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DNA content in species of *Vernonia* and *Vernonanthura* from South America: An approach to systematics and evolution of the Vernonieae (Asteraceae)

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Abstract
There are relatively few studies of DNA content in the Vernonieae (Asteraceae) tribe. The first studies were realized in the *Lessingianthus* genus and determined the DNA content of 25 species. After DNA content, ploidy level and the total karyotype were compared in 6 *Chrysolaena* species. The aim of this study was to present, for the first time, the DNA content values of *Vernonanthura* and *Vernonia* and to thereby expand knowledge of the Vernonieae tribe. A total of 19 natural populations belonging to the genera *Vernonanthura* and *Vernonia* were studied for the first time. The results were compared with other Vernonieae genera and with other Asteraceae tribes. Our results found that Vernonieae have the smallest range of 1C value variation in Asteraceae. Furthermore, there were differences in the DNA content of *Vernonia* and *Vernonanthura*. These results show that low DNA content and herbaceous habit in *Vernonia* are characters derived from the higher DNA content and woody habit present in *Vernonanthura*. These results could indicate a hybrid origin of one species and allow the determination of both the ploidy and chromosome number of other taxa. The results observed in *Vernonanthura* species showed a highly significant correlation between 1C-value and latitude.

Key words: *Asteraceae, evolution trends, genome size, hibrid origin, systematic, Vernonieae*

Introduction

Vernonieae Cassini (1819) is one of the largest tribes in the Asteraceae Berchtold and von Presl (1820) comprising approximately 1700 species distributed across the tropical regions of Asia, Africa, and America (Robinson 2007; Keeley & Robinson 2009). There are two major centers of Vernonieae diversification, one in southern Brazil and other in tropical Africa. The members of the tribe are grouped into 21 different subtribes based on inflorescence pattern, persistence of phyllaries, anther appendages, style base, achene crystals, pollen morphology, and chemical composition (Keeley & Robinson 2009).

The Vernonieae are considered one of the most complex groups within the Asteraceae from the taxonomic viewpoint (Keeley et al. 2007). Discussions have mainly centered on the delimitation of *Vernonia* Schreber (1791), the core genus of the tribe with that includes approximately 1200 species (Bremer 1994; Robinson 1999). Recent classification has restricted *Vernonia* to 22 North American and 2 South American species, while the remaining species have been reclassified into 16 new genera (Robinson 1999). *Vernonia* sens. str. has only two South American species, *V. incana*, which is diploid with $2n = 34$ (Dematteis & Fernandez 1998; Dematteis 2002) and *V. echioides*, which has two cytotypes, one diploid with $2n = 34$ and the other tetraploid with $2n = 68$ (Vega & Dematteis 2012).

One of these newly segregated genera is *Vernonanthura* H. Rob., which was established to separate the taxa previously assigned to *Vernonia* sect. *Lepidaploa* subsect. *Paniculatae* Benth. and Hook.. This genera has 90 species that are distributed from southern Mexico to central Argentina, but are mostly concentrated in southeast Brazil (Dematteis 2006; Vega & Dematteis 2011). This genus is closely related to *Vernonia s. str.*, but differs in the type of inflorescence, erect habit, xylopodial bases, and sometimes in the presence of tailed anther bases (Robinson 1992). However, this morphological differentiation remains unclear. Answering the need to clearly differentiate these genera, Redonda-Martínez et al. (2012) studied the diversity of trichomes on the leaves and flowers of *Vernonanthura* and *Vernonia* species from Mexico. This study concluded that the presence or absence and type of trichomes are insufficient to differentiate these genera. However, looking at multiple south

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American species, chromosome size was found to be quite different between groups (Vega & Dematteis 2011, in press), which could be reflected in the amount of DNA in both genera.

The *Vernonanthura* species are shrubs or small trees with thyrsoid to pyramidal inflorescences and individual cymose to corymbose branches, while *Vernonia* species are herbs with corymbiform inflorescences, irregular-length branches and few to many heads on peduncules of irregular length or some nearly sessile (Robinson 1992). In southern South America, the species are mainly concentrated in the mountains of northwest Argentina and in the fields and forests of Paraguay (Cristobal & Dematteis 2003, ~~2009~~; Vega & Dematteis 2008). Almost all *Vernonanthura* species are diploid with $2n = 34$, excluding *V. pinguis* (Griseb.) H. Rob., which constitutes the single tetraploid species of the genus with $2n = 68$ (Dematteis & Fernandez 1998; Dematteis 2002; Vega & Dematteis 2012).

