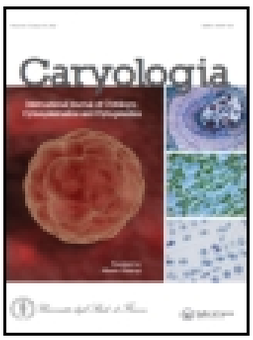

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Cytotaxonomy of some species of *Vernonanthura* and *Vernonia* (Asteraceae, Vernonieae) from South America

A.J. Vega[a] and M. Dematteis[a,b]

[a]Instituto de Botánica del Nordeste (UNNE-CONICET), Corrientes, Argentina; [b]Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Corrientes, Argentina

ABSTRACT

The number and behavior of meiotic chromosomes in four populations of three species of *Vernonanthura* were analyzed. It was observed that all stages of meiosis were stable without chromosomal irregularities. The somatic chromosome number is reported for four populations of three species of *Vernonanthura* and two populations of *Vernonia*. All species were diploid with $2n = 34$. The first chromosome count is presented for *Vernonanthura chaquensis* ($2n = 17II$ and $2n = 34$). The karyotypes were determined for *Vernonanthura lucida* (32m + 2sm), *V. oligactoides* (24m + 10sm) and *V. pseudolinearifolia* (28m + 6sm); they are presented for the first time. The total length of the karyotype was 31.67µ–40.48 µm. The average chromosome length was 1.86µ–2.38 µm and the centromeric index was 38.61–42.99. *Vernonanthura oligactoides* showed the smallest variation of chromosome length within the karyotype (1.42 µm) while *V. squamulosa* had the biggest (2.15 µm). The different karyotypic parameters were analyzed with two statistic tests. The resulting species arrangement from Unweighted Pair Group Method with Arithmetic Mean (UPGMA) grouping analysis fully fits with that obtained with principal component analysis (PCA). Both tests demonstrated congruence between karyotypic parameter and classification based on morphological characters.

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Vernonanthura; *Vernonia*; karyotypes; mitosis; meiosis

Introduction

The Vernonieae Cass. (Asteraceae) are a pantropical tribe of 89 genera which are concentrated around two major centers of diversification, the central region of Africa and southern Brazil. Traditional classification grouped the members of the tribe into six different subtribes based on inflorescence patterns, persistence of phyllaries, pollen morphology, chemical composition and chromosome numbers (Bremer 1994). The subtribe Vernoniinae Less constitutes the largest group within the Vernonieae, including approximately 1100 species. This group comprises almost all the species previously placed into the huge genus *Vernonia* Schreb.

Morphological and molecular phylogenetic studies had led to the recognition of 21 subtribes, 15 in the New World and six in the Old World. The new classification proposed significant taxonomic changes, resulting in the reduction in the size and distribution of the core genus *Vernonia* and increment of the new genera. Now, there are 125 genera, 50 of them are monotypic and 35 with fewer than three species (Keeley and Robinson 2009). Chromosome numbers have not been utilized in the proposed classification and less than 20% of the species from the New World have been analyzed (Dematteis 2002). However, the cytological data from South America Vernonieae show that chromosomes are widely variable in numbers and morphology, which suggest that can be useful in the taxonomic and evolutionary studies (Ruas et al. 1991; Dematteis and Fernández 1998, 2000; Angulo and Dematteis 2009a; Via do Pico and Dematteis 2012).

The genus *Vernonanthura* H. Rob. was established to separate the early taxa arranged under *Vernonia* sect. *Lepidaploa Paniculatae* Benth. and Hook., which consists of about 80 species distributed from southern Mexico to the central Argentina, but mostly concentrated in southeastern Brazil (Robinson 1992, 1995; Vega and Dematteis 2011a). This genus is closely related to *Vernonia* s. str. because they both show the same type of pollen and basic chromosome number (Vega and Dematteis 2011b, 2012), but differ in the inflorescence type, habitat and sometimes by the presence of tailed anther bases (Robinson 1992). The members of the new genus are shrubs or small trees having thyrsoid to pyramidal inflorescences, with individual branches cymose to corymbiform (Robinson 1992). In southern South America, the species are mainly concentrated in the mountains of northwest Argentina and the fields and forests of Paraguay and eastern Argentina (Cristóbal and Dematteis 2003; Vega and Dematteis 2008).

