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Karyotype analysis and DNA content in some species of *Chrysolaena* (Vernonieae, Asteraceae)

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Abstract
Cytogenetic characterization by karyotyping and determination of DNA content by flow cytometry of five species of *Chrysolaena* (Vernonieae, Asteraceae) was performed. This is the first study of nuclear DNA content realized in the genus. The 2C-values were compared with the ploidy level and the total karyotype length (TKL) of each species. Mitotic analysis revealed a base chromosome number $x = 10$ for all entities and different ploidy levels, from diploid ($2n = 2x = 20$) to octoploid ($2n = 8x = 80$). All species showed bimodal karyotypes composed of metacentric and submetacentric chromosomes. The average chromosome size (ML) varied from 1.86 μm to 2.70 μm, while the TKL ranged from 18.65 μm to 80.55 μm. The intrachromosomal asymmetry index (A_1) varied from 0.27 to 0.38, while the interchromosomal asymmetry index (A_2) ranged from 0.19 to 0.25. A new cytotype is reported for the first time for *C. propinqua*. Accessory chromosomes found in *C. verbascifolia*, *C. cognata*, *C. flexuosa*, and *C. propinqua* are also reported as new.

Keywords: *Chromosomes, Compositae, flow cytometry, Vernonia*

Introduction

The tribe Vernonieae Cass. is one of the largest groups of Compositae (Asteraceae) with ca. 1500 species distributed in America, Asia, and Africa (Keeley and Robinson 2009). It is considered one of the most complex groups in the family Asteraceae from the biological and taxonomic point of view (Keeley et al. 2007). The tribe has two important centers of diversification, one in southern Brazil and the other in tropical Africa (Jones 1979; Keeley & Jansen 1994). In a recent work, Keeley and Robinson (2009) recognize 21 subtribes, 15 from the New World and 6 from the Old World, mostly based on morphological and molecular phylogenetic studies. Recent studies have resulted in significant taxonomic changes in Vernonieae and particularly reduction in size and distribution of the core genus *Vernonia* Schreb. As a consequence, a total of 126 genera are now recognized for Vernonieae. In the New World, the tribe is represented by about 70 genera and approximately 600–700 species.

The subtribe Lepidaploinae S. C. Keeley & H. Rob. is one of the recently created subtribes, with 12 genera, 5 of them monotypic, and about 320 species that mainly grow in the Western Hemisphere (Keeley & Robinson 2009). It contains several genera segregated from *Vernonia*, such as *Lessingianthus* H.Rob., *Lepidaploa* (Cass.) Cass., and *Chrysolaena* H.Rob., which have been previously included in subtribe Vernoniinae Less. (Robinson 1999).

The genus *Chrysolaena* H.Rob. differs from other South American members of the tribe by the presence of a sericeous or velutinous indumentum, anthers with apical glandular appendages, styles without a basal node, and glandular cypselas. Also, the genus can be separated from other American groups by the morphology of the pollen grains and the chromosome number. The latter is one of the most important features because *Chrysolaena* is the only American member of the tribe with the base number $x = 10$, which is mainly present in the Old World Vernonieae.

Currently, *Chrysolaena* includes 18 species concentrated in southern Brazil, Paraguay, and northeastern Argentina, which present significant chromosomal and morphological variability. Chromosome studies reported previously reveal that species of the genus have a base number of $x = 10$, with chromosome numbers ranging from

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$2n = 20$ to $2n = 80$ and interpreted to be different ploidy levels or cytotypes (Ruas et al. 1991; Dematteis 1997a; Dematteis 2002; Dematteis et al. 2007; Dematteis 2009; Angulo & Dematteis 2009a, 2009b). Diploid and tetraploid populations have been found in *C. flexuosa* (Sims) H.Rob., whereas only diploid populations are known for *C. verbascifolia* (Less.) H. Rob., *C. propinqua* (Hieron.) H.Rob., and *C. lithospermifolia* (Hieron.) H.Rob. *C. cognata* (Less.) Dematt. and *C. platensis* (Spreng.) H.Rob. show a greater variation, having diploid, tetraploid, hexaploid, and octoploid populations, and even odd polyploids in *C. cognata* (Galiano & Hunziker 1987; Dematteis 1997a, 2002, 2009; Angulo & Dematteis 2009b). *Chrysolaena* is not well characterized cytologically and the studies are limited to chromosome counts of nine species (Dematteis 2009). Only the karyotypes of *C. flexuosa*, *C. simplex* Less., *C. platensis*, *C. cognata*, *C. verbascifolia*, and *C. lithospermifolia* have been analyzed (Ruas et al. 1991; Dematteis 1997a; Angulo & Dematteis 2009a, 2009b) and these analyses did not include all the cytotypes of the species.

The genomic size has not yet been estimated for *Chrysolaena*. DNA C-value is correlated with many biological characteristics, such as cell and nuclear volume, size of the chromosomes, and development of parameters such as duration of male meiosis, among others (Bennett 1987). It is also a useful trait in taxonomy and evolution (Godelle et al. 1993; Zoldos et al. 1998). However, the available information about DNA content in angiosperms is still limited; hence, there is a need for information of C-value in different plants (Bennett & Leitch 1995; Hanson et al. 2001).

In the present study, five species of *Chrysolaena*, *C. cognata*, *C. flexuosa*, *C. lithospermifolia*, *C. propinqua*, and *C. verbascifolia*, were examined in greater detail than prior reports. Variations in ploidy levels among different populations, the chromosome morphology, and DNA content are reported here.

Materials and methods

Mitosis and karyotype analysis

We examined nine populations belonging to five species of *Chrysolaena*. The source of the analyzed material is detailed in Table I. Voucher specimens are kept at the herbarium of the Instituto de Botánica del Nordeste (CTES).

The mitotic chromosomes were obtained from root tips of germinated seeds in Petri dishes. The rootlets were pretreated with 8-hydroxyquinoline solution 0.002 M for 4–5 hours and then fixed in absolute alcohol–acetic acid (3:1). The staining was performed using Feulgen's technique.

The nomenclature used to describe the chromosome morphology is the one proposed by Levan et al. (1964). For the preparation of the idiograms, 7–10 metaphase plates per each population were used. The measurement of the length of the chromosome arms and satellites was made by camera lucida drawings. The morphology of the chromosomes was determined using the centromeric index (CI = short arm × 100/ total length of the chromosome). Accordingly, the chromosomes were classified as metacentrics (m):

Table I. Specimens examined and somatic chromosome numbers ($2n$) observed in five species of *Chrysolaena*.