The DNA amount has been estimated in 7542 angiosperms species (Garcia et al. 2013), which represents about 2.5% of known flowering plants (*sensu* APG I 1998; APG II 2003). More Asteraceae species have been analyzed than any other family, reaching ca. 1219 species (Garcia et al. 2013). The Asteraceae data are biased to some degree, since the vast majority of species analyzed (96.74%) were concentrated in just five of the 29 tribes recognized (Anthemideae, Cichorieae, Cardueae, Senecioneae, Heliantheae). However, these five groups contribute around 50% of the family's species richness (Funk et al. 2009). *Artemisia* L. is one of the genera particularly well covered in this respect, with data available for 24.3% of the taxa (Vallès et al. 2011, 2012). Nuclear DNA content in this genus varies ninefold, from 2C = 3.5 in *A. annua* L. (Torrell & Vallès 2001) to 31.51 pg in *A. copa* Philippi (Pellicer et al. 2010).

The studies on DNA amount in the Vernonieae tribe are relatively scarce. The first studies were conducted in *Lessingianthus* H. Rob., where the DNA content of 25 species was determined (Angulo & Dematteis 2013). Via Do Pico and Dematteis (2013) compared the DNA content, ploidy level, and the total length of the karyotype in six species of *Chrysolaena* H. Rob. They found that in species with different cytotypes (*C cognata* and *C. flexuosa*), there is generally a positive correlation between increasing ploidy level, the total length of the karyotype, and amount of DNA. Moreover, they observed that polyploid populations have higher 1C value that related diploids.

The aim of this study was to present *Vernonanthura* and *Vernonia* DNA content for the first time and to expand the knowledge of the Vernonieae tribe. On the other hand, these results will contribute to the understanding of the taxonomy and evolution of both groups.

Material and methods

DNA amount measurements were obtained from the fresh leaves of live plants collected both in the field and from a greenhouse. To a lesser extent, DNA content measurements were obtained from newly germinated cotyledons in Petri dishes.

The 1C values of the tested species were estimated using flow cytometry. Measurements were calculated from three replicates per individual. s

The fresh leaves or newly germinated cotyledons were macerated together with in 0.5 mL of buffer Otto I. Subsequently, the core suspension was passed through a 30-microns nylon filter and poured into a plastic tube receiver, to which was added 1.5 mL of staining buffer Otto with IP (1 µ/µL).

All the samples were analyzed with a Partec PA II flow cytometer (Partec GmbH, Münster, Germany) with the detector operating at 355 nm. At least 5000 nuclei were counted per sample. The PA-II Partec FloMax software was used for the data analysis.

Paspalum intermedium Munro ex Morong. accession *Schinini 28857* (diploid, 2C = 1.417 pg, Vaio et al. 2007) leaves were used as an internal standard for the majority of the specimen, while *P. dilatatum* Poir. ssp. *flavescens* Roseng., B.R.Arrill. & Izag. (tetraploid, 2C = 2.43 pg, Vaio et al. 2007) was used as the standard for *V. incana*.

The absolute 2C-value of each sample's DNA was calculated employing the following formula, as outlined according to Dolezel and Bartoš (2005):

$$\text{Sample 2C DNA content} = \frac{\text{Sample G1 mean peak} \times \text{Standard 2C DNA content}}{\text{Standard G1 mean peak}}$$

To assess whether there were differences in DNA content between species of *Vernonanthura* and *Vernonia*, a randomization test was performed using two groups, one consisting of the fifteen populations of *Vernonanthura* and the second of three *Vernonia* populations. *V. chaquensis* was omitted and 1Cx-value was utilized to avoid bias from the inclusion of data from the cytotype tetraploid of *V. echioides*. This analysis was performed with EcoSim software version 7.72.

