubricaulis*, *L. laniferus*, and *L. selowii* were tetraploid with $2n = 64$, while *L. oxyodontus* was hexaploid with $2n = 96$. B chromosomes were observed in *L. coriaceus* and *L. varroniifolius*. *Lessingianthus macrocephalus* ($2n = 11x = 176$) is reported as the first case of an odd polyploid and the higher chromosome number of *Lessingianthus*. The significance of the results is discussed in relation to chromosomal data available for the genus.

Keywords Chromosome numbers · Polyploidy · Base number · B chromosomes · *Vernonia*

Introduction

The tribe Vernoniaeae Cass. (Asteraceae) comprises approximately 89 genera and 1,700 species concentrated

around two major centers of distribution, eastern Africa and non-Amazonian Brazil. From a taxonomic viewpoint, the Vernoniaeae have been considered one of the most complex group of Asteraceae (Robinson 2007; Keeley et al. 2007). The main dispute has commonly centered around the circumscription of the large genus *Vernonia* Schreb. (Cabrera 1944; Keeley 1978; Jones 1979a, 1981; Keeley and Turner 1990; Keeley and Robinson 2009). According to the traditional infrageneric classification, most South American species belong to the section *Lepidaploa* (Cass.) DC. (Bentham 1873; Baker 1873). However, Robinson (1999) revised the taxonomy of the New World species of the Vernoniaeae and segregates almost all the species of this section to new genera, leaving *Vernonia* primarily to North America.

As presently delimited, one of the largest genera of the Vernoniaeae in the New World is *Lessingianthus* H. Rob. with more than 120 taxa. This genus includes the species previously placed in *Vernonia* sect. *Lepidaploa* subsect. *Macrocephalae* Benth. & Hook. and some species belonging to subsect. *Axilliflorae* Benth. & Hook. (Bentham 1873). *Lessingianthus* species have a great range of morphological variation. Species are herbaceous perennials or shrubs with xylopodia, having medium- or large-sized (10–30 mm long) heads and seriate-cymose inflorescences (Robinson 1988b).

The taxa are widely distributed in Brazil, Paraguay, Uruguay, Bolivia, and Argentina, but are basically concentrated in southeastern Brazil (Dematteis and Almeida 2010). They can be distinguished from other members of the tribe by pollen type, anther appendages, chromosome number, and the shape of the crystals in the achene wall (Robinson 1988a, b, 1999; Dematteis 2006). All the species have “type B” grains, which are tricolporate, echinolate, with a discontinuous tectum, having very long

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germinal furrows, joined at poles and lacunae disposed in a regular pattern, lacking a polar lacuna (Keeley and Jones 1979; Angulo and Dematteis 2010). Among the most notable features of the genus the base chromosome number $x = 16$, which distinguishes it from the majority of American Vernoniae with $x = 17$ (Dematteis 2002; Angulo and Dematteis 2009a). Additionally, the genus also has the greatest number of polyploid taxa and the highest ploidy levels within the Vernoniae, with the most extreme case of a decaploid species having $2n = 160$ (Dematteis and Fernández 2000).

Some species of *Lessingianthus* have been previously analyzed cytologically (Jones 1979b; Turner et al. 1979; Galiano and Hunziker 1987; Ruas et al. 1991; Dematteis 1996, 1997, 1998, 2002; Dematteis and Fernández 2000; Dematteis et al. 2007; Oliveira et al. 2007a, b; Angulo and Dematteis 2009a, b). However, most of these studies focused on comparative analysis among species of *Vernonia* or between different genera of the tribe Vernoniae. At present, only 28 species of *Lessingianthus* (23%) have been evaluated cytologically. Despite the scarce cytological information available, the chromosomes have been shown to be useful in the taxonomy of the genus (Dematteis 2002).

In the present study, the chromosome numbers of 23 species of the genus *Lessingianthus* were determined to allow assessment of the relationship between the chromosomal data and taxonomy of the genus.

Materials and methods

The specimens were obtained from natural populations in Argentina, Paraguay, Bolivia, and Uruguay (Table 1).

Mitotic chromosome preparations were made from root meristems obtained from germinating seeds. The roots were pretreated for about 5 h in 0.002 M 8-hydroxyquinoline solution at room temperature, fixed in 3:1 absolute alcohol/acetic acid, and then stained using Feulgen's technique. Permanent microscope slides were prepared by mounting in Euparal. In all samples at least 20 counts of 7–10 individuals were made to verify the observations.

Results

The chromosome numbers of 32 populations belonging to 23 species of *Lessingianthus* were determined. The analyzed taxa and their chromosome numbers are given in Table 1. Eleven species were analyzed for the first time (marked with “*”), while new chromosome numbers (marked with “+”) were found in *L. sellowii*, *L. laniferus* (Fig. 1e), *L. rubricaulis*, and *L. oxyodontus* (Fig. 1g). The

chromosome numbers observed in the remaining taxa are in agreement with previous studies.

