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Karyotypes of some species of the genus *Lessingianthus* (Vernonieae, Asteraceae) and taxonomic implications

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We determined the karyotypes of nine species of *Lessingianthus*, eight of which are here analyzed for the first time. The results include the first chromosome count for *L. plantaginoides*, which is tetraploid with 2n = 64. All species showed a high proportion of metacentric chromosomes combined with a lower proportion of submetacentric pairs. Only *L. coriaceus* had a subtelocentric chromosome pair. B chromosomes were observed in *L. coriaceus* and *L. varroniifolius*, which were subtelocentric. Differences among the karyotypes of the studied species were small, suggesting that karyotype diversity in the genus evolved by small changes in the structure of chromosomes. Karyotype features appear useful to distinguish *Lessingianthus* from the closely related genera *Chrysolaena* and *Lepidaploa*.

The Asteraceae is the largest family of flowering plants with 1600–1700 genera and 24 000 species (Funk et al. 2009). This family has for long interested scientists due to a large variation in karyotype characteristics (Ruas et al. 2000, Mansanares et al. 2007). Within Asteraceae, Vernonieae is one of the most complex tribes characteristised by its high karyotypic diversity including a wide range of basic chromosome numbers: x = 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 23 and 31 (Turner 1981, Ruas et al. 1991, Dematteis 1998, Mansanares et al. 2007, Salles de Melo et al. 2010). Of these x = 9 or x = 10, reported for African and Asian species, are considered to be ancestral in the tribe (Jones 1979).

Most of the research in Vernonieae cytology has focused on *Vernonia* Schreb., one of the largest genera of the tribe including over 1000 species (Keeley and Jansen 1994). *Vernonia* has a complex taxonomic history due to different interpretations of its morphological, cytological and palynological data (Baker 1873, Cabrera 1944, Jones 1981, Robinson 1988a, 1988b, 1990). For instance, in Robinson's (1999) taxonomic revision of American Vernonieae, the large genus *Vernonia* was greatly reduced and most species retained in the genus are restricted to eastern North America as the south American species were placed in 16 new genera. Many of the new taxonomic circumscriptions were supported by recent molecular phylogenetic analyses (Keeley et al. 2007).

One large genus resulting from Robinson's (1999) revision is *Lessingianthus* H. Rob. (133 spp.), including species formerly recognized as *Vernonia* Schreb. sect. *Lepidaploa* subsect. *Macrocephalae* Benth. (Baker 1873). *Lessingianthus* is distributed in tropical South America, with a high concentration of species in eastern Brazil (Robinson 1988a, Dematteis 2006). The basic chromosome number x = 16, constant within the genus, distinguishes *Lessingianthus* from other genera of Vernonieae, such as the closely related *Chrysolaena* H. Rob. and *Lepidaploa* (Cass.) Cass. (Angulo and Dematteis 2012a), characterized by x = 10 and x = 14, 15 (and rarely x = 16), respectively (Robinson 1988a, 1988b, 1990, Dematteis 2002). Chromosome numbers in *Lessingianthus* are known from 51 species and range between 2n = 32 (diploid) to 2n = 176 (endecaploid) (Dematteis 1997, 1998, Dematteis and Fernández 2000, Oliveira et al. 2007, Angulo and Dematteis 2009, 2012a). In contrast, karyotypes are known for only 13 species (i.e. about 10% of the genus; Ruas et al. 1991, Dematteis 1997, 1998, Dematteis and Fernández 2000, Oliveira et al. 2007, Angulo and Dematties 2009). These few studies on karyotypes indicate that most chromosomes are metacentric and, in some taxa, are occasionally accompanied by a few submetacentric and subtelocentric chromosomes.

Chromosome characteristics of *Lessingianthus*, such as karyotype formula, total chromosome length and degree of asymmetry of chromosomes, have proved taxonomically useful to distinguish species from one another (Ruas et al. 1991, Dematteis 1997, 1998, Dematteis and Robinson 1997, Dematteis and Fernández 1998, Angulo and Dematteis 2012b). For example, the number and morphology of chromosomes are additional characters to distinguish species of the *L. saltensis* complex (Angulo and Dematteis 2012b). The taxonomically useful variability of karyotype features in *Lessingianthus* might be the result of an evolutionary history characterized by variations in chromosome number and size (Angulo and Dematteis 2012a). However, too few species

have been studied to infer any evolutionary trends within the genus. Therefore, to increase the knowledge of *Lessingianthus*, in this study we investigate the karyotypes of nine species of *Lessingianthus* and compare our results with published ones on the closely related *Chrysolaena* and *Lepidaploa* in order to determine whether karyotypic differences among these genera support Robinson's (1999) taxonomic proposal.

Material and methods

We examined 11 natural populations from nine species of *Lessingianthus* collected in Argentina, Bolivia, Paraguay and Uruguay. Information about the studied material and the voucher specimens is provided in Table 1. Vouchers are deposited at the herbarium of the Instituto de Botánica del Nordeste (CTES).

To study somatic chromosomes, root tips of germinating seeds were pretreated in 0.002 M 8-hidroxyquinoline for 4–5 h, then fixed in acetic acid:absolute alcohol (3:1) overnight, and stored in 70% aqueous ethanol. Root tips were stained according to the Feulgen technique, and meristems were macerated and squashed in a drop of lacto-propionic orcein (Dyer 1963). Permanent microscope slides were prepared in Euparal using Bowen (1956)'s method. Drawings of at least 10 metaphases per population (3–8 individuals) were made using a camera lucida, and the five best ones were selected to prepare the final idiograms.

To designate a chromosome morphology, the centromeric index (CI = short arm × 100/total length of the chromosome; Levan et al. 1964) was used with the following ranges: 50.0–37.5 for metacentric ('m'), 37.5–25.0 for submetacentric ('sm'), and 25.0–12.5 for subtelocentric ('st') chromosomes.