A Spearman correlation was carried out *in Vernonanthura* species to establish the relationship between the DNA amount and several abiotic factors (e.g., altitude, longitude, latitude). Furthermore, the

Table I. Species, voucher, locality, latitude (Lat.), longitude (Lon.), altitude (AMSL), 2C-value (pg) ± SE, of all the *Vernonanthura* and *Vernonia* studied.

Species	Voucher specimens	Lat.	Lon.	Alt.	2C
Vernonanthura H. Rob.					
V. amplexicaulis	Bolivia. Dept. Santa Cruz. Provincia Ñuflo de Chavez. 11.6 km N de San Javier, camino a la curtiembre. *Dematteis et al. 3846.* (CTES, SI).	−16.2727	−62.5444	577	3.85 (±0.03)
V. brasiliana	Paraguay. Dept. Central. Camino de Ypacarai a Luque. *Vega 67* (CTES).	−25.3889	−57.2710	71	3.28 (±0.14)
V. brasiliana	Bolivia. Dept. Santa cruz. 2,2 km N de Abra de Quiñe. *Vega 162* (CTES).	−18.0747	−64.3366	2120	3.51 (±0.08)
V. chamaedrys	Argentina. Province Misiones. Dept. Candelaria. Santa Ana, Base del cerro Santa Ana. *Vega et al. 160* (CTES).	−27.5280	−58.4325	61	2.93 (±0.05)
V. chamaedrys	Argentina. Province Corrientes. Camino desde Laguna Brava a Puente Pexoa. *Vega et al. 67* (CTES).	−27.4354	−55.5726	171	3.12 (±0.11)
V. chaquensis	Argentina. Province Corrientes. Dept. Santo Tomé. 40 km N de Santo Tomé, camino a Galarza. *Dematteis et al. 4274.* (CTES).	−28.4216	−56.2461	95	2.51 (±0.04)
V. ferruginea	Bolivia. Dept. Santa cruz. 2,2 km N de Abra de Quiñe. *Vega 163* (CTES).	−18.0747	−64.3366	2120	3.01 (±0.08)
V. loretensis	Argentina. Province Misiones. Dept. San Ignacio. Casa de Horacio Quiroga. *Dematteis et al. 3046* (CTES).	−27.2650	−55.5505	68	3.16 (± 0.03)
V. lucida	Argentina. Province Misiones. Dept. San Pedro. Parque Provincial Moconá. Embarcadero, costa del río. *Dematteis et al. 3095* (CTES, G, MBM).	−27.1552	−53.8911	145	3.34 (±0.01)
V. membranacea	Bolivia. Dept. Santa Cruz. Province Chiquitos. 7.2 km NW de Chochis, camino a San José. *Dematteis et al. 3945* (CTES, SI, G, OS).	−18.1091	−60.0861	515	4.14 (±0.05)
V. montevidensis	Argentina. Province Corrientes. Dept. San Roque. 8 km W de Chavarría, sobre ruta 12. *Dematteis et al. 4132* (CTES).	−28.9105	−58.6533	59	3.16 (±0.04)
V. nudiflora	Uruguay. Dept. Rivera. 16.9 km S de Tranqueras. Cerro Alegre. *Dematteis et al. 3792* (CTES, UTEP).	−31.3063	−55.7435	175	3.10 (±0.07)
V. oligactoides	Argentina. Province Misiones. Dept. General Manuel Belgrano. Campina de Americo. *Dematteis et al. 3077.* (CTES).	−26.2744	−53.6997	813	3.23 (±0.05)
V. petiolaris	Argentina. Province Misiones. Dept. General Manuel Belgrano. Detrás de la cancha de deportes de la escuela. *Vega et al. 31* (CTES).	−26.2802	−53.7052	791	2.74 (±0.03)
V. squamulosa	Argentina. Province Jujuy. Dept. El Carmen. Dique La Cienaga, borde de monte. *Dematteis et al. 2998.* (CTES).	−24.4175	−65.2894	1110	3.58 (±0.03)
V. tweediana	Argentina. Province Misiones. Dept. San Pedro, 8, 9 km NE de Cruce Caballero *Vega et al. 157* (CTES).	−26.5002	−53.8991	647	3.50 (±0.06)
Vernonia Schreb.					
V. echioides	Argentina. Province Corrientes. Dept. Alvear. 5,5 km N de Alvear camino a Santo Tomé. *Dematteis et al, 4135.* (CTES).	29.0538	56.5208	55	2.47 (±0.03)
V. echioides	Argentina. Province Misiones. Dept. Capital. Campos bajos cercanos al Arroyo Zaimán. *Dematteis et al. 3030.* (CTES).	27.4475	55.9044	59	5.29 (±0.22)
V. incana	Argentina. Province Corrientes. Dept. Empedrado. 19.4 km S de Riachuelo. *Dematteis et al. 4130* (CTES).	27.7555	58.7691	59	1.58 (±0.08)