Among the species counted, the chromosome numbers have a base of $x = 16$ but ranged widely between $2n = 32$ and $2n = 176$. Of these, only six species were diploid ($2n = 2x = 32$): *L. bardanoides*, *L. coriaceus*, *L. durus* (Fig. 1a), *L. lanatus*, *L. pusillus*, and *L. varroniifolius*. The remaining taxa were polyploids, i.e., tetraploid ($2n = 64$), hexaploid ($2n = 96$), octoploid ($2n = 128$), decaploid ($2n = 160$) or 1-decaploid ($2n = 176$). Two of the diploid species, *Lessingianthus coriaceus* and *L. varroniifolius*, also had B or accessory chromosomes (in somatic cells). Of the 15 individuals counted of the former species there was a range of 0–6 accessory chromosomes, 46.6% with no B chromosomes (Fig. 2a), 26.6% with 1 B, and the remaining 6.6% with 2, 4, 5, or 6 Bs (Fig. 2b). In *L. varroniifolius*, of the 10 individuals counted, 60% had no Bs (Fig. 2c), while 10% presented 2 Bs, 20% had 3 Bs, and the remaining 10% showed 5 Bs (Fig. 2b).

Lessingianthus laniferus was tetraploid ($2n = 4x = 64$) along with the previously reported *L. cataractarum* (Fig. 1d), *L. intermedius*, and *L. pseudoincanus*. *Lessingianthus oxyodontus* is hexaploid ($2n = 6x = 96$), as are *L. argenteus* (Fig. 1f), *L. niederleinii* (Fig. 2e), *L. profusus*, and *Lessingianthus* sp. nov. 1 (Fig. 2f). Octoploid species ($2n = 8x = 128$) are *Lessingianthus scabrifolius* (Fig. 2g) and *Lessingianthus* sp. nov. 2 (Fig. 1h), while *L. teyucuaensis* and *L. robustus* (Fig. 2h) are decaploid ($2n = 10x = 160$). The highest chromosome number was found in *L. macrocephalus* (Fig. 1i), a 1-decaploid with $2n = 11x = 176$. There was little variation in counts within a species except for *L. sellowii* (Fig. 1b–c) and *L. rubricaulis*, where both diploid and tetraploid populations were found. However, only one chromosome number was found within a given plant, and no variation was observed among populations.

Discussion

Polyploidy is a very common phenomenon in New World species of tribe Vernoniae (Jones 1979b). About 25% of the South American taxa are polyploids, most of them tetraploids or octoploids. Among the New World genera, *Lessingianthus* has the greatest proportion of polyploids known, with over 82.5% of a total of 39 taxa (Ruas et al. 1991; Dematteis 1996, 1997, 1998, 2002; Dematteis et al. 2007; Oliveira et al. 2007a; Angulo and Dematteis 2009a, b).

Among the analyzed species, five taxa presented diploid cytotypes: *L. durus*, *L. coriaceus*, *L. bardanoides*, *L. lanatus*, and *L. varroniifolius*. The results obtained in *L. bardanoides* disagrees with a prior analysis carried out by Jones (1979b) that recorded $2n = 34$ for this species.

Table 1 *Lessingianthus* species analyzed in this study, with their respective chromosome numbers, locations, and voucher specimens