Almost all the cytological studies of *Vernonanthur*a species were carried out under the old concept of

CONTACT A.J. Vega alvarojose_82@yahoo.com.ar

Vernonia (Hunter 1964; Jones 1979, 1982; Galiano and Hunziker 1987; Stutts 1988; Ruas et al. 1991; Dematteis 1996, 1997, 2002; Dematteis and Fernández 1998; Salles de Melo et al. 2010; Oliveira et al. 2007). The single analysis after the genus segregation reported chromosome numbers of 15 populations belonging to 10 taxa of *Vernonanthura* (Vega and Dematteis 2012). In this study, first chromosome counts were presented for three taxa: *V. lucida* (n = 17II, $2n$ = 34); *V. oligactoides* (n = 17II, $2n$ = 34) and *V. pseudolinearifolia* ($2n$ = 34). The genus *Vernonanthura* seems to be cytologically homogeneous with basic number x = 17 and almost all diploid taxa with $2n$ = 34.

The genus *Vernonia* sens. str. has only two South American species, *V. incana*, which is always diploid with $2n = 2x = 34$, and *V. echioides* with diploid and tetraploid cytotypes (Vega and Dematteis 2012).

The karyotype of seven species of *Vernonia* sensu lato were analyzed by Dematteis and Fernández (1998), six of them now included in *Vernonanthura.* These taxa show rather similar chromosomal features, although some differences in karyotype formula, chromosome size, and symmetry.

The chromosome number of about 30% of the species of *Vernonanthura* are known and the karyotypes of about 10% of these were analyzed (Hunter 1964; Jones 1974, 1979, 1982; Stutts 1988; Galiano and Hunziker 1987; Dematteis 1996, 1997, 2002; Dematteis and Fernández 1998; Carr et al. 1999; v2007; Salles de Melo et al. 2010; Vega and Dematteis 2012).

The purpose of this study was to determine the chromosome number and karyotype formula of some species of *Vernonia* and *Vernonanthura*. The cytological data are discussed in relation to the taxonomy and the chromosome evolution of both genera.

Material and methods

The populations studied were collected in the field. Voucher specimens are deposited in the herbarium of Instituto de Botánica del Nordeste (CTES). The sources of examined species and somatic numbers are presented in Table 1.

The meiotic analyses were carried out from flower buds previously fixed in ethanol and lactic acid in the proportion of 5:1; they were then stored in ethanol 70° at 4°C. Squashes of pollen mother cells were made using acetocarmine at 3%. Mitotic chromosome preparations were obtained from root-tips of germinating seeds. The material was pre-treated with 8-hydroxiquinoline 0.002 M for 4 h and fixed in ethanol:acetic acid (3:1); then for microscope observations it was stained following the Feulgen technique.

Nomenclature used for the karyotype description was that suggested by Levan et al. (1964). The chromosome morphology was determined using the centromeric

Table 1. Specimens examined, meiotic number (n) and mitotic number ($2n$) observed in a population of *Vernonanthura* and two populations of *Vernonia incana.*