DNA amount of the species studied in this work was also analyzed with respect to 19 bioclimatic variables (annual mean temperature, mean monthly temperature range, isothermality, temperature seasonality, max temperature of warmest month, min temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter) to gain insights into the ecological significance of the variation observed. Data for bioclimatic variables were extracted from the WorldClim database (Hijmans et al. 2005). This procedure was carried out with Xlstat 2004 software. The locations analyzed, latitude, longitude, and altitude of each population is detailed in Table I.

The ploidy level and chromosome number for the majority of the populations studied were extracted

Table II. Species, chromosome number (2***n***), ploidy level, 1C-value (pg), 1Cx-value (pg), and coefficient of variation (HPCV) of all the *Vernonanthura* and *Vernonia* studied.

Species	Chromosome numbers	Ploidy	1C	Cx	HPCV (%) of samples	Reference[a]
Vernonanthura H. Rob.						
V. amplexicaulis	34	2x	1.92	1.92	0.018	1
V. brasiliana I	34	2x	1.64	1.64	0.089	2
V. brasiliana II	34	2x	1.75	1.75	0.047	3
V. chamaedrys I	34	2x	1.46	1.46	0.035	2
V. chamaedrys II	34	2x	1.56	1.56	0.076	3
V. chaquensis	34	2x	1.25	1.25	0.032	2
V. ferruginea	34	2x	1.50	1.50	0.053	3
V. loretensis	34	2x	1.58	1.58	0.019	1
V. lucida	34	2x	1.67	1.67	0.007	1
V. membranacea	34	2x	2.07	2.07	0.025	3
V. montevidensis	34	2x	1.58	1.58	0.029	3
V. nudiflora	34	2x	1.55	1.55	0.048	1
V. oligactoides	34	2x	1.61	1.61	0.031	1
V. petiolaris	34	2x	1.37	1.37	0.046	3
V. squamulosa	34	2x	1.79	1.79	0.018	1
V. tweediana	34	2x	1.75	1.75	0.036	3
Vernonia Schreb.						
V. echioides	34	2x	1.23	1.23	0.024	1
V. echioides	68	4x	2.64	1.32	0.084	1
V. incana	34	2x	0.79	0.79	0.10	1

[a]Ploidy and chromosome number obtained from.
1: Vega and Dematteis (2012).
2: Vega and Dematteis (in press).
3: Reported here.
V. brasiliana I: population from Paraguay.
V. brasiliana II: population from Bolivia.
V. chamaedrys I: population from Argentina, Misiones.
V. chamaedrys II: population from Argentina, Corrientes.

from Vega and Dematteis (2012), in which the authors analyzed the same samples, while others were reported from previous chromosome counts (Dematteis 1996, 2002; Dematteis & Fernandez 1998) and DNA content data obtained here (Table I).

Results

The *Vernonanthura* specimen studied were as follows: *V. amplexicaulis* (R.E. Fr.) H. Rob, *V. brasiliana* (L.) H. Rob., *V. chaquensis* (Cabrera) H. Rob., *V. chamaedrys* (Less.) H. Rob., *V. ferrugiena* (Less.) H. Rob., *V. loretensis* (Hieron.) H. Rob., *V. lucida* (Less.) H. Rob., *V. membranacea* (Gardn.) H. Rob., *V. montevidensis* (Spreng.) H. Rob., *V. nudiflora,* (Less.) H. Rob. *V. oligactoides* (Less.) H. Rob., *V. petiolaris* (DC.) H. Rob., *V. squamulosa* (Hook. & Am.) H. Rob., y *V. tweediana* (Baker) H. Rob., while the *Vernonia* taxa analyzed were *V. echioides* Less. and *V. incana* Less. All of the populations analyzed were diploid, except for a single *V. echiodes* sample that was tetraploid.