Species	2n	Location, voucher
* <i>L. argenteus</i> (Less.) H.Rob.	2n = 6x = 96	Paraguay. Dept. Amambay. Chirigüelo, 2 km W of P.J. Caballero. <i>Dematteis et al.</i> 3396 (CTES)
<i>L. bardanoides</i> (Less.) H.Rob.	2n = 2x = 32	Paraguay. Dept. Amambay. Chirigüelo, 2 km W of P.J. Caballero. <i>Dematteis et al.</i> 3393 (CTES)
* <i>L. cataractarum</i> (Hieron.) H.Rob.	2n = 4x = 64	Argentina. Misiones. Dept. San Pedro. National Park Moconá. <i>Dematteis et al.</i> 3096 (CTES)
* <i>L. centaurosideus</i> (Hieron.) Dematt.	2n = 6x = 96	Argentina. Salta. Dept. Santa Victoria. 6 km S of Los Toldos, on the road to Lipeo. <i>Dematteis et al.</i> 2937 (CTES)
<i>L. coriaceus</i> (Less.) H.Rob.	2n = 2x = 32 + 0–6 Bs	Bolivia. Dept. La Paz, Nor Yungas Province. Climb to Coroico, 1.4 km SE of the city. <i>Dematteis et al.</i> 4062 (CTES)
<i>L. durus</i> (Mart. ex DC.) H.Rob.	2n = 2x = 32	Bolivia. Dept. Santa Cruz, Chiquitos Province 5.7 km S of Santiago, on the road to Roboré. <i>Dematteis et al.</i> 3921 (CTES)
* <i>L. intermedius</i> (DC.) Dematt.	2n = 4x = 64	Uruguay. Dept. Maldonado. Piriápolis, San Antonio Mount. <i>Dematteis et al.</i> 3807 (CTES)
<i>L. intermedius</i> (DC.) Dematt.	2n = 4x = 64	Uruguay. Dept. Artigas, Route 3, 20 km W of Artigas, on the road to Tomás Gomensoro. <i>Dematteis et al.</i> 3704 (CTES)
* <i>L. lanatus</i> (Cabrera) Dematt.	2n = 2x = 32	Paraguay. Dept. Paraguari. 3 km N of Paraguari, in front of Cerro Hú. <i>Dematteis et al.</i> 2803 (CTES)
+ <i>L. laniferus</i> (Cristóbal & Dematt.) M.B.Angulo	2n = 4x = 64	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. <i>Dematteis et al.</i> 3076 (CTES)
* <i>L. macrocephalus</i> (Less.) H.Rob.	2n = 11x = 176	Uruguay. Dept. Rivera, road from Tranqueras to Paso Ataques, 4 km off route 30. <i>Dematteis et al.</i> 3731 (CTES)
* <i>L. macrocephalus</i> (Less.) H.Rob.	2n = 11x = 176	Uruguay. Dept. Rivera, 9 km S of Tranqueras, on the road to Cerro Alegre. <i>Dematteis et al.</i> 3735 (CTES)
<i>L. niederleinii</i> (Hieron.) H.Rob.	2n = 6x = 96	Argentina Misiones. Dept. General Manuel Belgrano. Campina de Américo. <i>Dematteis et al.</i> 2610 (CTES)
<i>L. niederleinii</i> (Hieron.) H.Rob.	2n = 6x = 96	Paraguay. Dept. Amambay. Colonia Lorito Picada. <i>Dematteis et al.</i> 2893 (CTES)
<i>L. niederleinii</i> (Hieron.) H.Rob.	2n = 6x = 96	Argentina Misiones. Dept. General Manuel Belgrano. Campina de Américo. <i>Dematteis et al.</i> 3052 (CTES)
+ <i>L. oxyodontus</i> Malme	2n = 6x = 96	Paraguay. Dept. Canindeyú. 3.4 km N of Igatimí, on the road to Ypé- Jhú. <i>Dematteis et al.</i> 2843 (CTES)
* <i>L. profusus</i> (Dematt. & Cabrera) M.B.Angulo	2n = 6x = 96	Paraguay. Dept. Canindeyú. 3.4 km N of Igatimí, on the road to Ypé-Jhú. <i>Dematteis et al.</i> 2843 (CTES)
<i>L. pseudoincanus</i> (Hieron.) Dematt.	2n = 4x = 64	Argentina. Corrientes. Dept. Mercedes. 8 km N of Felipe Yofre. <i>Dematteis & Seo</i> 2463 (CTES)
<i>L. pusillus</i> (Dematt.) M.B.Angulo	2n = 2x = 32	Argentina. Corrientes. Dept. Capital. Perichón. <i>Dematteis et al.</i> 2769 (CTES)
<i>L. pusillus</i> (Dematt.) M.B.Angulo	2n = 2x = 32	Paraguay. Dept. Concepción. 16 km N of Paso Barreto, Estancia Rosalía. <i>Dematteis et al.</i> 3234 (CTES)
* <i>L. robustus</i> (Rusby) H.Rob.	2n = 10x = 160	Bolivia. Dept. La Paz, Nor Yungas Province. Climb to Coroico, 1.4 km SE of the city. <i>Dematteis et al.</i> 4063 (CTES)
<i>L. rubricaulis</i> (Humb. & Bonpl.) H.Rob.	2n = 2x = 32	Argentina. Corrientes. Dept. San Roque. Route 12, 2 km N of route 123. <i>Dematteis et al.</i> 2756 (CTES)
<i>L. rubricaulis</i> (Humb. & Bonpl.) H.Rob.	2n = 2x = 32	Paraguay. Dept. Amambay. 25 km S of Bella Vista, on the road to route 5. <i>Dematteis et al.</i> 3375 (CTES)
+ <i>L. rubricaulis</i> (Humb. & Bonpl.) H.Rob.	2n = 4x = 64	Bolivia. Dept. Santa Cruz, Chiquitos Province. 25 km N of San José, on the road to San Rafael. <i>Dematteis et al.</i> 3567 (CTES)
+ <i>L. sellowii</i> (Less.) H.Rob.	2n = 2x = 32	Uruguay. Dept. Tacuarembó. Gruta de los Helechos, 10 km NW of Tacuarembó. <i>Dematteis et al.</i> 3760 (CTES)
+ <i>L. sellowii</i> (Less.) H.Rob.	2n = 4x = 64	Argentina. Misiones. Dept. Concepción. On the road to Puerto Azara, 6 km E of Azara. <i>Dematteis et al.</i> 3315 (CTES)