Species	$2n$	Location and voucher specimens
C. cognata (Less.) Dematt.	40	Argentina. Misiones. Dept. San Ignacio. House of Horacio Quiroga, stony fields. 27°15′54″S–55°33′02″W. *Dematteis et al. 3040* (CTES)
C. cognata (Less.) Dematt.	40	Uruguay. Dept. Tacuarembó, Gruta de los Helechos, 10 km NW of Tacuarembó, wet hill with rocks outcropping. 31°38′22″–56°02′07″W. *Dematteis et al. 3761* (CTES)
C. cognata (Less.) Dematt.	80	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo, Cementery, high fields with *Araucaria*, red soils. *Dematteis et al. 3053* (CTES)
C. flexuosa (Sims) H.Rob.	40	Uruguay. Dept. Artigas. Route 3, 20 km W of Artigas, on the road to Tomás Gomensoro. 30°26′ 57″S–56°36′57″W, high fields, stony soils. *Dematteis et al. 3703* (CTES)
C. flexuosa (Sims) H.Rob.	40	Uruguay. Dept. Tacuarembó. 14 km NE of Tacuarembó, 1,7 km after Gruta de los Helechos, hill with rocks outcropping. 31°37′30″S– 55°02′16″W. *Dematteis et al. 3750* (CTES)
C. flexuosa (Sims) H.Rob.	20	Argentina. Corrientes. Dept. Itá Ibaté, field near to Route 12. 27°26′47″S–57°20′01″W. *Via do Pico et al. 45* (CTES)
C. lithospermifolia (Hieron.) H.Rob.	20	Argentina. Corrientes. Dept. San Roque. 12 km N of San Roque, Route 12. 28°27′51″S–58°43′16″W. *Via do Pico et al. 4* (CTES)
C. propinqua (Hieron.) H.Rob.[a]	40	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo, Cementery, high fiels with *Araucaria*, red soils. *Dematteis et al. 3078* (CTES)
C. verbascifolia (Less.) H.Rob.	20	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo, Cementery, high fiels with *Araucaria*, red soils. *Dematteis et al. 3080* (CTES)

[a] New cytotype for the taxon.

50–37.5, submetacentrics (sm): 37.5–25, and subtelocentrics (st): 25–12.5.

The following karyological parameters were evaluated: total length of karyotype (TKL), average centromeric index (CI) and mean chromosome length (ML). The karyotype asymmetry was estimated using intrachromosomal (A_1) and interchromosomal (A_2) indexes suggested by Romero Zarco (1986).

DNA content

The nuclear DNA content was estimated in populations where material was available, using flow cytometry according to the protocol of Doležel and Göhde (1995). For this purpose, young leaves of living plants maintained under greenhouse conditions at the Instituto de Botánica del Nordeste were utilized. Voucher specimens are deposited at the herbarium of the Instituto de Botánica del Nordeste (CTES).

Small pieces of leaves from each specimen to be tested were incorporated together with the standard in a Petri dish. The leaf material was mechanically dispersed with a scalpel to which 1.5 mL of extraction buffer (Partec) was added, followed by incubation for 5 minutes at room temperature. Next, the extracts were filtered through a nylon membrane (CellTrics) to 5 mL cytometry tubes, and after addition of staining buffer with propidium iodide, plant cells were immediately analyzed by flow cytometry (FACSVantage, Becton Dickinson, San José, CA). The plots of absorption and the quantitative results were stored in data files. Three estimates were made of DNA content per individual (5000–10,000 nuclei per analysis). Three patterns were used as reference standards: *Paspalum intermedium* Sch 28857 (diploid, 2C = 1.417 pg), *P. dilatatum ssp. flavescens* (tetraploid, 2C = 2.43 pg), and *P. dilatatum* Chirú (hexaploid, 2C = 3.57 pg). Nuclear DNA content (2C) was calculated as: (sample peak mean/standard peak mean) × 2C DNA content of standard (pg). Also, we calculated the Cx value (2C DNA content/ploidy level).

Results

Chromosome numbers and karyotypes

The species analyzed, somatic chromosome numbers, and voucher specimens are listed in Table I. The ploidy level, karyotype formula, mean chromosome length (ML), total karyotype length (TKL), CI, and asymmetry indexes (A_1 and A_2) of the specimens tested are detailed in Table II.

In all species analyzed, the same chromosome number base of $x = 10$ was found. Of the nine studied populations, three were diploid ($2n = 2x = 20$), five

Table II. Somatic chromosome number (2n), ploidy level, karyotype formula, mean chromosome length (ML), total karyotype length (TKL), average centromeric index (CI), and asymmetry indexes (A_1 and A_2) of *Chrysolaena* populations analyzed.

Species	2*n*	Ploidy	Karyotype formula	TKL ± SE (μm)	ML (μm)	CI ± SE	A1	A2	2C (pg) ± SE	Cx (pg)
C. cognata 3040	40	4*x*	2*n* = 18m + 22sm	52.96 ± 0.12	2.65	39.06 ± 1.20	0.35	0.21	–	–
C. cognata 3761	40	4*x*	2*n* = 26m + 14sm	48.05 ± 0.12	2.40	39.53 ± 1.06	0.34	0.22	6.42 ± 0.08	1.605
C. cognata 3053	80	8*x*	2*n* = 54m + 26sm + B	80.55 ± 0.08	2.01	40.42 ± 0.91	0.31	0.24	12.27 ± 0.12	1.53
C. flexuosa 3703	40	4*x*	2*n* = 22m + 18sm + B	50.80 ± 0.11	2.54	39.55 ± 1.15	0.33	0.20	–	–
C. flexuosa 3750	40	4*x*	2*n* = 20m + 20sm	44.52 ± 0.12	2.23	38.24 ± 1.08	0.37	0.24	7.03 ± 0.11	1.75
C. flexuosa 45	20	2*x*	2*n* = 10m + 10sm	24.39 ± 0.19	2.44	38.06 ± 1.41	0.38	0.25	3.26 ± 0.026	1.63
C. lithospermifolia 4	20	2*x*	2*n* = 10m + 10sm	18.65 ± 0.14	1.86	39.41 ± 1.29	0.34	0.23	3.61 ± 0.06	1.805
C. propinqua 3078	40	4*x*	2*n* = 20m + 20sm + B	48.86 ± 0.11	2.44	38.88 ± 1.19	0.35	0.19	12.98 ± 0.19	3.24
C. verbascifolia 3080	20	2*x*	2*n* = 12m + 8sm + B	29.71 ± 0.18	2.70	39.31 ± 1.55	0.27	0.23	3.39 ± 0.028	1.69

Note: SE, standard error.

tetraploid ($2n = 4x = 40$), and one octoploid ($2n = 8x = 80$). In *C. verbascifolia* (Figure 1(h)–(j)), *C. cognata* (Figure 1(b)), *C. flexuosa*, and *C. propinqua*, the presence of accessories or B chromosomes that were unstable at mitosis was observed (Figure 1). The number of accessory chromosomes found ranged from 0 to 7 and this variation was observed between individuals of the same population and even within the same individual. In all species, accessory chromosomes were characterized as small metacentric elements with similar size. Because the accessory chromosomes were so small, the location of the centromere (Figure 1) was not always visible.