Vernonanthura 1C values varied from 1.25 pg in *V. chaquensis* to 2.07 pg in *V. membaranacea*. In *Vernonia*, the 1C values ranged from 0.79 pg in *V. incana* to 2.64 pg in the tetraploid cytotype of *V. echioides*. The tetraploid cytotype of *V. echioides* had higher 1Cx values than the diploid sample (Table II).

Most *Vernonanthura* species presented small genomes (1C < 3.5 pg), excluding *V. chaquensis* (1C = 1.25 pg) and *V. petiolaris* (1C = 1.37 pg), which presented very small genomes. In *Vernonia*, *V. incana* (1C = 0.79 pg) and the diploid cytotype of *V. echioides* (1C = 1.23 pg) presented a very small genome, while the tetraploid cytotype presented a small genome (1C = 2.64).

Both the *Vernonanthura brasiliana* and *V. chamaedrys* populations analyzed demonstrated that there is a little intraspecific variation (6.28 and 6.60%, respectively).

The randomization test did not show significant differences in 1Cx value between the *Vernonanthura* and *Vernonia* species.

The results of the Spearman correlation analyses show significant correlation between 1C value (pg) and latitude ($r(s) = -0.68$; $n = 16$; $p =< 0.005$) in *Vernonanthura*, while the DNA content and bioclimatic variables considered did not show a significant correlation. The distribution of the analyzed populations is represented in Figure 1.

Discussion

The DNA amount has been estimated for 7542 angiosperms species (Garcia et al. 2013), about 2.5% of the total flowering plant species (*sensu* APG I 1998; APG II 2003). At present, 1219 species from the Asteraceae family have been studied, making it the most abundantly represented family in these studies (Garcia et al. 2013).

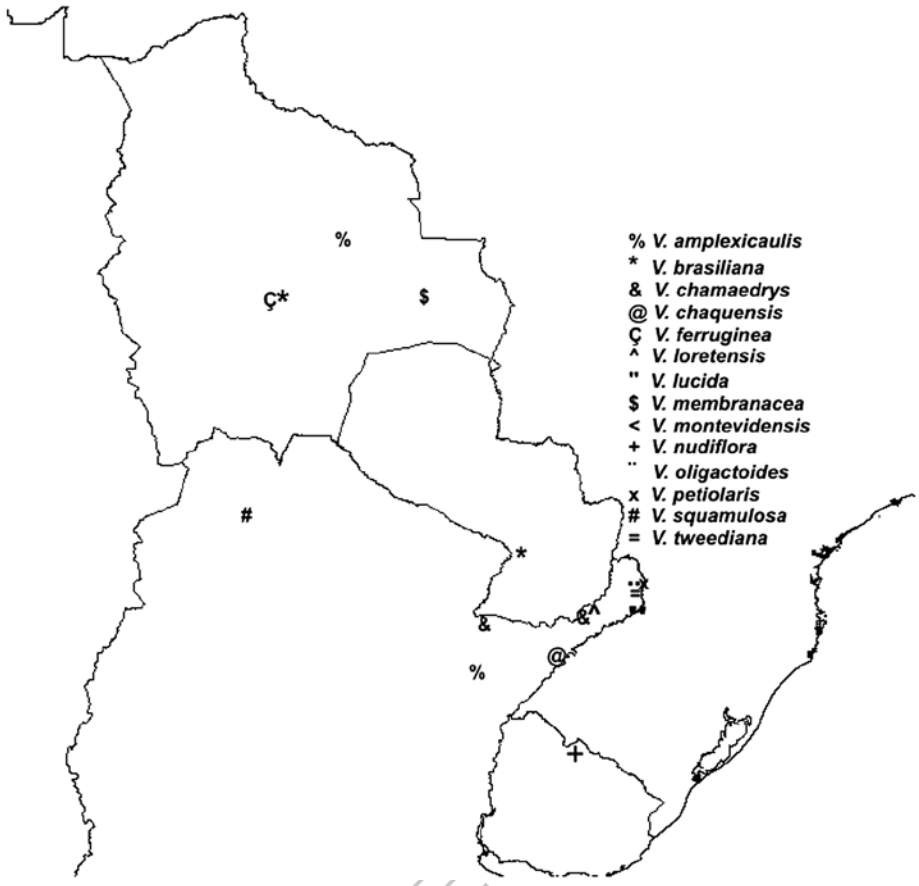

Figure 1. Distribution of the populations of *Vernonanthura* analyzed in Spearman correlation analyses.