Table 1 continued

Species	2n	Location, voucher
<i>L. scabrifolius</i> (Hieron) H.Rob.	$2n = 8x = 128$	Bolivia. Dept. Santa Cruz, Velasco Province, 67 km from Concepción, on the road to San Ignacio. <i>Dematteis et al.</i> 3856 (CTES)
<i>L. scabrifolius</i> (Hieron) H.Rob.	$2n = 8x = 128$	Bolivia. Dept. Santa Cruz, Velasco Province, 5 km from San Ignacio, on the road to San Rafael. <i>Dematteis et al.</i> 3865 (CTES)
<i>L. teyucuaensis</i> (Cabrera) Dematt.	$2n = 10x = 160$	Argentina. Misiones. Dept. San Ignacio. Teyú Cuare. <i>Dematteis</i> 476 (CTES)
* <i>L. varroniifolius</i> (DC.) H.Rob.	$2n = 2x = 32 + 0-5$ Bs	Bolivia. Dept. Santa Cruz, Velasco Province. 67 km E of Concepción, on the road to San Ignacio. <i>Dematteis et al.</i> 3860 (CTES)
* <i>Lessingianthus</i> sp. nov. 1	$2n = 6x = 96$	Bolivia. Dept. Santa Cruz, Chiquitos Province. Natural Reserve of Tuca Vaca Valley. <i>Dematteis et al.</i> 3930 (CTES)
* <i>Lessingianthus</i> sp. nov. 2	$2n = 8x = 128$	Paraguay. Dept. Concepción, 1 km E of Huguá Poí. <i>Dematteis et al.</i> 3230 (CTES)

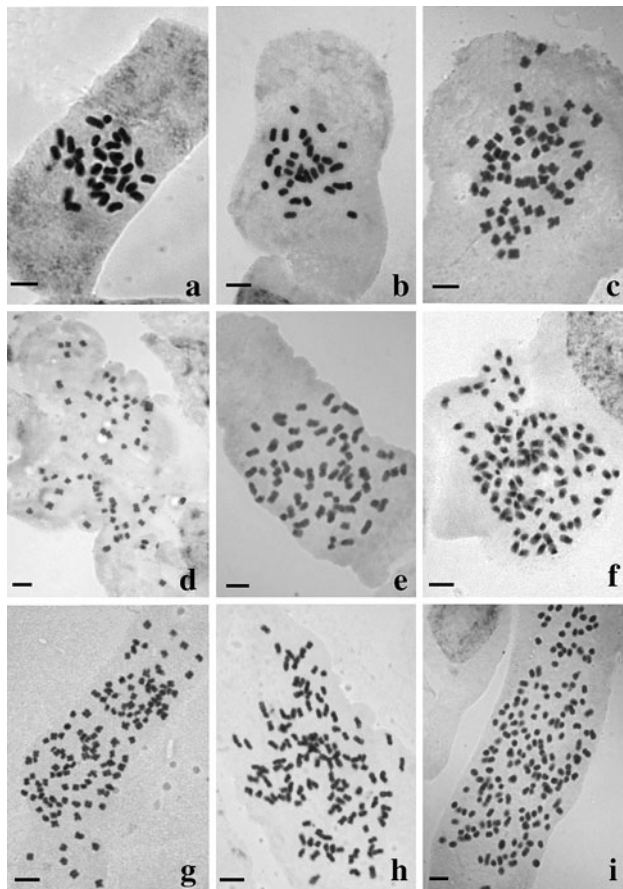


Fig. 1 Mitotic chromosomes of *Lessingianthus*. **a** *L. durus* ($2n = 2x = 32$), *L. sellowii*, **b** diploid cytotype ($2n = 2x = 32$), **c** tetraploid cytotype ($2n = 4x = 64$), **d** *L. cataractarum* ($2n = 4x = 64$), **e** *L. laniferus* ($2n = 4x = 64$), **f** *L. argenteus* ($2n = 6x = 96$), **g** *L. oxyodontus* ($2n = 6x = 96$), **h** *Lessingianthus* sp. nov. 2 ($2n = 8x = 128$), **i** *L. macrocephalus* ($2n = 4x = 64$). Scale 5 μ m

This may have been due to the small size of the chromosomes or, most likely, an incorrect species identification. In some cases, the disparity in chromosome numbers could be attributed to the small size of plant chromosomes (Guerra 1988). In addition, incorrect botanical identification can lead to incorrect reports of the chromosome numbers of species. The taxonomic complexity of this group because of their diversity and/or the existence of hybrids and polyploids that can generate individuals or species with intermediate morphological characters can create serious problems in specimen identification (Stace 1989).

Dematteis (1998) determined $2n = 32$ for Brazilian populations of *L. coriaceus* and *L. durus*. In the present study we examined populations from Bolivia of both entities, which also were diploids, hence our results agree with those found previously.