All taxa showed relatively symmetrical and bimodal karyotypes, which consisted of metacentric and submetacentric chromosomes, varying only in proportion among the different species (Figures 2 and 3). The average chromosome length (ML) ranged from 1.86 μm in *C. lithospermifolia* to 2.70 μm in *C. verbascifolia*. All species showed spherical satellites, whose diameters ranged from 0.28 μm to 0.51 μm, always placed on the short arm of metacentric pairs. At the intra-individual level, the presence of satellites on both members of a chromosome pair was not always visible.

The diploid sample of *C. flexuosa* (10m + 10sm) showed the most asymmetrical karyotype with an asymmetry index A_1 of 0.38 and an average CI of 38.06 (Figure 4). *C. verbascifolia* (12m + 8sm + Bs) presented a more symmetrical karyotype with an A_1 index of 0.27 and CI of 39.31. The interchromosomal asymmetry index (A_2) ranged from 0.19 in *C. propinqua* N°3078 to 0.25 in *C. flexuosa* N°45. The relationship between A_1 and A_2 indexes of the species analyzed is presented in Figure 4.

Of the three populations of *C. cognata* analyzed, two were tetraploid ($2n = 40$) and the remaining octoploid ($2n = 80$). In the latter, accessory chromosomes were detected at a frequency of 0–4 per cell, and had an average length of 1.27 μm. In *C. flexuosa*, two populations were tetraploid ($2n = 40$) and one diploid ($2n = 20$). In the population N°3703 from 0 to 2

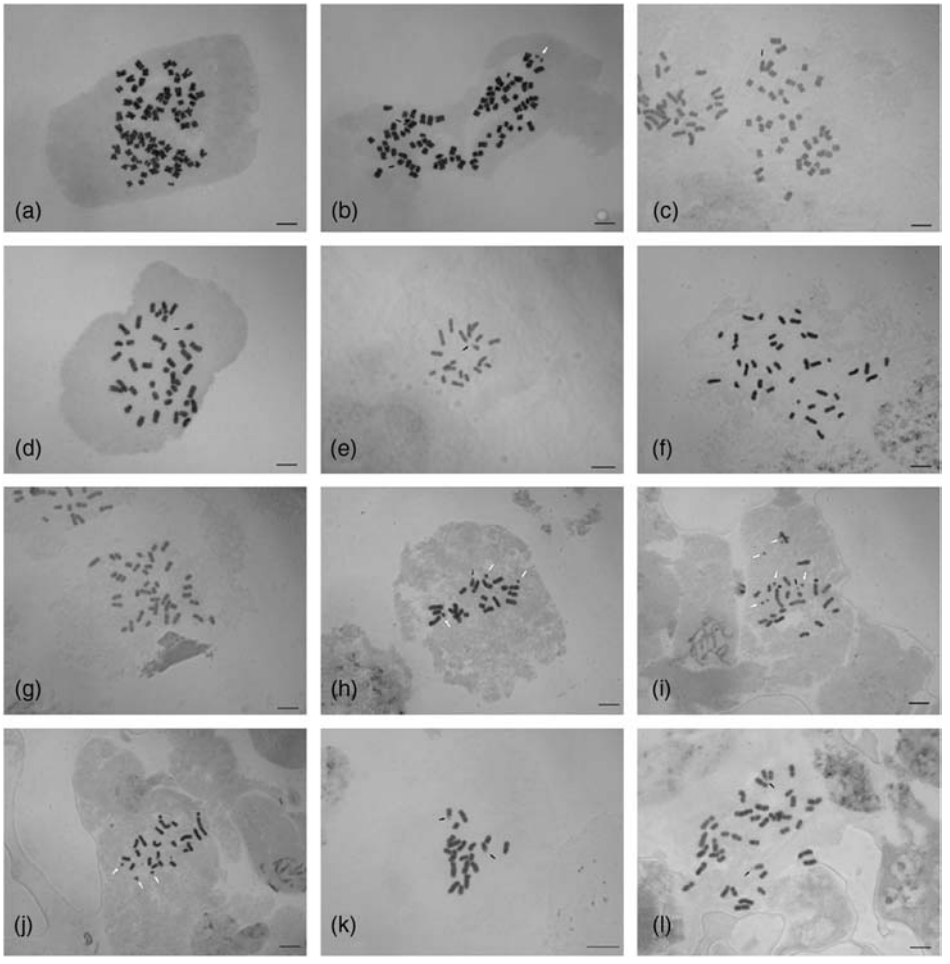

Figure 1. Mitotic chromosomes of *Chrysolaena*. (a) *C. cognata* N°3053, $2n = 8x = 80$; (b) *C. cognata* N°3053, $2n = 8x = 80 + 2$Bs; (c) *C. cognata* N°3761, $2n = 4x = 40$; (d) *C. cognata* N°3040, $2n = 4x = 40$; (e) *C. flexuosa* 45, $2n = 2x = 20$; (f) *C. flexuosa* N°3703, $2n = 4x = 40$; (g) *C. flexuosa* N°3750, $2n = 4x = 40$; (h) *C. verbascifolia* N°3080, $2n = 2x = 20 + 3$Bs; (i) *C. verbascifolia* N°3080, $2n = 2x = 20 + 7$Bs; (j) *C. verbascifolia* N°3080, $2n = 2x = 20 + 3$Bs; (k) *C. lithospermifolia*, $2n = 2x = 20$; (l) *C. propinqua* N°3078, $2n = 4x = 40$. Scale bar = 5 μm. Empty arrows show accessory chromosomes. Filled arrows show satellites. Asterisk symbols show heteromorphic chromosome pair.