Holoploid nuclear DNA content (1C) in Asteraceae ranges from 1C = 0.4 pg in *Leontodon longirostris* (Finch & P.D. Sell) Talavera to 1C = 28.3 pg in *Coreopsis nuecensis* A. Heller, which represents a nearly 70.75-fold variation within the family, whereas monoploid values (1Cx) vary from 0.18 pg in *Pericallis appendiculata* (L.f.) B. Nord. to 10.45 pg in *Chrysanthemum carinatum* Schousb. (Garcia et al. 2013). Within the Asteraceae, 208 species of the Anthemidae tribe have been analyzed, making Anthemidae the most studied tribe in terms of DNA amount (Vallès et al. 2012). This tribe has the highest mean genome size (1C = 5.97 pg), with the values ranging 14.19-fold. Despite the fact that more genome size records are available for the Anthemideae tribe than for other tribes, its range is considerably smaller than the range for the Senecioneae and Cichorieae tribes, which vary between 47.55-fold and 39.13-fold, respectively (Garcia et al. 2013).

Within the Vernonieae tribe, the DNA content of several *Chrysolaena* and *Lessingianthus* species is known (Angulo & Dematteis 2013; Vía do Pico & Dematteis 2013). Including the *Vernonia* and *Vernonanthura* species analyzed here, 46 species and 51 samples from this tribe were evaluated. The mean 1C genome size is 2.33 pg, ranging 9.07-fold from 1C = 0.79 pg in *Vernonia incana* to 1C = 14.17 pg in *Lessingianthus macrocephalus* (Less.) H. Rob. These results show that Vernonieae have the smallest 1C value variation range within the Asteraceae.

One of the main objectives of this work was to establish whether there is difference in DNA content between *Vernonia* and *Vernonanthura*. Observing the range of 1C value variation of each genus (*Vernonia* 1C = 0.79–2.64 pg, *Vernonanthura* 1C = 1.25–2.07 pg), we can see that the results overlap. But when 1Cx-value was used, to avoid the bias due to inclusion of data from the cytotype tetraploid of *Vernonia echioides*, and *Vernonanthura chaquensis* was omitted (per possible hybrid origin), the range of variation in *Vernonia* (1Cx = 0.79–1.32) and *Vernonanthura* (1Cx = 1.37–2.07) does not overlap. However, this result was not found to be statistically significant by the randomization test.

There are several genera in Asteraceae that have the same number of chromosomes but a different DNA amount; e.g., in *Helianthus* L., 18 diploid species with $2n = 18$ show a fourfold difference between the highest (*H. agrestis* Pollard, 1C = 12.95 pg) and the lowest (*H. neglectus* Heiser, 1C = 3.20 pg) 1C value (Ohri 1998). However, in diploid *Lessingianthus* species, the 1C value varied from 1.02 pg

in *L. pussilus* (Dematt.) M. B. Angulo to 1.30 pg in *L. coriaceus* (Less.) H. Rob. (Angulo & Dematteis 2013). In diploid *Chrysolaena* species, the 1C value ranged from 1.63 pg in *C. flexousa* (Sims) H. Rob. to 1.80 pg in *C. lithospermifolia* (Hieron.) H. Rob. (Via do Pico & Dematteis 2013). *Vernonanthura* ranges from 1C = 1.25 pg in *V. chaquensis* to 1C = 2.07 pg in *V. membranacea*. Within the Vernonieae, the genus *Lessingianthus* had up to 11x ploidy levels and had a sevenfold 1C genome size variation (Angulo & Dematteis 2013), whereas in *Chrysolaena* with polyploid series up to 8x, it showed 4.05-fold range in 1C value (Via do Pico & Dematteis 2013). On the other hand, *Vernonia* with ploidy levels reported up 4x (Vega & Dematteis 2012), ranges 3.29-fold in 1C value, whereas in *Vernonanthura,* all species were diploid and they show a 1C genome size variation of 1.64-fold. However, if we consider the monoploid genome size (1Cx), the variation range in Vernonieae is lowest than the observed in 1C values (*Lessingianthus* 1.43-fold; *Chrysolaena* 2.11-fold, *Vernonia* 1.67-fold, *Vernonanthura* 1.64-fold).