In this paper, a Bolivian population of *L. varroniifolius* was analyzed cytologically for the first time, being diploid with $2n = 32$. This entity along with *L. coriaceus* showed accessory or B chromosomes. B chromosomes are extra chromosomes of the complement that occur in many organisms. They have been described in more than 1,300 plant and almost 500 animal species (Jones and Rees 1982; Jones 1995).

Many Vernoniaeae have been reported with one or more B chromosomes, accessory or extra chromosomes relative to the normal haploid set. The occurrence of these accessory chromosomes in the Asteraceae family is relatively frequent (Jones 1995), including species of *Vernonia* (Jones 1979b; Galiano and Hunziker 1987; Dematteis 1997, 1998; Angulo and Dematteis 2009b), *Mikania* (Ruas et al. 2000), *Crepis* (Jamilena et al. 1994), and *Haplopappus* (Jackson and Newmark 1960), among other genera. B chromosomes can arise in diverse ways, including

Fig. 2 Somatic chromosomes of *Lessingianthus*. *L. coriaceus*.

a Diploid cytotype ($2n = 2x = 32$), **b** diploid cytotype with B chromosomes ($2n = 2x = 32 + 5 \text{ Bs}$).

L. varroniifolius

($2n = 2x = 32$). **c** Diploid cytotype ($2n = 2x = 32$),

d diploid cytotype with B chromosomes ($2n = 2x = 32 + 5 \text{ Bs}$).

e *L. niederleinii*

($2n = 6x = 96$).

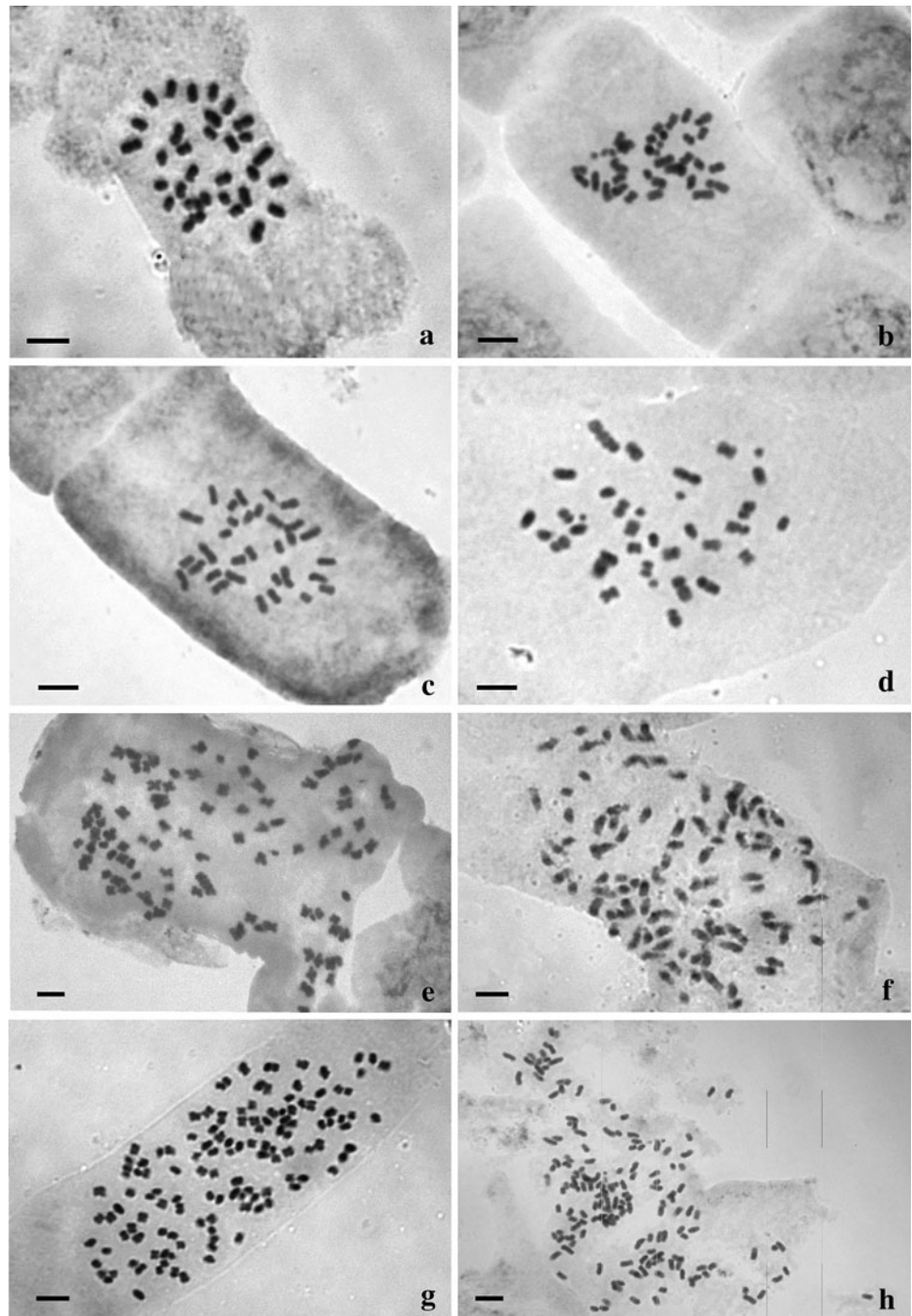
f *Lessingianthus* sp. nov. 1

($2n = 6x = 96$).