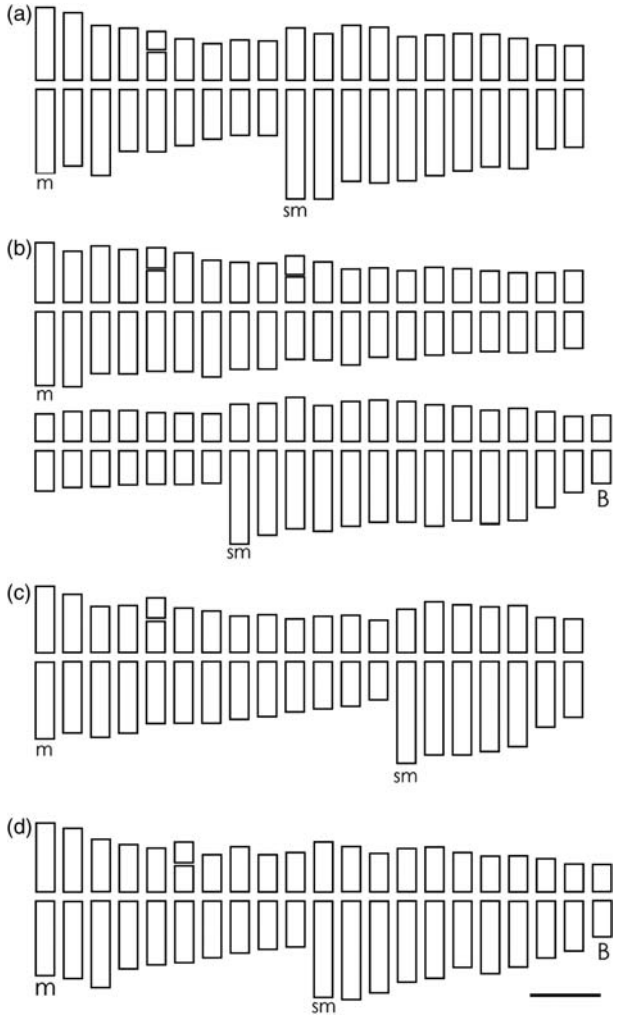

Figure 2. Idiograms of some species of *Chrysolaena*. (a) *C. cognata* N°3040, $2n = 40 = 18m + 22sm$; (b) *C. cognata* N°3053, $2n = 80 = 54m + 26sm + B$; (c) *C. cognata* N°3761, $2n = 40 = 26m + 14sm$; (d) *C. propinqua* N°3078, $2n = 40 = 20m + 20sm + B$. Scale bar = 1.5 μm.

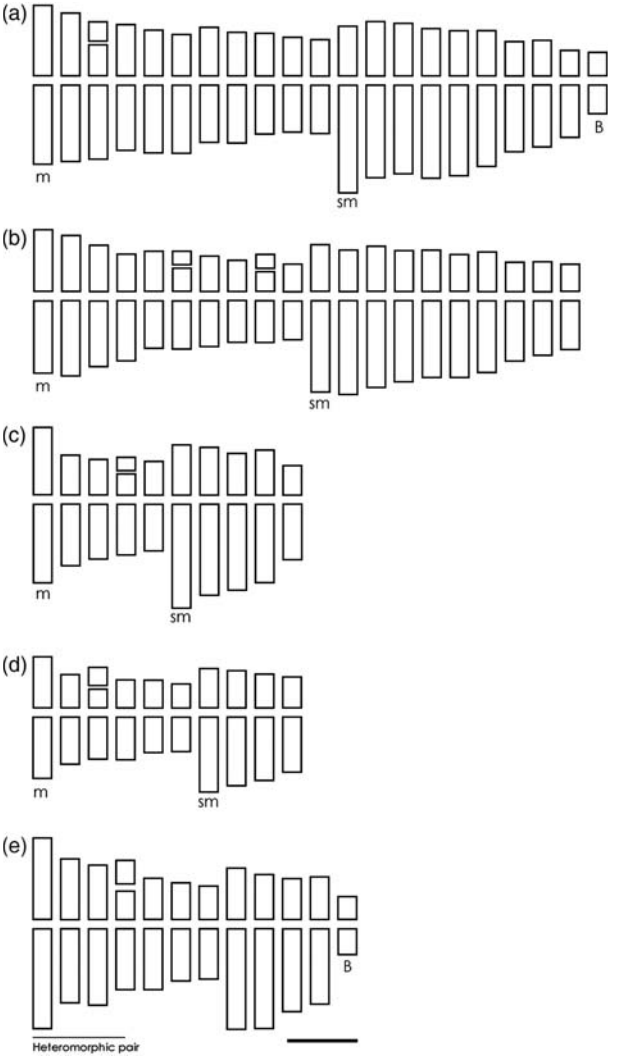

Figure 3. Idiograms of some species of *Chrysolaena*. (a) *C. flexuosa* N°3703, $2n = 40 = 22m + 18sm + B$; (b) *C. flexuosa* N°3750, $2n = 40 = 20m + 20sm$; (c) *C. flexuosa* N°45, $2n = 20 = 10m + 10sm$; (d) *C. lithospermifolia* N°4, $2n = 20 = 10m + 10sm$; (e) *C. verbascifolia* N°3080, $2n = 20 = 12m + 8sm + B$. Scale bar = 1.5 μm.

accessory chromosomes per cell were observed with an average length of 1.15 μm. *C. propinqua* was tetraploid ($2n = 40$) and also presented from 0 to 2 Bs with an average length of 1.37 μm. The remaining taxon, *C. verbascifolia*, was diploid ($2n = 20$) and showed between 0 and 7 accessory chromosomes per cell, with an average length of 0.94 μm. In addition, the karyotype analysis performed for this species revealed a heteromorphic chromosome pair (Figure 3(e)). This pair is characterized by having both chromosomes be metacentric, one of small size (2.37 μm) and the other one larger (4.61 μm).

Genomic DNA content

The genomic DNA content of the species of *Chrysolaena* analyzed is shown in histograms in Figure 5. The amount of DNA 2C-values calculated and Cx are shown in Table II. The 2C DNA content ranged from 3.26 pg in the diploid population of *C. flexuosa* N°45 ($2n = 20$) to 12.98 pg in the tetraploid sample of *C. propinqua* (tetraploid, $2n = 40$). The Cx value, which indicated the DNA content per genome, ranged from 1.605 pg in *C. cognata* N°3703 to 3.24 pg in *C. propinqua*. Diploid species showed 2C-values between 3.26 pg and 3.61 pg. Tetraploid species ranged from 6.42 pg to 12.98 pg and the only octoploid taxon (*C. cognata* 3053) showed 12.27 pg. The dispersion diagram in Figure 6 shows clearly that the diploid species are grouped showing similar 2C-values, and similarly the tetraploids grouped together, except for *C. propinqua* whose DNA content was similar to the octoploid population of *C. cognata*.

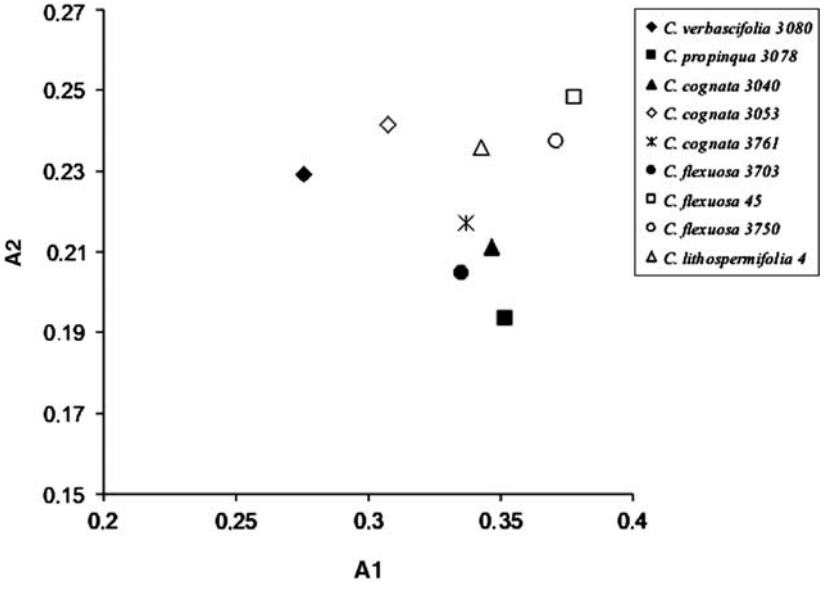

Figure 4. Dispersion diagram representing the relations between the A_1 and A_2 karyotype asymmetry indexes.