To characterize karyotypes numerically the following parameters were determined: mean chromosome length (ML), mean centromeric index (CI), total haploid chromosome length (HCL), total karyotype length (TKL). Karyotype asymmetry was determined using the intra-and inter chromosomal asymmetry indexes A_1 and A_2, respectively (Romero Zarco 1986) in which $A_1 = 1 - [\sum (b/B)/n]$ and $A_2 = s/x$, where b and B are the mean length of short and long arms of each pair of homologues, respectively, n is the number of homologues, s is the standard deviation, and x is the mean chromosome length. The Infostat software (Di Rienzo et al. 2013) was used for comparative analyses of karyotypes within *Lessingiantus* and among *Lessingianthus*, *Chrysolaena* and *Lepidaploa*. A scatter diagram was calculated to evaluate the relationship between asymmetry indices A_1 and A_2. Karyotypes of *Lessingianthus*, *Chrysolaena* and *Lepidaploa* species were additionally analyzed using a principal component analysis (PCA). A data matrix of 19 operational taxonomic units (OTUs) and nine variables was constructed using our and published results (Dematteis 1998, Angulo and Dematteis 2010, Via do Pico and Dematteis 2013; Table 3). The nine karyotype variables analyzed were: TKL; CI; A_1 and A_2 indices; number of m, sm, and st chromosomes; and basic numbers x and 2n. We excluded HCL from these analyses, because it was unknown for the species taken from the literature.

Table 1. *Lessingianthus* species analyzed in this study and respective collection information.

Species	Location and voucher specimens
L. bardanoides (Less.) H. Rob.	Paraguay. Dept Amambay. Chirigüelo. 2 km west of P. J. Caballero. Dematteis et al. 3393 (CTES)
L. coriaceus (Less.) H. Rob.	Bolivia. Dept La Paz, Prov. Nor Yungas, subida a Coroico, 1.4 km southeast de la ciudad. Dematteis et al. 4062 (CTES)
L. laniferus (Cristóbal & Dematt.) M. B. Angulo	Uruguay. Dept Tacuarembó. Gruta of the Helechos, 15 km northwest of Tacuarembó. Dematteis and Schinini 1833 (CTES)
	Argentina Misiones. Dept General Manuel Belgrano. Campina de Américo. Dematteis et al. 2613 (CTES)
	Argentina. Misiones. Dept General Manuel Belgrano. Campina de Américo. Dematteis et al. 3076 (CTES)
L. plantaginoides (Less.) H. Rob.	Argentina. Corrientes. Dept San Martín. Paraje Tres Cerros, Est. Las Marías, Cerro Chico. Medina and Salas 378 (CTES)
L. pseudoincanus (Hieron.) Dematteis & Angulo	Argentina. Corrientes. Dept Mercedes. 8 km north of Felipe Yofre. Dematteis and Seo 2463 (CTES)
	Argentina. Misiones. Dept Capital. Ayo. Zaiman. Angulo 19 (CTES)
L. pusillus (Dematt.) M. B. Angulo	Argentina. Corrientes. Dept Capital, Perichón. Dematteis et al. 2769 (CTES)
	Paraguay. Dept Concepción 16 km north of Paso Barreto, Est. Rosalía. Dematteis et al. 3234 (CTES)
L. rubricaulis (Humb. & Bonpl.) H. Rob.	Argentina. Corrientes. Dept San Roque. Ruta 12, 2 km north of Ruta 123. Dematteis et al. 2756 (CTES)
	Argentina. Corrientes. Dept Bella Vista. Ayo Toropí. Angulo 9 (CTES)
	Paraguay. Dept Amambay, 25 km south of Bella Vista, road to Ruta 5. Dematteis et al. 3375 (CTES)
	Bolivia. Dept Santa Cruz, Prov. Chiquitos 25 km north of San José, road to San Rafael. Dematteis 3567 (CTES)
L. sellowii (Less.) H. Rob.	Uruguay. Dept Tacuarembó, Gruta of the Helechos, 10 km northwest of Tacuarembó. Dematteis et al. 3760 (CTES)
L. varroniifolius (DC.) H. Rob.	Bolivia. Dept Santa Cruz, Velasco Province 67 km east of Concepción, road to San Ignacio. Dematteis et al. 3860 (CTES)

Results

Karyotype formulae and parameters of species are summarized in Table 2. Mitotic chromosomes are illustrated in Fig. 1 and their idiograms in Fig. 2 and 3. All species had a basic chromosome number of x = 16. Five species were diploids with 2n = 32 and two were tetraploids with 2n = 64, while two species (*L. laniferus* and *L. rubricaulis*) included both populations with 2x and 4x cytotypes (Table 2). The chromosome number and karyotype of *L. plantaginoides* (Fig. 1D) are here presented for the first time.

All studied species had small chromosomes with a mean length of 1.66 µm, ranging from 1.33 µm, in *L. pusillus*, to 2.03 µm, in the diploid cytotype of *L. laniferus*. All species had only m and sm chromosomes (Fig. 2–3), except *L. coriaceus* which had also one st pair (20m + 10sm + 2st, Fig. 2B, Table 2). In most species, satellites were observed on one m pair of the cromosomic complement, whereas, in *L. coriaceus*, they were found on the long arm of the st pair (pair 16). Satellites of the m pairs were observed on the short chromosome arm in *L. bardanoides* (pair 6) and *L. plantaginoides* (pair 2) and on the long arm in *L. sellowii* (pair 9) and in *L. rubricaulis* (both cytotypes: pair 1 in diploid individuals, and pair 2 in tetraploid individuals). B chromosomes (i.e. accesory chromosomes) were observed in *L. coriaceus* and *L. varroniifolius*. The two species had, up to 6 and up to 5 B chromosomes, respectively; with numbers varying among individuals of a population and also within an individual. These B chromosomes were subtelocentric, with a mean chromosome length of 1.46 µm in *L. coriaceus* and 1.36 µm in *L. varroniifolius.*

Karyotypes of the studied *Lessingianthus* species were generally symmetrical with formulae that showed many m chromosomes and few sm chromosomes. *Lessingianthus varronifolius* (CI = 38.80) had the most asymmetrical karyotype, while the 4x cytotype of *L. laniferus* (CI = 43.82) showed the most symmetrical karyotype. The scatter diagram of A_1 and A_2 asymmetry indices (Fig. 4) showed that *L. varronifolius,* with the highest A_1 value, presented the largest difference in the length of chromosome arms, whereas *L. coriaceus*, with the highest A_2 value, showed a large variation in length among chromosomes. *Lessingianthus laniferus* (4x) and *L. bardanoides* had the lowest values of A_1 and A_2 and the most symmetrical karyotypes.