g *L. scabrifolius*

($2n = 8x = 128$). **h** *L. robustus*

($2n = 10x = 160$). Scale 5 μm



derivation from autosomes or sex chromosomes (Camacho et al. 2000). Most B chromosomes are generally heterochromatic and variable in number between individuals of the same species (Guerra 1988). The general idea of these elements is that they are genetically inert; i.e., they represent extra genetic material that has little or no effect on phenotype. However, there is evidence that they can affect a multitude of cellular and physiological processes, including characters associated with vigor, fertility, and

fecundity (Camacho et al. 2000). While their effects rarely manifest in the phenotype, in *Haplopappus gracilis* the B chromosomes influence the color of the achenes (Jackson and Newmark 1960). B chromosomes show irregular mitotic and/or meiotic behaviors which generally constitute the basis of accumulation in the germinal line determining non-Mendelian modes of inheritance which involve transmission rates higher than those of normal chromosomes (Camacho et al. 2000). The irregular inheritance of B

chromosomes is due to variations in the levels of pairing, to degrees of meiotic elimination, and to various drive processes. Drive is mostly caused by directed nondisjunction of sister chromatids at the first pollen mitosis; for example, in Gramineae, the generative nucleus carries the unreduced number, which then forms the sperm (Jones and Houben 2003). In maize, the nondisjunction happens at the second pollen mitosis, followed by preferential fertilization by the B-chromosome-containing sperm. Meiotic drive and accumulation at earlier developmental stages in the germline operate in a few cases. These irregularities in transmission generate a numerical polymorphism in populations, with a variety of B chromosome numbers, including individuals with none (Jones and Houben 2003).

In *L. coriaceus* and *L. varroniifolius*, the accessory chromosomes are present in some individuals but not in others of the same population, therefore it is clear that their presence is not necessary for normal development of these individuals.

The chromosome numbers for *L. cataractarum* and *L. intermedius* have been determined here for the first time. Both species were tetraploids having $2n = 4x = 64$. *Lessingianthus intermedius* is morphologically closely related to *L. plantaginoides*, which is also tetraploid with $2n = 64$ (Dematteis 1997). However, the two entities can be distinguished by the type of inflorescence and the shape and pubescence of the phyllaries.

Lessingianthus argenteus, *L. centaurosideus*, *L. profusus*, and *Lessingianthus* sp. nov. 1 were hexaploid ($2n = 6x = 96$). These entities have not been previously analyzed and consequently these are the first records of chromosome numbers for these taxa. *Lessingianthus* sp. nov. 1 is distributed in eastern Bolivia and seems to be closely related to *L. scabrifolius*. However, both species can be distinguished by some morphological features and the chromosome number. The new species differs from *L. scabrifolius* in leaf apices, size of the heads, and achenes trichomes. Regarding chromosome number, *Lessingianthus* sp. nov. 1 is hexaploid with $2n = 96$, while *L. scabrifolius* is octoploid with $2n = 128$. The counts in the two Bolivian populations of *L. scabrifolius* confirm the single previous record for this species (Angulo and Dematteis 2009a).

Lessingianthus oxyodontus also presents $2n = 96$, but it is distributed in southern Brazil, Paraguay, and Uruguay. A prior analysis in this species reported $2n = 128$ (Dematteis 1997). The results obtained here indicate that it has two different ploidy levels. The chromosome count of $2n = 128$ could represent an octoploid population, while the sample analyzed here having $2n = 96$ is hexaploid and constitutes the first report of that ploidy level for the species. This taxon is closely related to *L. glabratus* and *L. niederleinii*, from which it differs in the shape and size

of leaves, among other morphological features. These taxa also have certain resemblance to *L. teyucarensis* that was decaploid with $2n = 160$, which agrees with a prior analysis realized by Dematteis and Fernández (2000). *Lessingianthus niederleinii* was hexaploid with $2n = 6x = 96$, a report that differs from a determination carried out by Dematteis (1996), who described $2n = 90$ for the species. All these species have high chromosome numbers, however *L. niederleinii*, *L. teyucarensis*, and *L. oxyodontus* can be distinguished by morphological features, as well as by the chromosome number.

Lessingianthus sp. nov. 2 is octoploid with $2n = 128$. This new species is distributed in Paraguay and closely resembles *L. niederleinii* but differs in the disposition of heads, pubescence of leaves, and chromosome number. A Bolivian population of *L. robustus* was decaploid with $2n = 10x = 160$, which constitutes the first chromosome count for the species and the second report of the decaploid ploidy level in the genus.