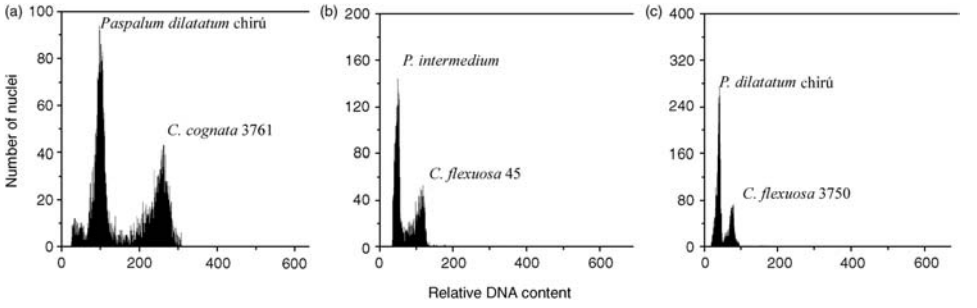

Figure 5. Histograms showing the relative DNA content at 2C of *Paspalum* (standard) and *Chrysolaena* species obtained by flow cytometry analysis of propidium iodide stained nuclei isolated from young leaves. (a) *C. cognata* N°3761, $2n = 4x = 40$; (b) *C. flexuosa* N°45, $2n = 2x = 20$; (c) *C. flexuosa* N°3750, $2n = 4x = 40$.

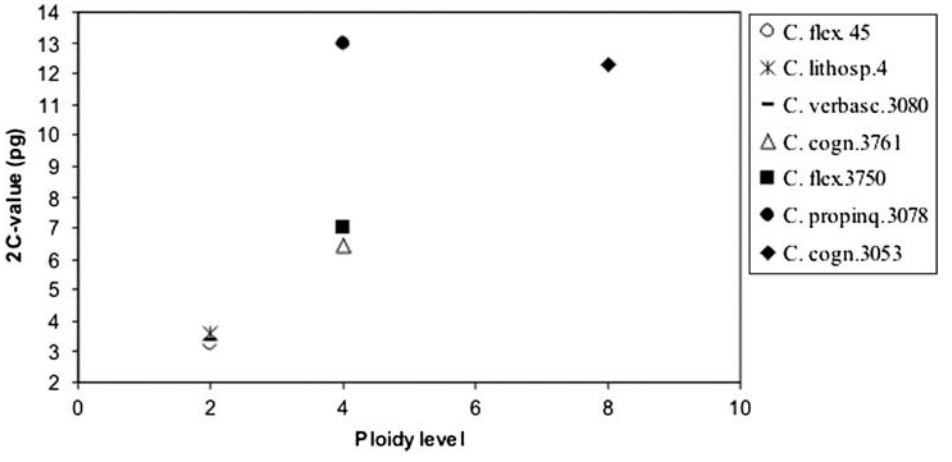

Figure 6. Dispersion diagram representing the relationship between ploidy levels and DNA 2C-values in *Chrysolaena* species analyzed.

Discussion

The five species of *Chrysolaena* analyzed in this paper all had a base chromosome number of $x = 10$, which is consistent with previous studies (Dematteis 1997a, 1997b, 1998, 2002, 2009; Dematteis et al. 2007; Angulo & Dematteis 2009a, 2009b). However, the results of this study broadened the cytological information by reporting new cytotypes and karyotypes for *Chrysolaena* species. The chromosome counts obtained for *C. cognata* showed a great

variation in chromosome number, including diploid, tetraploid, hexaploid, and octoploid populations and odd polyploids, as previously mentioned for specimens from Argentina and Paraguay (Angulo & Dematteis 2009a, 2009b; Dematteis 2009). In this paper, we report the first chromosome count for a population of *C. cognata* from Uruguay, which was tetraploid with $2n = 4x = 40$. The chromosome numbers found in populations of *C. flexuosa* ($2n = 2x = 20$, $2n = 4x = 40$) disagreed with the count of $n =$ ca. 17 determined by Jones (1979) in samples from Uruguay and the count of n = ca. 30–32 found by Hunziker et al. (1990) on plants from Argentina. However, the numbers observed are consistent with other studies that analyzed specimens from Brazil (Ruas et al. 1991), Argentina, Uruguay, and Paraguay (Dematteis 1997a, 1997b, 2002; Dematteis et al. 2007; Angulo & Dematteis 2009a). *C. lithospermifolia* and *C. verbascifolia* were found to be diploid ($2n = 2x = 20$) and both chromosome counts are in concordance with previous studies (Dematteis 1998; Angulo & Dematteis 2009a, 2009b). For these species, only Argentine specimens have been analyzed and only diploid populations have been found. The karyotype formula of *C. verbascifolia* is consistent with that reported by Angulo and Dematteis (2009b). However, our analysis found a pair of heteromorphic chromosomes. There are several processes through which the heteromorphism may have arisen: pericentric inversion, intra-chromosomal translocations, and "re-translocations" (Walters 1952). Other cytogenetic studies should be conducted on this species to understand better the origin of these elements. This issue goes beyond the goals of the current study.

For *C. propinqua*, previous studies reported only diploid populations from Argentina and Paraguay; so this is the first report of a tetraploid ($2n = 4x = 40$) population in Argentina. Also, this is the first report of the karyotype of the species, which was similar to other species of *Chrysolaena* that were analyzed.

The data obtained in the present paper along with those previously reported provide evidence that *Chrysolaena* species have numerous ploidy levels. Within even a single species, it was possible to observe different cytotypes ranging from diploid to other ploidy levels as high as octoploid. The available data on South American Vernonieae suggest that the most frequent chromosomal changes between populations are due to polyploidy (Galiano & Hunziker 1987; Dematteis 2002). According to Jones (1979) and Ruas et al. (1991), the New World Vernonieae show greater diversity in chromosome numbers and higher proportions of polyploid species than Old World species. Ruas et al. (1991) and Dematteis (1996, 1997a) argued that the more primitive species ($x = 10$ and $x = 17$) have longer chromosomes, while derived species ($x = 14$ and $x = 15$) have smaller chromosomes. High chromosome numbers in New World Vernonieae are consistent with a pattern of members invading new geographical areas. Since New World taxa are clearly derived, part of their success may be due to increased genetic diversity achieved through higher chromosome number and hybridization (Keeley & Robinson 2009).