The PCA of the karyotypic variables of *Lessingianthus*, *Chrysolaena* and *Lepidaploa* showed that the first two principal components account for 75.5% of the total variation (see Fig. 5 for a bidimensional projection of the axes). Component one (46.7%) emphasized differences in the number of m chromosomes and total chromosome length, while component two (28.8%) accentuated variation in the number of sm chromosomes. The species arrangement resulting from this analysis was consistent with the current circumscription of the three genera.

Discussion

Karyotype characteristics

The present study provides full descriptions of the karyotypes of nine *Lessingianthus* species, completing the data from previous studies on only three species (*L. bardanoides*, *L. coriaceus,* and the diploid cytotype of *L. rubricaulis*; Dematteis 1998, Oliveira et al. 2012) and; therefore, broadening our understanding of the cytology in this genus.

All nine *Lessingianthus* species analyzed here had a basic chromosome number of x = 16, agreeing with previous studies (Dematteis 1998, Angulo and Dematteis 2012a). However, the karyotype formula of a single taxon studied here disagrees with a previous analysis while karyotypes of other species agree with those from the literature. The analyzed Paraguayan population of *L. bardanoides* differs from that reported by Oliveira et al. (2012) in samples from Brazil (22m + 10sm). The disparity in the karyotype formulae could probably be attributed to differences in the measurement technique applied or to the particular appreciation of the chromosome morphology. The karyotype formula of Bolivian specimens of *L. coriaceus* analyzed here coincides with that of a Brazilian population studied by Dematteis (1998). Our karyotype formula of the diploid cytotype of *L. rubricaulis* was already recorded by Dematteis (1998), whereas the formula of the tetraploid cytotype is presented here for the first time.

Table 2. Somatic chromosome number (2n), karyotype formula (KF), mean chromosome length (ML), range of chromosome length, total haploid chromosome length (HCL), total chromosome length (TKL), average centromeric index (CI), intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices of studied *Lessingianthus* species.

Species	2n	KF	ML	Range (µm)	HCL	TKL	CI ± SE	A_1	A_2
L. bardanoides	32	26m + 6sm	1.81	1.64–2.34	28.96	57.92 ± 0.15	42.70 ± 0.12	0.246	0.152
L. coriaceus	32	20m + 10sm + 2st + B	1.72	1.28–2.15	27.20	54.40 ± 0.20	40.03 ± 0,54	0.333	0.278
*L. laniferus**	32	24m + 8sm	2.03	1.34–2.52	32.98	65.96 ± 0.05	42.47 ± 0.52	0.243	0.197
*L. laniferus**	64	48m + 16sm	1.83	1.22–2.55	29.38	117.55 ± 0.19	43.82 ± 0.27	0.221	0.173
*L. plantaginoides**†	64	42m + 22sm	1.38	1.26–2.19	22.17	90.89 ± 0.14	39.61 ± 0.32	0.324	0.225
*L. pseudoincanus**	64	52m + 12sm	1.47	1.00–1.98	22.56	96.57 ± 0.03	43.57 ± 0.42	0.223	0.180
*L. pusillus**	32	28m + 4sm	1.33	1.00–1.82	21.78	43.57 ± 0.08	43.46 ± 0.32	0.227	0.172
L. rubricaulis	32	20m + 12sm	1.68	1.24–2.15	26.99	53.99 ± 0.14	42.33 ± 0.25	0.270	0.176
*L. rubricaulis**	64	52m + 12sm	1.51	0.88–2.35	23.78	92.14 ± 1.20	42.34 ± 0.31	0.230	0.258
*L. sellowii**	32	24m + 8sm	1.71	1.54–2.67	26.13	52.27 ± 0.20	41.56 ± 0.23	0.317	0.186
*L. varroniifolius**	32	20m + 12sm + B	1.81	1.39–2.50	28.93	43.00 ± 0.45	38.80 ± 0.30	0.364	0.203

*First karyotypic analysis, †First chromosome count.