Lessingianthus includes closely related species that are morphologically similar but with different ploidy level, constituting polyploid complexes, such as *L. glabratus* (Less.) H. Rob., *L. mollissimus*, *L. rubricaulis*, and *L. saltensis* (Hieron.) H. Rob. (Angulo and Dematteis 2009a). The *L. rubricaulis* complex consists of four related species: *L. rubricaulis*, *L. pseudoincanus*, *L. pusillus*, and *L. laniferus*. These entities are distinguished by their habit, underground system, indumentum type, size of leaves, and leaf shape (Dematteis 2004). However, the ploidy level is not useful to distinguish these species. *Lessingianthus rubricaulis* has both diploid ($2n = 32$) and tetraploid ($2n = 64$) cytotypes. A previous analysis on populations from Argentina and Paraguay reported $2n = 32$ (Dematteis 2002), while the Bolivian specimens of *L. rubricaulis* examined here were tetraploids with $2n = 64$. *Lessingianthus pseudoincanus* is tetraploid with $2n = 64$, while *L. laniferus* has two ploidy levels: diploid and tetraploid. Previously, in specimens from Uruguay, $2n = 32$ has been recorded (Angulo and Dematteis 2009a), while in one Argentinean population $2n = 64$ was found, which constitutes a new chromosome number for the species. Finally, the count of $2n = 32$ for *L. pusillus* confirms the single previous record for this entity (Dematteis 2002 sub nom. *Vernonia pseudoincana*). Another species that showed diploid and tetraploid cytotypes was *L. sellowii*. The ploidy level found in this work suggests that the taxon has the basic number $x = 16$, since the numbers $2n = 32$ and 64 are multiple of 16 . Our analysis differs from the count of $2n = 62$ reported by Dematteis (1997).

Several species of *Lessingianthus* present different ploidy levels. These cytotypes could have arisen by several cytological mechanisms that induce polyploidy in plants. Sexual polyploidization is a pathway of polyploidy formation involving gametic “nonreduction” during micro- and

megasporogenesis. This process includes fusion of unreduced gametes, also referred to as “ $2n$ gametes,” which contain the full somatic chromosome number (Darlington 1965; Harlan and De Wet 1975; Bretagnolle and Thompson 1995). The union of reduced and unreduced gametes, or two $2n$ gametes, can generate polyploid embryos (Ramsey and Schemske 1998). Also, the production of unreduced gametes would promote the establishment of polyploids in diploid populations. Sexual polyploidization can occur in unilateral or bilateral forms, which involve different stages in the origin of polyploids in diploid population (Ramsey and Schemske 1998). The first mechanism includes the production of odd polyploidy by fusion of the unreduced and reduced gametes. These polyploids could act as an intermediary bridge for the origin of even-ploidy cytotypes, because they can produce unreduced balanced gametes and these become fused with unreduced gametes from other diploid or triploid individuals; for example, *Crepis capillaries* (L.) Wallr. has triploid and pentaploid cytotypes, which result from union of reduced and unreduced gametes (Navashin 1925). The second mechanism includes the production of par polyploidy by union of a microspore and megaspore unreduced in one step, without the formation of intermediary odd polyploid. Also, the formation of higher ploidy levels can be explained by this mechanism. The union of reduced and unreduced gametes generates a new cytotype of higher ploidy; for example, in the progeny of open-pollinated *Beta vulgaris* L., 2% hexaploid cytotypes were products of the union of reduced ($2x$) and unreduced ($4x$) gametes (Hornsey 1973).

The chromosome numbers of *Lessingianthus* varied from $2n = 32$ to $2n = 176$. The highest number in the genus has been recorded in populations from Uruguay of *L. macrocephalus* with $2n = 11x = 176$. This species constitutes the first case of odd polyploidy in the genus. In *Chrysolaena cognata*, another species of the Vernoniae tribe, pentaploid populations ($5x$) have been established (Dematteis 2009). However, odd cytotypes greater than $5x$ have never been observed. The high ploidy level observed in *L. macrocephalus*, with odd chromosome complement, suggests that an irregular meiosis behavior could lead to sterility. Polyploidy is frequently connected to asexual reproduction. Apomixis is a type of asexual reproduction in which plants reproduce seeds without fertilization. Almost all plants that reproduce seeds through apomixis are polyploid (Otto and Whitton 2000). These odd polyploid cytotypes could have originated by aneuploid reduction. Aneuploidy is hypothesized to contribute to phenotypic evolution and speciation in some genera, and may, in some cases, enable transition between euploid chromosome numbers (Ramsey and Schemske 2002). Some polyploids have high occurrence of aneuploid

progeny. The high frequency of aneuploidy in the gametes of polyploids has two possible outcomes. First, aneuploidy may be lethal at the gamete or embryo development stages. Second, aneuploid gametes may function similarly to euploid gametes, and generate viable gametes. In this case, polyploid progeny will be more numerous but include a high percentage of aneuploid individuals (Ramsey and Schemske 2002).