Species	n	$2n$	Location, voucher	Reference*
Vernonanthura H. Rob.				
V. brasiliana (L.) H. Rob.		34	Paraguay. Camino de Ypacarai a Luque. *Vega 67* (CTES)	1
V. chamaedrys (Less.) H. Rob.		34	Argentina. Corrientes. Departament Ituzaingó. National Route 12, 3 km NE from junction with Provincial Route 120. *Vega & Gomez Lutz 35* (CTES).	1
V. chamaedrys (Less.) H. Rob.		34	Argentina. Corrientes. Departament Ituzaingó. 53 km N from Galarza, way to Ituzaingó. *Dematteis et al. 4270.*(CTES).	1
V. chaquensis (Less.) H. Rob.		34	Argentina. Corrientes. Departament Santo Tomé. 40 km N from Santo Tomé, way to Galarza. *Dematteis et al. 4274.* (CTES).	1
V. loretensis (Hieron.) H. Rob.		34	Argentina. Misiones. Departament San Ignacio. House of Horacio Quiroga. *Dematteis et al. 3046* (CTES).	2
V. lucida (Less.) H. Rob.		34	Argentina. Misiones. Departament San Pedro. Provincial park Moconá. Pier, river coast. *Dematteis et al. 3095* (CTES, G, MBM).	2
V. nudiflora (Less.) H. Rob.		34	Uruguay. Departament Rivera. Tranqueras, high fields near to A° Sauzal, on Route 30. *Dematteis et al. 3721* (CTES).	2
V. nudiflora (Less.) H. Rob.	17		Uruguay. Departament Rivera. 5 km N from Valley of Lunarejo. *Dematteis et al. 4145* (CTES).	1
V. nudiflora (Less.) H. Rob.	17		Argentina. Corrientes. Departament Santo Tomé. 40 km N from Santo Tomé, way to Galarza. *Dematteis et al. 4275* (CTES).	1
V. oligactoides (Less.) H. Rob.		34	Argentina. Misiones. Departament General Manuel Belgrano. Campina of Américo. *Dematteis et al*. 3077 (CTES).	2
V. pseudolinearifolia (Hieron.) A. J. Vega & Dematt.		34	Uruguay. Departament Tacuarembó. Gruta de los Cuervos, ca. 20 km NW from Tacuarembó. *Dematteis et al. 3784*. (CTES, CORD).	2
V. squamulosa (Hook. & Arn.) H. Rob.		34	Argentina. Jujuy. Departament El Carmen. Pozo Verde. *Sato* 25 (CTES).	2
V. tweedieana (Baker) H. Rob.		34	Paraguay. Departament Canindeyú. Ñanderakoi, cerrado degraded, sandy soil. *Dematteis et al. 2854*. (CTES, FCQ, G)	2
V. tweedieana (Baker) H. Rob.	17		Argentina. Corrientes. Departament Santo Tomé. 8 km N from Santo Tomé, way to Galarza. *Dematteis et al. 4277* (CTES).	1
Vernonia Schreb				
V. incana Less.		34	Argentina. Corrientes. Departament Capital. Laguna Brava, way to Puente Pexoa. *Vega 37* (CTES).	1
V. incana Less.		34	Argentina. Formosa Departament Pilcomayo. 20 km S from Clorinda. *Dematteis & Vega 4294* (CTES).	1

*1 = present study; 2 = Vega and Dematteis (2012)

index (CI = short arm × 100/total chromosomal length). The chromosomes were classified as metacentric (m): 50–37.5 or submetacentric (sm): 35.5–25. The ideograms and chromosome measures were estimated from 10 metaphases plates of 7–10 individuals per species.

The karyotypes of *V. loretensis* (2*n* = 34), *V. lucida* (2*n* = 34), *V. nudiflora* (2*n* = 34), *V. oligactoides* (2*n* = 34), *V. pseudolinearifolia* (2*n* = 34), *V. squamulosa* (2*n* = 34), and *V. tweediana* (2*n* = 34) were carried out from a population analyzed by Vega and Dematteis (2012).

The cluster analysis of karyotypic data was carried out in order to examine karyotype similarity among the species. A data matrix of eight OTUs (operational taxonomic units) and 10 variables was constructed. The variables were: total length of karyotype (TKL), the mean chromosome length (ML), size of the smallest (S) and size of the longest (L) chromosomes, the average CI and the ratio between the longest and shortest chromosome pair (R), and the number of m and sm chromosomes. The karyotype asymmetry has been determinate using intrachromosomal (A_1) and interchromosomal (A_2) index as suggested by Romero Zarco (1986).