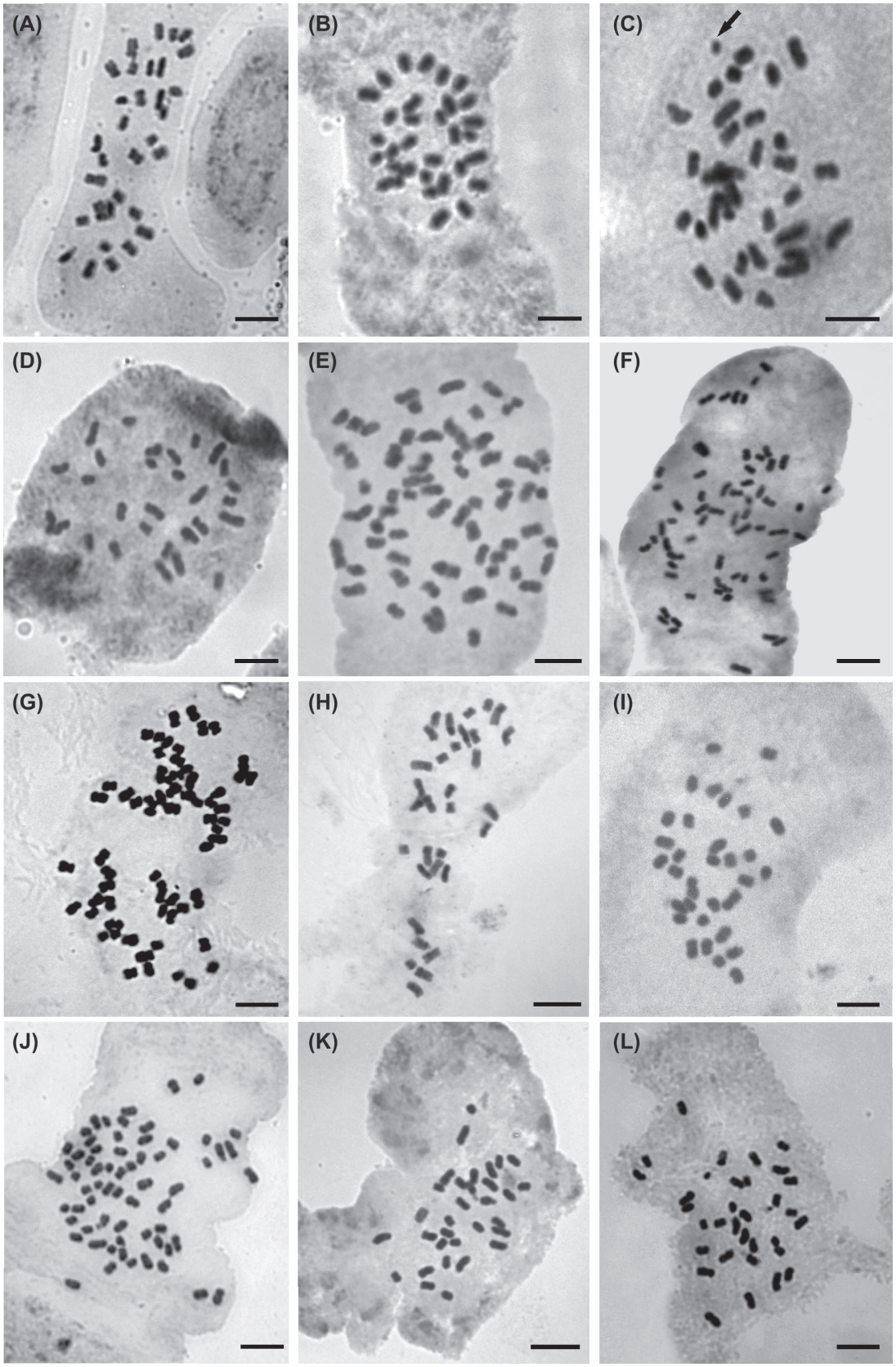

Figure 1. Somatic chromosomes of *Lessingianthus* species. (A) *L. bardanoides*, (2n = 2x = 32), (B)–(C) *L. coriaceus*: (B) 2n = 2x = 32, (C) 2n = 2x = 32 + 1B (D)–(E) *L. laniferus*: (D) diploid cytotype (2n = 2x = 32), (E) tetraploid cytotype, 2n = 4x = 64, (F) *L. pseudoincanus*, 2n = 4x = 64, (G) *L. plantaginoides*, 2n = 4x = 64, (H) *L. pusillus*, 2n = 2x = 32, (I)–(J) *L. rubricaulis*: (I) diploid cytotype (2n = 2x = 32), (J) tetraploid cytotype, (2n = 4x = 64), (K) *L. sellowii*, (2n = 2x = 32), (L) *L. varroniifolius*, 2n = 2x = 32. Arrow indicates B chromosome. Scale bars = 5 μm.