With respect to the karyotypes, the predominance of metacentric and submetacentric chromosomes is consistent with previous reports for the tribe Vernonieae (Ruas et al. 1991; Dematteis 1997a, 1997b, 1998; Dematteis & Fernández 1998, 2000; Oliveira et al. 2007a, 2007b; Angulo & Dematteis 2009b). The karyotype symmetry was "moderate type" in all entities (Ruas et al. 1991), due to prevalence of metacentric chromosomes and the lack of marked differences among larger and smaller chromosomes. The A_1 and A_2 indexes, used preferably when the chromosomes are similar in size and morphology, differ little between the karyotypes of species analyzed (Table II). However, small differences in karyotypes were observed between different populations of *C. cognata* and *C. flexuosa*. In both species, tetraploid populations differed in the proportion of metacentric and submetacentric chromosomes. In *C. flexuosa*, the location of secondary constrictions was also different in diploid and tetraploid cytotypes. The average chromosome length (ML) and TKL were similar. Small variations in the degree of condensation of chromosomes can lead to calculation of different CIs when values are very close to the limit between one morphological type and another. These differences may be due to small divergences in the structure of chromosomes that involve gain and/or loss of chromatin, or structural changes without loss of chromatin that occurred in each population, or also due to small variations in the pretreatment technique. In addition, in one population of *C. flexuosa*, accessory chromosomes were observed. It has been shown that these types of chromosomes are instable during meiosis and show greater stability in mitosis. However, by causing high rates of non-disjunction during the latter, chromosomal instability can occur. This can lead, for example, to alterations in the morphology of chromosomes as a result of genomic rearrangements (Techio et al. 2010) and thus to the karyotype differences observed between populations. Small differences in karyotype formula and asymmetry indexes found among species suggest that small structural changes may have contributed to the diversification of the genus.

Polyploidy is an important mechanism that has contributed to adaptation and speciation in many organisms, mainly in plants (Masterson 1994; Coghlan et al. 2005), and it has been recognized as

a major force acting on the evolution of angiosperms. It is common in Asteraceae and occurs in most of the clades (Semple & Watanabe 2009). This mechanism is characterized by a variation in the amount of nuclear DNA (C-value) and simultaneous changes in number and structure of chromosomes (Murray 2005). Thus, polyploidy is clearly a possible contributor to the change in C-values.

DNA C-values are currently available for more than 7000 plant species (Bennett & Leitch 2010, http://data.kew.org/cvalues/). According to the available literature and the Plant DNA C-values database (Bennett & Leitch 1995, 2010), this is the first study of nuclear DNA content performed in the genus *Chrysolaena.* The estimates 2C and Cx calculated for *Chrysolaena* species in this study are within ranges of variation found in Angiosperms and Asteraceae (Leitch & Bennett 2004; Garnatje et al. 2010). The 1C-value ranges from 0.00648 pg in the carnivorous plant *Genlisea margaretae* (Greilhuber et al. 2006) to 152.23 pg in the monocot *Paris japonica* (Pellicer et al. 2010). Compared to values reported by Leitch et al. (1998) and Soltis et al. (2003), the genomic size of species of *Chrysolaena* falls into the categories "small" (1C values $>$ 1.4–3.5 pg) to "medium" ($>$ 3.5 to $>$ 14.0 pg). Within a taxon, DNA content is very often positively correlated with ploidy level. In species with different cytotypes (*C. cognata* and *C. flexuosa*), in general, there was a positive correlation between the increase in ploidy level and the TKL with respect to DNA content (see Figure 6). Polyploid taxa or populations always have a higher DNA amount than related diploid ones, and diploid accessions exhibited almost exactly half the DNA amount of their corresponding tetraploid strains. Similar observations have been made in other vascular plant genera, such as *Oxalis* (De Azkue & Martínez 1988) and *Artemisia* (Torrel & Vallés 2001). In general, there is a small diminution in DNA content per haploid genome (Cx) in polyploids in comparison with their diploid relatives. This has also been reported in other genera and has been interpreted as a balance mechanism to reduce some nucleotypic effects of DNA gain due to polyploidy (Bennett 1972; Poggio et al. 1989; Murray et al. 1992; Dimitrova & Greilhuber 2000). The results obtained in *C. propinqua* for 2C-values and Cx were unexpected given the similar karyotypes found among species of the genus. The increase in DNA content with no significant changes in karyotype may be caused by non-random distribution of these changes (Poggio et al. 2007). These phenomena occur in various sections of the genus *Vicia* (Raina & Rees 1983; Naranjo et al. 1998), *Hippeastrum* (Poggio et al. 2007), and some species of *Aloe*, which have different genomic sizes but maintain the bimodal karyotypes with similar proportions among species (Brandham 1983). DNA amount can increase not only by gain in individual chromosomes but also by polyploidy, both mechanisms often occurring in one plant group (Ohri 1996). This general rule is also valid for *Chrysolaena*, a genus in which polyploidy plays an important role. *Chrysolaena propinqua* is clearly distinguishable from the other members of the genus by the presence of a single stem with basal leaves. In addition to these morphological differences, DNA content can be a useful tool to differentiate this species from the remaining taxa of the genus.

The presence of accessory chromosomes or B chromosomes has been reported in many groups of plants. It is a very common phenomenon in the Compositae, which together with Poaceae is among the families with the greatest number of species that have been reported to have accessory chromosomes (Jones 1995). Accessory chromosomes have been found in the Eupatorieae (Waisman & Rozenblum 1986), *Senecio* (Dematteis & Fernández 1998), several species of *Baccharis* (Wulff et al. 1996) and *Brachycome dichromosomatica* (Houben et al. 1999), among others. In the tribe Vernonieae, numerous cases have been reported, such as *V. brevifolia* (Angulo & Dematteis 2009b), *V. sellowii* (Dematteis 1998), *V. canescens* (Galiano & Hunziker 1987), *V. geminata* (Oliveira et al. 2007a), *L. varroniifolius*, and *L. coriaceus* (Angulo & Dematteis 2012). The cytological analysis conducted in this paper reported as new the occurrence of accessory chromosomes in four species of *Chrysolaena: C. cognata*, *C. flexuosa*, *C. propinqua*, and *C. verbascifolia*. Jones (1995) refers to B chromosomes as extra chromosomes that do not clearly fit in the category of aneuploids or polyploids of a standard basic set. Their presence leads to the enlargement of the genome and creates polymorphisms in DNA content in natural populations (Jones et al. 2007). There is no doubt that B chromosomes originated from A chromosomes as a result of errors in meiosis that generate fragments of A chromosomes (Jones & Houben 2003). From this assumption, there are some possible ideas and models about how they escaped to complement and became autonomous elements and "parasites". One of the possible origins raised is genomic rearrangements following interspecific hybridization, e.g., in the derivatives of *Coix gigantea* ($2n = 2x = 20$), which hybridizes naturally with *C. aquatica* ($2n = 2x = 10$) (Sapre & Deshpande 1987 in Jones et al. 2007). This mechanism could explain the occurrence of B chromosomes in some *Chrysolaena* species. In *C. cognata*, the occurrence of populations with odd chromosome numbers ($2n = 50$) has been reported and it has been postulated that this could be a result of interspecific hybridization between individuals with different cytotypes (Dematteis 2002, 2009).