Species with large genomes are restricted to the more derived families, and phylogenetic reconstructions indicate that a very small genome size was the ancestral condition for most major Angiosperm clades (Leitch et al. 1998; Soltis et al. 2003). Although evolution of genome size in Angiosperms is dynamic, undergoing both increases and decreases (Bennett & Leitch 2005), most of the *Vernonanthura* species have small genomes (1C < 1.4 > 3.5), with the exception of *V. chaquensis* and *V. petiolaris*, which presented very small genomes (both species are discussed below). These results corroborate the information recorded for diploid species of *Chrysolaena* (Via do Pico & Dematteis 2013). However, the diploid taxa of *Vernonia* have very small genomes (1C < 1.4), except for the tetraploid cytotype of *V. echiodes* (2.64 pg). These results are similar to those found for the diploid species of *Lessingianthus* (Angulo & Dematteis 2013). In the phylogenetic analysis by Keeley et al. (2007), *Vernonia* and *Vernonanthura* are reported as sister groups. However, *Vernonia* is phylogenetically distant from *Lessingianthus* like *Vernonanthura* of *Chrysolaena*. Due to the similarity in the amount of DNA among these phylogenetically distant groups, we can assume that this character was acquired independently in these phylogenetically distant genera.

The two South American species of *Vernonia* with herbaceous habitats have lower DNA (very small) content than *Vernonanthura* species (small) with woody habitats. Based on this result and the phylogeny of the Vernonieae tribe (Keeley et al. 2007), we can conclude that low DNA content and herbaceous habit in *Vernonia* are characters derived from the higher DNA content and woody habit present in Vernonanthura.

One of two species of *Vernonanthura* with a very small genome is *V. chaquensis*, which had 1C = 1.25 pg. This specimen was described by Cabrera (1944) and presents a chromosome number of $2n = 2x = 34$ (Vega & Dematteis in press). Cabrera (1944), who described the species, considered this taxon a possible hybrid of *V. chamaedrys* and *V. incana*. The results obtained here could effectively provide indications of a possible hybrid origin (*V. chamaedrys* × *V. incana*); because the sum of the 1C values of *V. incana* (0.79 pg) and *V. chamaedrys* (1.56 pg) is 2C 2.35 pg, very close to the 2C value for *V. chaquensis* (2C = 2.51 pg). This could indicate the occurrence of an intergeneric hybrid, a phenomenon previously reported in species of both genera in Mexico that grow in sympatry (Jones 1976). However, to confirm the hybrid origin of this taxon, additional analysis should be performed.

The other species with very small genome is *Vernonanthura petiolaris*, which is the single species included in this study for which the chromosome number is unknown. Most of the species analyzed here are diploid with chromosome number $2n = 2x = 34$. The DNA amount of this species is 1C = 1.37, which suggests that it is diploid with $2n = 34$ because the genome size is within the range of variation for diploid *Vernonanthura* species (1.25–.07 pg).

Early studies of genome size established the concept of DNA constancy within a species, given a basic chromosome number. This was supported by several early studies which found no significant difference in 1C values between cultivars of several crops (Bennett & Smith 1976) and their wild relatives (Furuta et al. 1978). Prior to the 1980s, the genomes of Angiosperm species were thought to have strong DNA constancy, with only rare exceptions (Bennett & Leitch 1995). However, in the mid 1980s, Price et al. (1981a) found variation of more than 20% in the 1C values of two *Microseris* D. Don. species. Subsequently, Bennett (1985) listed 24 angiosperm species in which intraspecific variation was between 4 and 288%. In *Artemisia*, one of the most studied genera, the somatic chromosome number is $2n = 18$ and the rank of variation within *A. arborescens* L is up to 8.8% in wild populations. In the populations of *A. absinthium* L. analyzed, intraspecific variation is even lower (6%). On the other hand, some endemic species of the *Trinadentatae* subgenus show small differences in the majority of species (most ranging from 1 to 2%), with the notable exceptions of *A. pygmaea* A. Gray (5.98%) and *A. tridentata* Nutt. ssp. *spiciformis* (Osterh) Kartesz & Gandhi (10.02%), (Torrell & Vallès 2001; Garcia et al. 2004, 2006, 2008).