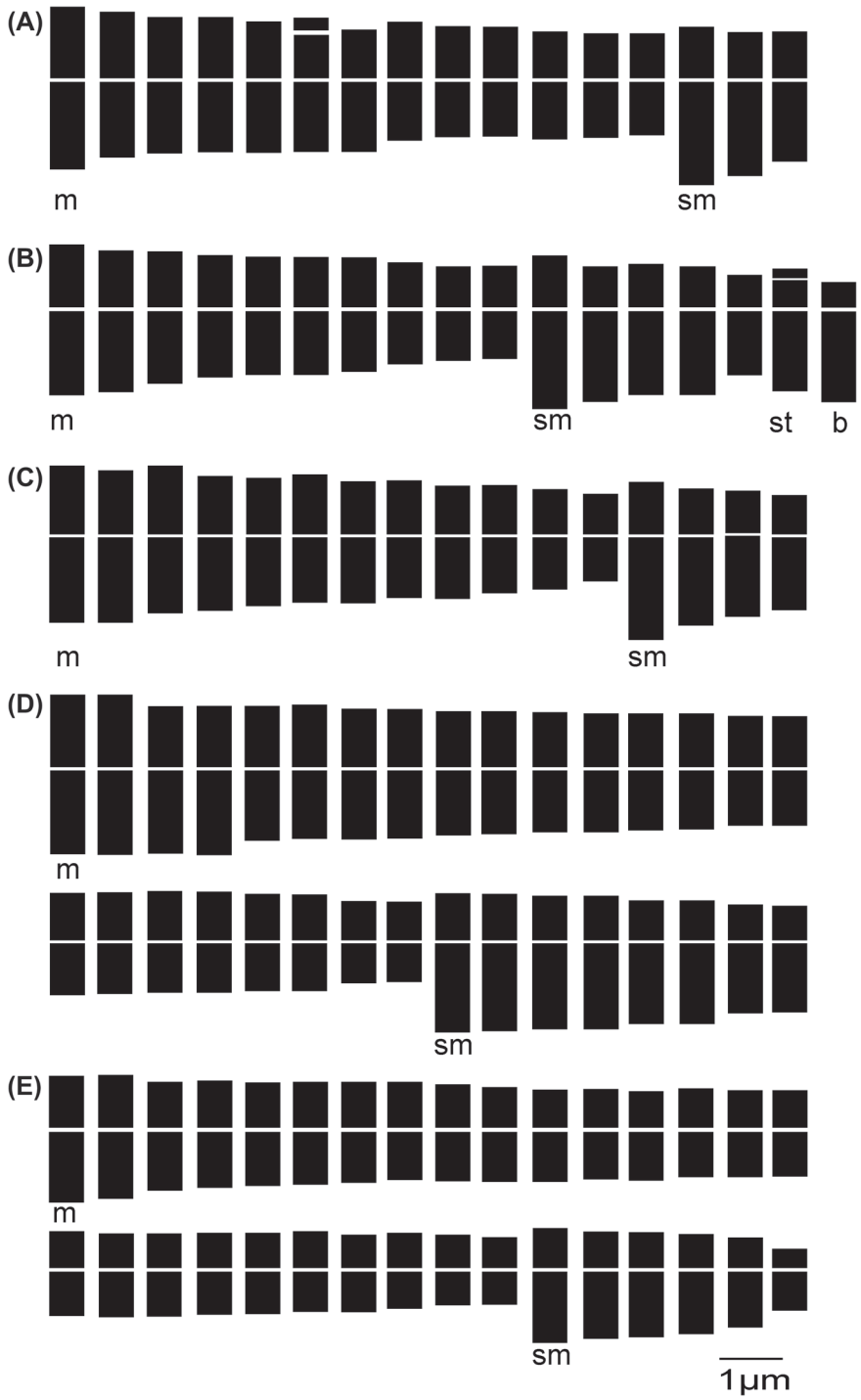

Figure 2. Idiograms of species of *Lessingianthus.* (A) *L. bardanoides*, 2n = 32 = 26m + 6sm, (B) *L. coriaceus,* 2n = 32 = 20m + 10sm + 2st + B, (C)–(D) *L. laniferus*, (C) diploid cytotype, 2n = 32 = 24m + 8sm, (D) tetraploid cytotype, 2n = 64 = 48m + 16sm, (E) *L. pseudoincanus*, 2n = 64 = 52m + 12sm.

Chromosomes of the studied species are small (according to the classification of chromosome length by Lima de Faria 1980), varying between 1.33 µm and 2.03 µm. Our results agree with previous reports of chromosome lengths in the genus (Dematteis 1997, 1998, Dematteis and Fernández 2000, Oliveira et al. 2007, Angulo and Dematteis 2009).

The *Lessingianthus* karyotypes here studied consist mainly of m chromosomes and a lower proportion of sm chromosomes, as those previously reported for the genus (Dematteis 1997, 1998, Angulo and Dematteis 2009). *Lessingianthus coriaceus* was the only species with a st pair, as was found by Dematteis (1998) in a Brazilian population. Subtelocentric chromosomes are generally rare in tribe Vernonieae. Only a few other species of *Lessingianthus* have one st pair, such as *L. onopordioides* (Baker) H. Rob. and the 4x cytotype of *L. sellowii* (Dematteis 1998). Elsewhere in the