The ability of closely related *Vernonia* species to hybridize was demonstrated in North American and African species (Keeley & Robinson 2009). The highest ploidy levels of all analyzed species of the genus have been reported in *C. cognata*, and in this work, it was one of the species with the highest DNA content, so it shows the general tendency of groups with large genomes to be more likely to have Bs. However, this trend was not observed in *C. verbascifolia* (diploid, $2n = 2x = 20$) for which B chromosomes were found even more often.

Apparently, the B chromosomes found in *Chrysolaena* species are genetically stable and they have no effect on the phenotype or development of the individuals. In addition, B chromosomes do not contribute significantly to the genome enlargement, since the 2C-values of populations with Bs were very similar to populations without B chromosomes.

The new chromosome reports for the *Chrysolaena* species analyzed in this paper confirm the base number of $x = 10$ proposed for the genus, and help to validate the use of chromosome numbers to recognize the genus as distinct. There are many important characters taxonomic weight for separating *Chrysolaena* from other Vernonieae groups, such as the morphology of the pollen grains and morphological characters. However, there is overlap of different groups having species with shared characters. This is one of the issues that make this group so complex.

Morphologically, *Chrysolaena* species differ from each other by the arrangement of leaves on the stem, density and shape of phyllaries, type of indumentum, and floret number, among other features. However, the results obtained in this study did not allow separate species by the observed karyotypic differences because the estimated parameters were very similar between the entities. The only species clearly distinguishable karyotypically was *C. verbascifolia*, by the presence of a heteromorphic chromosome pair and numerous B chromosomes. Regarding the chromosome numbers in some species, a variation in the ploidy level is accompanied by an increase in plant size, as occurs in some species of *Conyza* (Urdampilleta et al. 2005) and *Stemodia* (Sosa et al. 2011). Among the species analyzed here, no correlation was observed between ploidy level and morphological features. Species with different ploidy levels were morphologically similar. In consequence, it was not possible to distinguish one cytotype from another on the basis of morphological characteristics of the plant. In general, with the exception of *C. propinqua*, the 2C-values could be used to estimate ploidy level of the *Chrysolaena* species. For example, the tetraploid cytotype of *C. propinqua* can be distinguished by having almost twice the DNA content of the tetraploid cytotypes of other species. For this reason, studies in nuclear DNA content realized for the first time in *Chrysolaena* species could be useful to resolve taxonomic problems in the genus.

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References

Angulo MB, Dematteis M. 2009a. Karyological analysis of South American species of *Vernonia* (Vernonieae, Asteraceae). Plant Biosyst 143: 20–24.

Angulo MB, Dematteis M. 2009b. Karyotype analysis in eight species of *Vernonia* (Vernonieae, Asteraceae) from South America. Caryologia 62: 81–88.

Angulo MB, Dematteis M. 2012. Cytotaxonomy of some species of the South American genus *Lessingianthus* (Asteraceae, Vernonieae). Plant Syst Evol 298: 277–285.

Bennett MD. 1972. Nuclear DNA amount and minimum generation time in herbaceous plants. Proc R Soc Lond B Biol Sci 191: 109–135.

Bennett MD. 1987. Variation in genomic form in plants and its ecological implications. New Phytol 106: 177–200.

Bennett MD, Leitch IJ. 1995. Nuclear DNA amounts in angiosperms. Ann Bot 76: 113–176.

Bennett MD, Leitch IJ. 2010. Plant DNA C-values database, Available: http://www.kew.org/cvalues/. Accessed Sep 28 2010.

Brandham PE. 1983. Evolution in a stable chromosome system. In: Brandham PE, Bennett MD, editors. Kew chromosome conference II. London: Allen & Unwin. pp. 251–260.

Coghlan A, Eichler EE, Oliver SG, Paterson AH, Stein L. 2005. Chromosome evolution in eukaryotes: A multi-kingdom perspective. Trend Genet 21(12): 673–682.

De Azkue D, Martínez A. 1988. DNA content and chromosome evolution in the shrubby *Oxalis*. Genome 30: 52–57.

Dematteis M. 1996. Estudios cromosómicos en especies argentinas de *Vernonia* (Asteraceae). Bonplandia 9: 103–103.

Dematteis M. 1997a. Cromosomas en *Vernonia platensis* y especies afines (Asteraceae). Bonplandia 9(3-4): 259–264.

Dematteis M. 1997b. Números cromosómicos y cariotipos de algunas especies de *Vernonia* (Asteraceae). Bol Soc Argent Bot 33(1–2): 85–90.

Dematteis M. 1998. Chromosome studies on *Vernonia flexuosa* and *V. lithospermifolia*. Compos Newslett 32: 10–16.

Dematteis M. 2002. Cytotaxonomic analysis of South American species of *Vernonia* (Vernonieae: Asteraceae). Bot J Linn Soc 139(4): 401–408.

Dematteis M. 2009. Revisión taxonómica del género sudamericano *Chrysolaena* (Vernonieae, Asteraceae). Bol Soc Argent Bot 44(1–2): 103–170.

Dematteis M, Fernández A. 1998. Estudios cromosómicos en dos especies de *Senecio* (Asteraceae). Bol Soc Argent Bot 33(3–4): 181–184.

Dematteis M, Fernández A. 2000. Chromosome studies on nine South American species of *Vernonia* (Vernonieae, Asteraceae). Caryologia 53(1): 279–288.

Dematteis M, Molero J, Angulo MB, Rovira AM. 2007. Chromosome studies on some Asteraceae from South America. Bot J Linn Soc 153(2): 221–230.

Dimitrova D, Greilhuber J. 2000. Karyotype and DNA content evolution in ten species of *Crepis* (Asteraceae) distributed in Bulgaria. Bot J Linn Soc 132: 281–297.

Doležel J, Göhde W. 1995. Sex determination in dioecious plants *Melandrium album* and *M. rubrum* using high-resolution flow cytometry. Cytometry 19: 103–106.

Galiano NG, Hunziker JH. 1987. Estudios cariológicos en Compositae IV. Vernonieae y Eupatorieae. Darwiniana 28 (1–4): 1–8.