This phenomenon has been described in over 400 flowering plant taxa, representing over 40 families. Among the plants that show apomixis, 75% belong to three families: Asteraceae, Rosaceae, and Poaceae, which constitute only 10% of flowering plant species (Bhat et al. 2005). The expression of apomictic trait was found to be ploidy dependent. Recently, Quarin et al. (2001) proposed in *Paspalum notatum* that an unexpressed gene for apomixis exists at the diploid level. A rise in ploidy level induces the expression of apomixis, which was attributed to either the influence of ploidy on the locus controlling apomixis through some transcription factors or via a secondary locus which requires a higher allele dosage to affect the expression of the main locus. Hence, a possible development by apomixis in *L. macrocephalus* should be analyzed.

The segregation of different sections and subsections of *Vernonia* to new genera was based on several morphological features, chemical composition, and pollen type (Robinson 1988b). However, chromosome number also constitutes a useful tool to determine phylogenetic relationships in the Vernoniae tribe. Several genera segregated from *Vernonia* can be characterized by their basic chromosome number. The species belonging to the genus *Chrysolaena* H. Rob. (= *Vernonia* sect. *Lepidaploa* subsect. *Oligocephalae* Benth. & Hook.) presents exclusively base number $x = 10$, a number mainly present in Old World species of the tribe (Dematteis 2009). The genus *Vernonanthura* H. Rob. (= *Vernonia* sect. *Lepidaploa* subsect. *Paniculatae* Benth. & Hook.) always has $x = 17$, while *Lessingianthus* H. Rob. (= *Vernonia* sect. *Lepidaploa* subsect. *Macrocephalae*) presents base number $x = 16$. The single heterogeneous group is the genus *Lepidaploa* Cass. (= *Vernonia* sect. *Lepidaploa* subsect. *Axilliflorae*) that shows four different base numbers $x = 14, 15, 16$, and 17 (Dematteis 2002).

The data obtained in the present paper along with those counts previously reported suggest that *Lessingianthus* presents exclusively basic chromosome number $x = 16$ (Ruas et al. 1991; Dematteis 1996, 1997, 1998, 2002; Oliveira et al. 2007a, b; Angulo and Dematteis 2009a, b). However, a recent review of all available chromosome numbers found in tribe Vernoniae suggested $x = 20$ for *Lessingianthus* (Salles de Mello et al. 2010). This base number is absent in the tribe Vernoniae, but some species from Africa and South America show base $x = 10$. In the

New World, the base number $x = 10$ only occurs in *Chrysolaena*, a genus closely related to *Lessingianthus* that differs by its pollen type, inflorescence pattern, and chemical composition (Robinson, 1988a; Dematteis 2009). Nevertheless, the basic chromosome number $x = 10$ has been never found in species of *Lessingianthus*, as presently delimited.

Although all the species examined in the present study present $x = 16$, two previous studies have reported other base chromosome numbers. Jones (1979b) recorded $n = 17$ for *L. linearis* (Spreng.) Less., and the same result was found by Galiano and Hunziker (1987) in *L. lorentzii* (Hieron.) H. Rob. These are the only two species of *Lessingianthus* in which $x = 17$ has been reported. However, both taxa have all the diagnostic features of the genus *Lessingianthus*, including the distinctive “type B” pollen (Angulo and Dematteis 2010), due to which new cytological studies should be realized in these species in order to confirm its chromosome number.

The tribe Vernoniae present a great diversity of basic numbers, especially in the *Vernoniinae* subtribe with $x = 9, 10, 14, 15, 16, 17$, and 19 . Polyploidy and aneuploidy have been important in the evolution of the New World Vernoniae because from these processes arise the higher chromosome number (Jones 1979b). Many authors accept the hypothesis that the Vernoniae from the Old World are based on $x = 9$, which might have originated $x = 10$ by ascending aneuploidy (Jones 1979b). Higher chromosome numbers present in most American members of the tribe Vernoniae would be derived from $x = 9$ by duplication to 18 and posterior aneuploid reduction (Jones 1979b; Dematteis et al. 2007).

The results obtained in the present study combined with those reported previously confirm that the *Lessingianthus* genus has basic number $x = 16$ (Ruas et al. 1991; Dematteis 1996, 1997, 1998, 2002; Dematteis et al. 2007; Oliveira et al. 2007a; Angulo and Dematteis 2009). This number was considered by Jones (1979b) as uncommon for New World species of the tribe, being $x = 17$ the most frequent number. However, new chromosome studies (Ruas et al. 1991; Dematteis 1998, 2000, 2002; Oliveira et al. 2007a, b) have shown that the base number $x = 16$ is relatively frequent in South America.

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