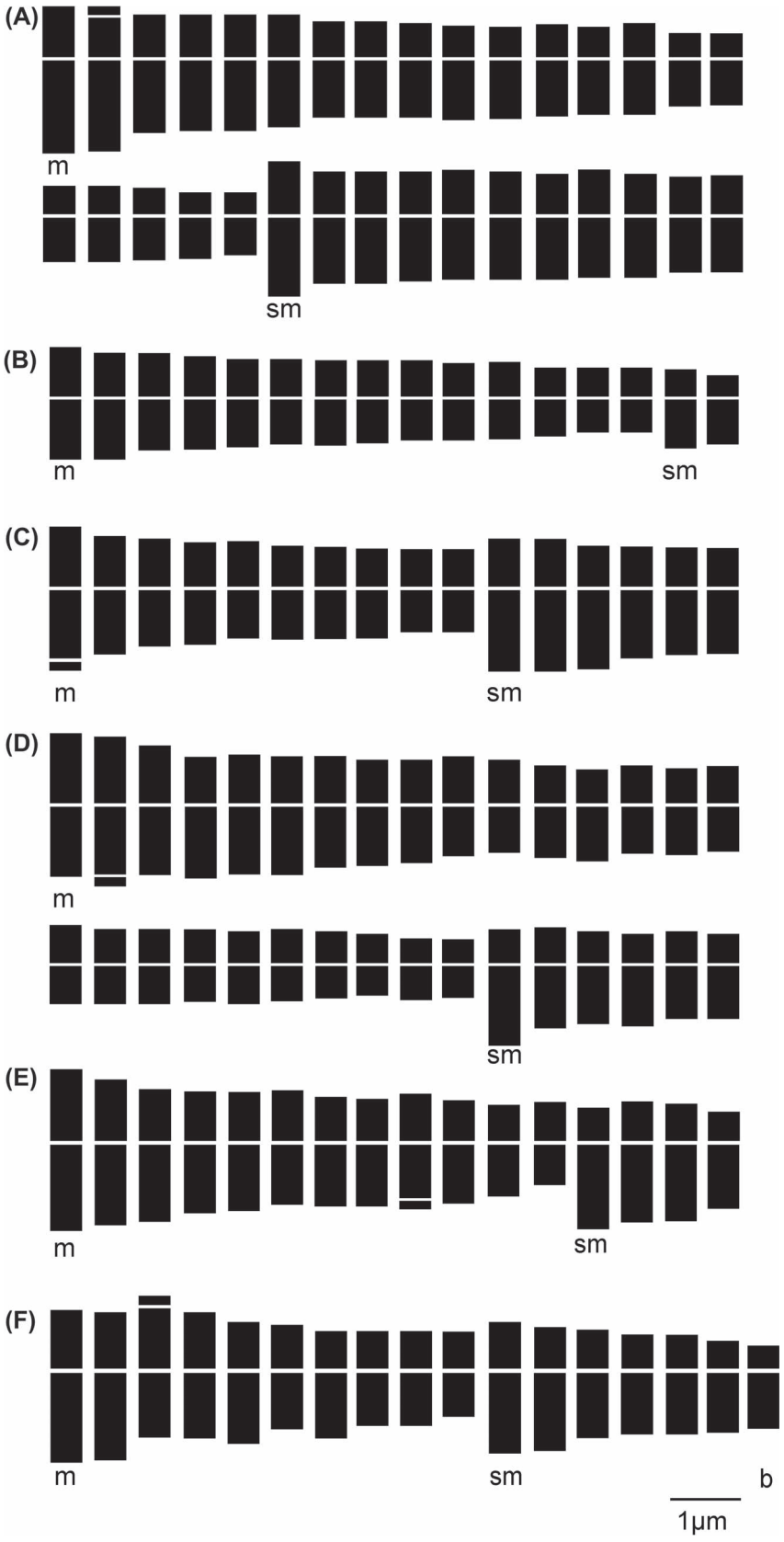

Figure 3. Idiograms of species of *Lessingianthus.* (A) *L. plantaginoides*, 2n = 64 = 42m + 22sm, (B) *L. pusillus*, 2n = 32 = 28m + 4sm, (C)–(D) *L. rubricaulis,* (C) diploid cytotype, 2n = 32 = 20m + 12sm, (D) tetraploid cytotype, 2n = 64 = 52m + 12sm, (E) *L. sellowii*, 2n = 32 = 24m + 8sm, (F) *L.varroniifolius*, 2n = 32 = 20m + 12sm + B.

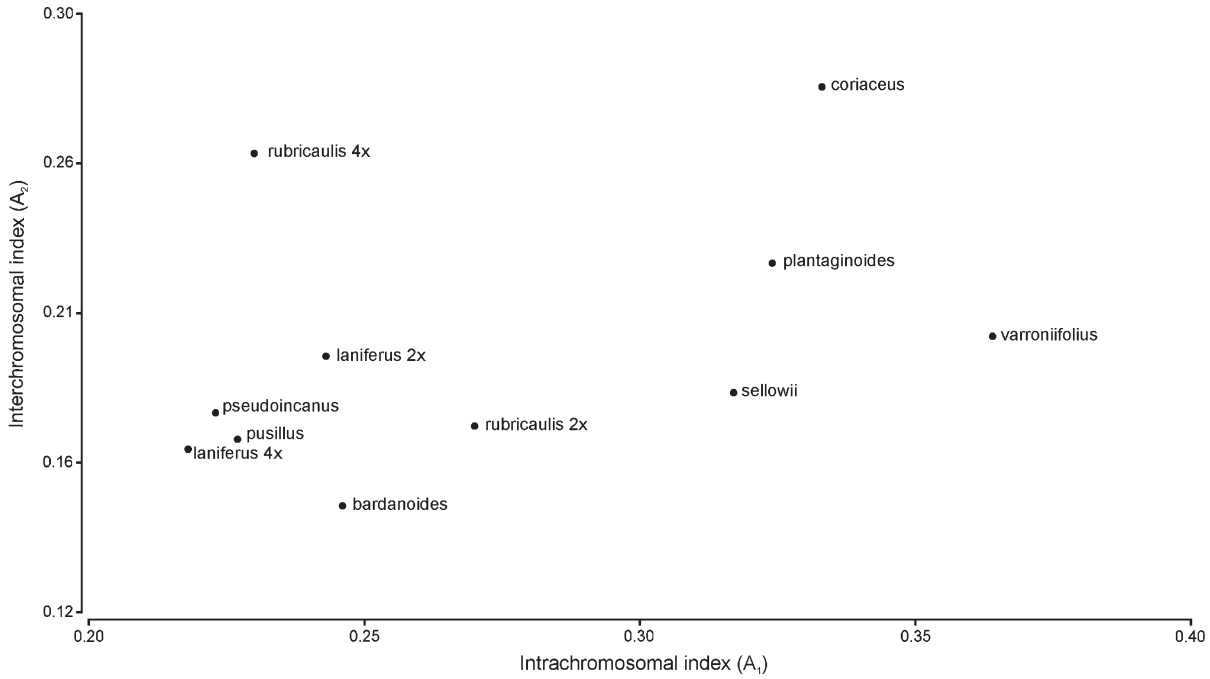

Figure 4. Scatter diagram representing the karyotype symmetry.

tribe, st chromosomes have been reported to our knowledge only in the genus *Lepidaploa*: *L. eriolepis* (Gardner) H. Rob. (one pair; Dematteis 1998) and *L. lilacina* (Mart. ex DC.) H. Rob. (one pair; Dematteis and Fernández 2000).

Chromosome numbers in *Lessingianthus* are highly variable (Angulo and Dematteis 2012a). Our results confirm that *L. plantaginoides* is tetraploid, as suggested by Angulo and Dematteis (2013) based on DNA content. Furthermore, it is frequent to find different cytotypes within a single species, as in *L. sellowii*, which has diploid (2n = 32) and tetraploid (2n = 64) cytotypes (Angulo and Dematteis 2012a). While we here analyzed the karyotype of the 2x