Garnatje T, Canela MA, García S, Hidalgo O, Pellicer J, Sánchez-Jiménez I, Siljak-Yakolev S, Vitales D, Vallés J. 2010. GSAD: A database on genome size of the family Asteraceae, Available: http://www.entobiofic.cat/gsad/. Accessed Sep 30 2010.

Godelle B, Cartier D, Viarie D, Brown SC, Siljak-Yakovlev S. 1993. Heterochromatin study demonstrating the non-linearity of fluorimetry useful for calculating genomic base composition. Cytometry 14: 618–626.

Greilhuber J, Borsch T, Müller K, Worberg A, Porembski S, Barthlott W. 2006. Smallest angiosperm genomes found in Lentibulariaceae with chromosomes of bacterial size. Plant Biol 8: 770–777.

Hanson L, McMahon KA, Johnson MAT, Bennett MD. 2001. First nuclear DNA C-values for 25 Angiosperm families. Ann Bot 87: 251–258.

Houben A, Thompson N, Ahne R, Leach CR, Verlin D, Timmis JN. 1999. A monophyletic origin of the B chromosomes of *Brachycome dichromosomatica* (Asteraceae). Plant Syst Evol 219: 127–135.

Hunziker JH, Escobar A, Xifreda CC, Gamerro JC. 1990. Estudios cariológicos en Compositae VI. Darwiniana 30: 115–121.

Jones SB. 1979. Chromosome numbers of Vernonieae (Compositae). Bull Torrey Bot Club 106: 79–84.

Jones NR. 1995. B chromosomes in plants. Review. New Phytol 131: 411–434.

Jones NR, Houben A. 2003. B chromosomes in plants: Escapees from the A chromosome genome? Trend Plant Sci 8: 417–423.

Jones NR, Viegas W, Houben A. 2007. A century of B chromosomes in plants: So what? Ann Bot 101: 767–775.

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 Phylog Evol 44: 89–103.

Keeley SC, Jansen RK. 1994. Choloroplast DNA restriction site variation in the Vernonieae (Asteraceae), an initial appraisal of the relationships of the New and Old World taxa and the morphology of *Vernonia*. Plant Syst Evol 193: 249–265.

Keeley SC, Robinson H. 2009. Vernonieae. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ, editors. Systematics, evolution and biogeography of Compositae. Vienna: IAPT. pp. 439–469.

Leitch IJ, Bennett MD. 2004. Genome downsizing in polyploid plants. Biol J Linn Soc 82: 651–663.

Leitch IJ, Chase MW, Bennett MD. 1998. Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. Ann Bot 82: 85–94.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

Masterson J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. Science 26: 421–423.

Murray BG. 2005. When does intraspecific C-value variation become taxonomically significant? Ann Bot 95: 119–125.

Murray BG, Cameron EK, Stardring LS. 1992. Chromosome numbers, karyotypes, and nuclear DNA variation in *Pratia* Gaudin (Lobeliaceae). New Zeal J Bot 30: 181–187.

Naranjo CA, Ferrari MR, Palermo AM, Poggio L. 1998. Karyotype, DNA content and meiotic behavior in five South American species of *Vicia* (Fabaceae). Ann Bot 82: 757–764.

Ohri D. 1996. Genome size and polyploidy variation in the tropical hardwood genus *Terminalia* (Combretaceae). Plant Syst Evol 200: 225–232.

Oliveira VM, Forni-Martins ER, Semir J. 2007a. Cytotaxonomy of species of *Vernonia*, section *Lepidaploa*, group *Axilliflorae* (Asteraceae, Vernonieae). Bot J Linn Soc 154: 99–108.

Oliveira VM, Forni-Martins ER, Semir J. 2007b. Cytotaxonomic studies in six species of *Vernonia* (Asteraceae: Vernonieae). Caryologia 60(1–2): 37–47.

Pellicer J, Fay MF, Leitch IJ. 2010. The largest eukaryotic genome of them all? Bot J Linn Soc 165(1): 10–15.

Poggio L, Burghardt AD, Hunziker JH, Nuclear DNA. 1989. variation in diploid and polyploid taxa of *Larrea* (Zygophyllaceae). Heredity 63: 321–328.

Poggio L, González G, Naranjo CA. 2007. Chromosome studies in *Hippeastrum* (Amaryllidaceae): Variation in genome size. Bot J Linn Soc 155: 171–178.

Raina SN, Rees H. 1983. DNA variation between and within chromosome complements of *Vicia* species. Heredity 51: 335–346.

Robinson H. 1999. Generic and subtribal classification of American Vernonieae. Smithsonian Contrib Bot 89: 1–116.

Romero Zarco C. 1986. A new method for estimating karyotype asymmetry. Taxon 35: 526–530.

Ruas PM, Ruas CF, Vieira AOS, Matzenbacher NI, Martins NS. 1991. Cytogenetics of genus *Vernonia* Schreber (Compositae). Cytologia 56: 239–247.

Semple JC, Watanabe K. 2009. A review of chromosome numbers in the Asteraceae with hypotheses on chromosome base number evolution. In: Funk VA, Susanna A, Stuessy T, Bayer R, editors. Systematics, evolution and biogeography of the Compositae. Vienna: IAPT. pp. 21–32.

Soltis DE, Soltis PS, Bennett MD, Leitch IJ. 2003. Evolution of genome size in the angiosperms. Amer J Bot 90: 1596–1603.

Sosa MM, Panseri AF, Fernández A. 2011. Karyotype analysis of the southernmost South American species of *Stemodia* (Scrophulariaceae). Plant Biosyst 145(2): 472–477.

Techio VH, Mittelmann A, Marció S, Pereira AV. 2010. Meiotic and mitotic behavior of B chromosomes of ryegrass. Cienc. Rural [online] 40 (1): 83–88.

Torrell M, Vallès J. 2001. Genome size in 21 *Artemisia* L. species (Asteraceae, Anthemideae): Systematic, evolutionary, and ecological implications. Genome 44: 231–238.

Urdampilleta JD, Amat AG, Bidau CJ. 2005. Karyotipic studies and morphological analysis of some reproductive features in five species of *Conyza* (Astereae: Asteraceae) from Northeastern Argentina. Bol Soc Argent Bot 40(1–2): 91–99.

Waisman CE, Rozenblum E. 1986. Estudios cariológicos en Compositae III. Darwiniana 27: 179–189.

Walters JL. 1952. Heteromorphic chromosome pairs in *Paeonia californica*. Amer J Bot 39(2): 145–151.

Wulff AF, Hunzinker JH, Escobar A. 1996. Estudios cariológicos en Compositae VII. Darwiniana 34(1–4): 213–231.

Zoldos V, Papes D, Brown SC, Panaud O, Siljak-Yakovlev S. 1998. Genome size and base composition of seven *Quercus* species: Inter- and intra-population variation. Genome 41: 162–168.