This study is the first record of intraspecific variation in the Vernonieae tribe. The intraspecific variation in *V. chamaedrys* and *V. brasiliana* was around 6%. In both taxa, the populations distributed in

higher altitudes had the highest DNA content. In a general sense, the extent of intraspecific genome size variation is controversial, as the C value is thought to be constant for a given species, and some authors have successfully attributed meaningful intraspecific differences to methodological errors and, in some cases, taxa misidentification (Greilhuber 1998, 2005; Ohri 1998). However, Dolezel and Bartoš (2005) stated that differences of 5% should be considered acceptable in some groups and, consequently, the low intraspecific variation in both *V. chamaedrys* and *V. brasiliana* supports the constancy of the 1C-value of both species.

In Vernonieae, increases in 1C-value in *Chrysolaena* and *Lessingianthus* species are related to increases in ploidy level, and the 1Cx value in most *Lessingianthus* species and *C. cognata* (Less.) Dematt. decreases as ploidy level increases (Angulo & Dematteis 2013; Via do Pico & Dematteis 2013). This genome downsizing after polyploidization appears to be a general trend in Angiosperms (Kellogg & Bennetzen 2004; Leitch & Bennett 2004), though the tetraploid cytotype of *V. echioides* had higher 1Cx-values than the diploid. These results corroborate the findings of Via do Pico and Dematteis (2013) in diploid and tetraploid populations of *Chrysolaena flexuosa*. Several factors may generate this increase in genome size. Bennetzen and Kellogg (1997) suggest the possibility of "genome obesity" in plants. This increase in size could follow the need for mechanisms to remove superfluous DNA. An alternative explanation is that polyploidization in these species may have occurred relatively recently and there has not yet been time for substantial reductions in nuclear DNA content. Alternatively, mechanisms causing major reductions in other polyploid plants may be inactive or have curtailed activity in these species (Jakob et al. 2004).

Many plant species show striking variations in genome size, which was previously thought to occur only between species (Ohri 1998). It is increasingly accepted that changes in genome size may not be restricted to species divergence but may also be associated with various environmental conditions and developmental stages affecting different populations or individual plants. To confirm this theory, several researchers have correlated DNA content with altitude and latitude, abiotic selection pressures that act on genome size (Knight et al. 2005). A meta-analysis of multiple studies relating altitude and DNA content revealed that nine interpopulation and interspecific analyses showed a positive correlation, eight negative correlation, and six found no correlation (Knight et al. 2005). In *Zea mays* L., Rayburn (1990) has found 23 positive and negative correlations when comparing the altitude and genome size. He suggests that species with large genomes are at intermediate elevations and species with small genomes tend to occur in the lowest and highest elevations. On the other hand, the results of most studies of genome size with respect to latitude are similar to those obtained with altitude: Five studies found a positive correlation, seven found a negative correlation, and five found a nonsignificant correlation (Knight et al. 2005). The results observed in *Vernonanthura* species showed a highly significant correlation between 1C-value and latitude ($r(s) = -0.69$; $n = 16$; $p =< 0.01$).

Environmental factors that covary with altitude or latitude may present selective pressures on nuclear DNA content, but have been incorporated in few studies. For example, Wakamiya et al. (1993) reported a positive correlation between 1C-value and local measurements of annual precipitation and a negative correlation between maximum temperature and 1C value. Price et al. (1981b) reported a positive correlation between annual precipitation and 2C DNA, and MacGillivray and Grime (1995) found that frost-resistant species tended to have greater 2C DNA content. The DNA content and bioclimatic variables considered were not significantly. This non-correlation could be explained by (1) sample sizes that are small for these analyses, or (2) bioclimatic factors that do not exert selective pressure on *Vernonanthura*, because all *Vernonanthura* species have small and very small DNA content, which are found in widely varying habitats.

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