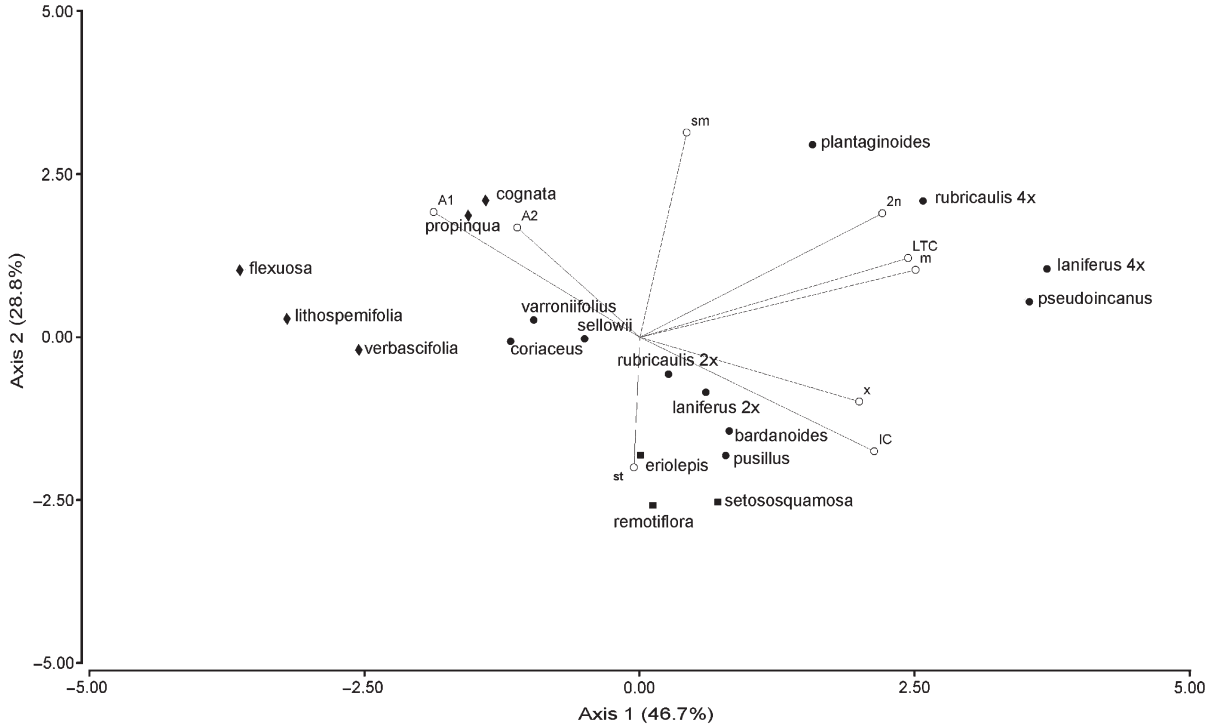

Figure 5. Graph resulting from the PCA showing the distribution of *Lessingianthus*, *Chrysolaena* and *Lepidaploa* species based on karyological data. Symbols: circle = *Lessingianthus* species, diamond = *Chrysolaena* species, square = *Lepidaploa* species.

cytotype from Uruguay and found the expected 2n = 32, Dematteis (1998) analyzed an Argentinian 4x population with an unusual 2n = 62. It is possible that these unusual karyotypes resulted from dysploidy. For instance, similar divergences in chromosome numbers in populations of *Vernonia scabra* Pers. from different places were attributed by Salles de Melo et al. (2010) to dysploidy in combination with polyploidy. Dysploidy is actually well documented in many Asteraceae (Watanabe et al. 1995, Weiss et al. 2003), and appears to have played a role in the diversification of tribe Vernonieae. The wide range of basic chromosome numbers in the tribe have lead to the suggestion of several hypotheses about their origin. For example, Jones (1979) and Turner (1981) suggested that the higher basic numbers of the tribe, like x = 16 in *Lessingianthus*, resulted from a complex combination of polyploidy and aneuploidy. Recently, Salles de Melo et al. (2010) suggested that dysploidy could be the main mechanism explaining the diversity of basic numbers in Vernonieae, since several authors has established a clear relationship between dysploidy and type of habitat and life cycle in various genera of Asteraceae. Watanabe et al. (1999) interpreted dysploidy as an adaptation to arid habitats favouring shorter life cycles in *Pogonolepis* Steetz in Lehm., *Sondottia* P. S. Short and *Trichanthodium* Sond. & F. Muell. Also, Uysal et al. (2009) suggested that the dysploidy in Centaureinae could be correlated to adaptation of mesophyllous taxa to the xeric conditions of the Mediterranean. Our results show that the variation in chromosome number of a single species of *Lessingianthus* (*L. sellowii*) is probably a case of dysploidy and, thus, it supports the suggestions by Salles de Melo et al. (2010) that dysploidy occurrs in genus *Vernonia* as well as in Vernonieae tribe.

B chromosomes are uncommon and have been reported only in some *Lessingianthus* species (Dematteis 1997, 1998, Angulo and Dematteis 2009, 2012a). Among these species, *L. coriaceus* and *L. varroniifolius* display a variable number of B chromosomes within populations as well as within individuals. Some individuals have none and others have up to six B chromosomes with variable numbers in cells of the same root meristem. This intraspecific variability could be due to variation in the level of paring, the degree of meiotic elimination, or nondisjunction of sister chromatids during pollen mitosis (Angulo and Dematteis 2012a). In another Asteraceae, *Mikania* Willd., the variability of B chromosomes in cells of root meristems appears to have resulted from nondisjunction during mitotic division of the meristematic cells (Ruas et al. 2000).

B chromosomes are frequently found as m and sm chromosomes (Stebbins 1971, Jones and Rees 1982), but there are some examples of plants with st and t B chromosomes (Mochizuki 1960). In Asteraceae, B chromosomes have been reported as telocentric in *Hypochaeris maculata* L. (Parker 1976). Dematteis (1998) found st B chromosomes in a 4x population of *L. sellowii*, and we found them in *L. coriaceus* and *L. varroniifolius.* B chromosomes are usually smaller than the smallest A chromosomes (Jones 1995), and indeed, in our studied *Lessingianthus* they ranged between 1.35 µm (in *L. varroniifolius*) and 1.60 µm (in *L. coriaceus*).

Karyotype and systematics

Lessingianthus not only comprises species with a broad range of ploidy levels, but also species with a broad morphological variation. Such broad morphological variation has prompted the recognition of not one but several species (Jones 1979, Robinson 1999). For example, *L. rubricaulis* is currently known as the *L. rubricaulis* complex, consisting of the four species *L. rubricaulis*, *L. laniferus*, *L. pseudoincanus* and *L. pusillus*, which differ in habit, underground system, indumentum type, leaf size and shape, and chromosome number (Dematteis 2004, Angulo and Dematteis 2012a). Our results support this view (Table 2) and suggest that karyotype features can be used to distinguish all four species of the complex, even though *L. pseudoincanus* and 4x *L. rubricaulis* have the same karyotype formula (52m + 12sm). Therefore, the broad morphological and cytological variation in the *L. rubricaulis* complex is best interpreted as characterizing four species rather than one.

Molecular and morphological data suggest that *Lessingianthus* is closely related to *Chrysolaena* and *Lepidaploa* (Robinson 1999, Dematteis 2007, 2009, Keeley et al. 2007). Karyotype characteristics are useful to distinguish *Lessingianthus* from these two genera, since our PCA identified three species groups that coincide with the three genera. Although the PCA suggests that the three genera have similar karyotypes, these differ in their total chromosome length and the number of m and sm chromosomes. Our results thus support current generic circumscriptions (Robinson 1999).

Table 3. Karyotype parameters in *Chrysolaena* and *Lepidaploa* species. Chromosome number, basic number (x), karyotype formula (KF), mean chromosome length (ML), average centromeric index (CI), intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indices.

Species	2n	x	KF	ML	TKL	CI	A_1	A_2	References*
Chrysolaena cognata (Less.) Dematt.	40	10	18m + 22sm	2.65	52.96	39.96	0.35	0.21	1
Chrysolaena flexuosa (Sims) H. Rob	20	10	10m + 10sm	2.44	29.34	38.06	0.38	0.25	1
Chrysolaena lithospermifolia (Hieron.) H. Rob	20	10	10m + 10sm	1.86	18.65	30.41	0.34	0.23	1
Chrysolaena propinqua (Hieron.) H. Rob.	40	10	20m + 20sm + B	2.44	48.86	38.88	0.35	0.19	1
Chrysolaena verbascifolia (Less.) H. Rob.	20	10	12m + 8sm + B	2.70	29.71	39.31	0.27	0.23	1
Lepidaploa remotiflora (Rich.) H. Rob.	28	14	22m + 4sm + 2st	1.65	46.20	43.43	1.19	0.19	2
Lepidaploa setososquamosa (Hieron.) M. B. Angulo & Dematt.	30	15	22m + 6sm + 2st	1.92	57.60	43.19	0.22	0.15	2
Lepidaploa eriolepis (Gardner) H. Rob.	32	16	22m + 8sm + 2st	1.30	41.60	41.39	0.245	0.183	3

*(1) Via do Pico and Dematteis (2013), (2) Angulo and Dematteis (2010), (3) Dematteis (1998).

Karyotype evolution of South American Vernonieae seems to be characterized by a decrease in chromosome size (Dematteis 1998). Chromosomes are in fact small in *Lessingianthus*, as well as in *Chrysolaena* and *Lepidaploa.* Based on available karyological data in the tribe, it seems that species considered as 'ancestral', i.e. with low basic numbers, have larger chromosomes than taxa considered as 'derived', i.e. with higher basic number and smaller chromosomes (Ruas et al. 1991, Dematteis 1997, 1998, Dematteis and Fernández 1998). According to this interpretation, *Chrysolaena* (x = 10) should have larger chromosomes than *Lessingianthus* (x = 16) and *Lepidaploa* (x = 14, 15 and 16) (Dematteis 1997, 2009, Angulo and Dematteis 2010, Via do Pico and Dematteis 2013). However, this is supported only in part, because mean chromosome length ranges overlap among the three genera (Table 2–3). For example, *L. brevifolius* (ML = 2.52 µm; Angulo and Dematteis 2009) and *L. laniferus* 2x (ML = 2.03 µm) are included in the range of *Chrysolaena* (ML = 1.86–2.70 µm), whereas *C. lithospermifolia* has a mean chromosome size (ML = 1.86 µm) similar to the mean values of *Lessingianthus* and *Lepidaploa*. Therefore, the variation in chromosome length in Vernonieae may not be unidirectional, and during the evolution and diversification of the tribe both increases and decreases of genome size occurred.

Lessingianthus is characterized by a moderate degree of karyotype symmetry, as are *Chrysolaena* and *Lepidaploa* (Dematteis 1998, Angulo and Dematteis 2010, Via do Pico and Dematteis 2013). Chromosome symmetry is one of the cytological parameters used to study plant evolution. It is common to consider, for a particular group of angiosperms, that the karyotypes with more asymmetry have a derived status compared with those more symmetrical (Stebbins 1971). However, this rule is not constant because reverse cases have been observed (Jones 1970, Seijo and Fernández 2003, Lavia et al. 2009). Our results and previously published data on Vernonieae also suggest that there is no correlation between karyotype asymmetry and derived taxa (Dematteis 1997, 1998, Dematteis and Fernández 2000, Angulo and Dematteis 2009, Oliveira et al. 2012, Via do Pico and Dematteis 2013). For example, species with x = 10, supposedly the ancestral state, have slightly more asymmetrical karyotypes than species with higher basic numbers (x = 16, 15, 14), supposedly the derived state (Dematteis 1998, Angulo and Dematteis 2009, 2010, Via do Pico and Dematteis 2013). Therefore, asymmetry indices should not be used to infer relationships and evolutionary trends within the tribe.

In conclusion, the present study shows that differences exist among karyotypes of *Lessingianthus* species. Such differences are small, probably because of small changes in chromosome structure during karyotype evolution in the genus, but are useful to distinguish *Lessingianthus* from *Chrysolaena* and *Lepidaploa* and thus support the taxonomic classification by Robinson (1999).

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