To calculate the average taxonomic distance Euclidia coefficent was used and to generate clustering method UPGMA was performed. Additionally, principal component analysis (PCA) was used to evaluate the contribution of each karyotypic parameter to the ordination of species.

Results

The number and behavior of meiotic chromosomes were determined for two populations of *V. nudiflora* (2*n* = 17II) and one population of *V. chaquensis* (2*n* = 17II) and *V. tweediana* (2*n* = 17II). The somatic chromosome numbers are reported for *Vernonanthura brasiliana* (2*n* = 34), *V. chamaedrys* (2*n* = 34), *V. chaquensis* (2*n* = 34) and *Vernonia incana* (2*n* = 34). The first chromosome count is presented for *V. chaquensis* (2*n* = 17II, 2*n* = 34). Figure 1 shows photographs of the somatic and meiotic chromosomes.

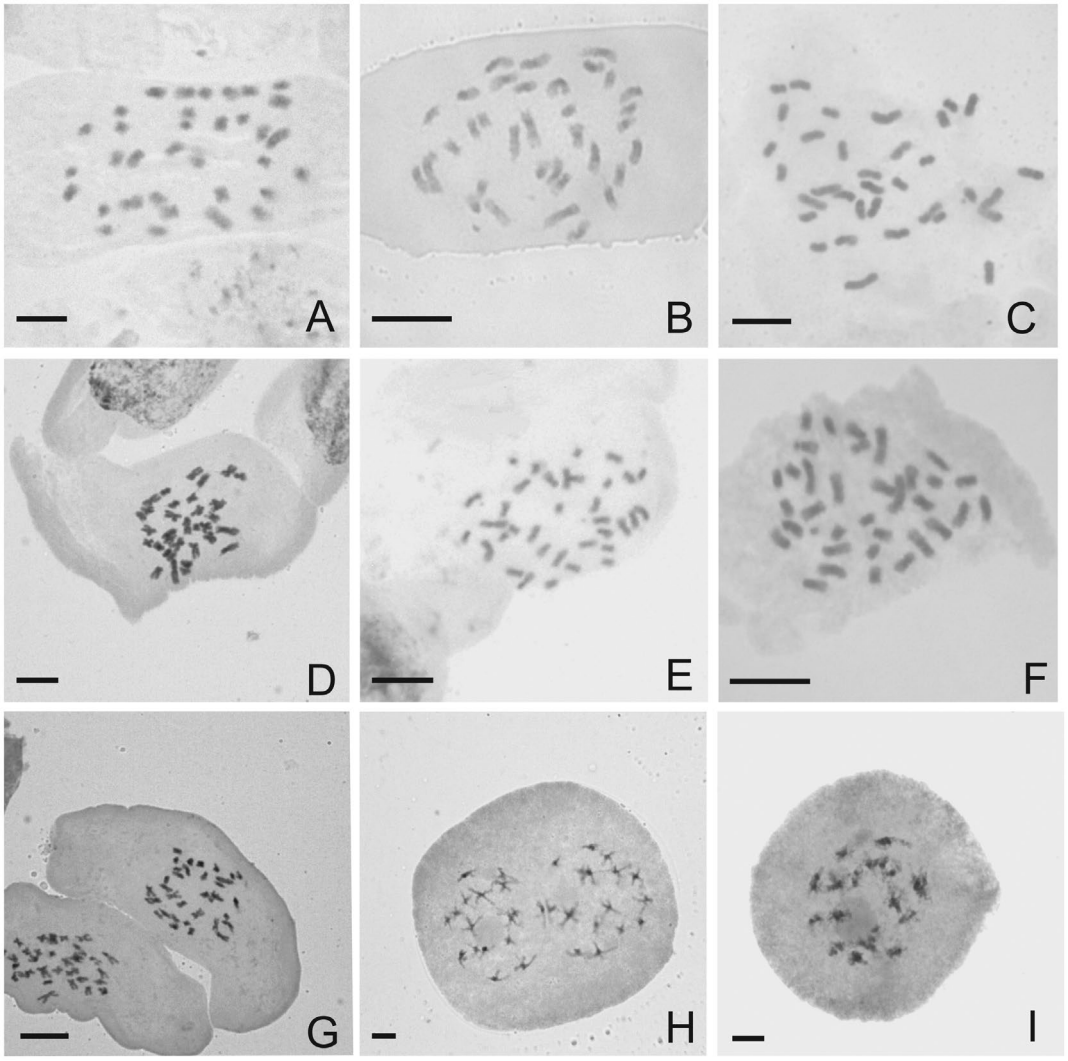

Figure 1. (A–G) Somatic chromosomes of *Vernonanthura* and *Vernonia incana*; (H–I) meiotic chromosomes of *Vernonanthura*. (A) *V. brasiliana*, 2*n* = 2*x* = 34; (B) *V. chaquensis*, 2*n* = 2*x* = 34; (C) *V. nudiflora*, 2*n* = 2*x* = 34; (D) *V. chamaedrys*, 2*n* = 2*x* = 34; (E) *V. squamulosa*, 2*n* = 34; (F) *V. incana*, 2*n* = 34; (G) *V. pseudolinearifolia*, 2*n* = 34; (H) P II of *V. chaquensis* with 17I-17I chromosome; (I) diakinesis of *V. tweediana* with 17 II.

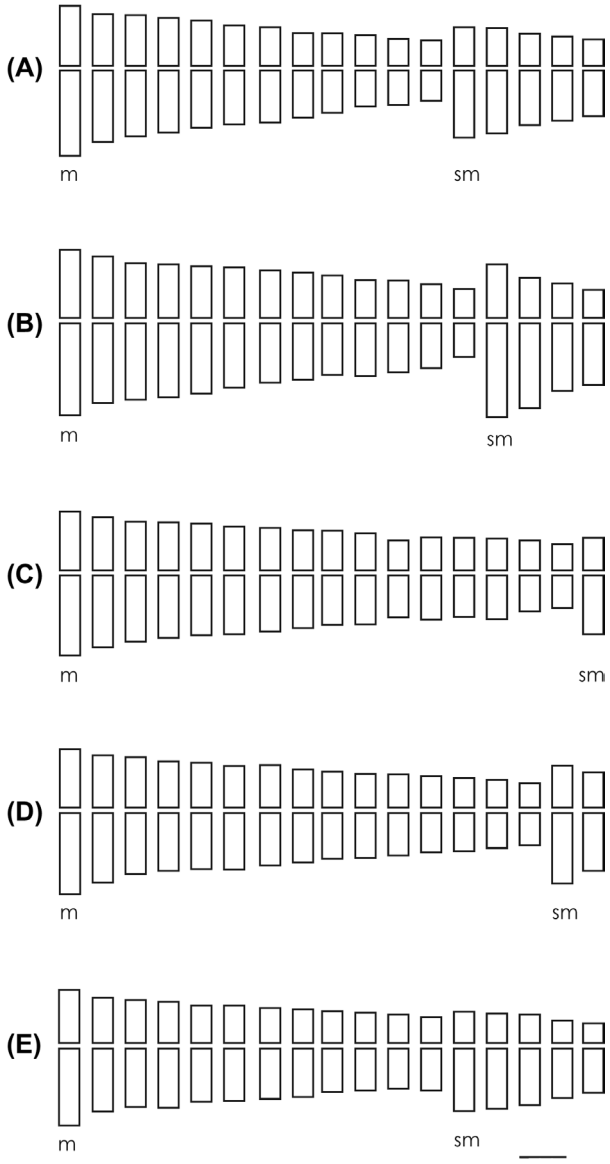

Figure 2. Idiograms of *Vernonanthura* species. (A) *V. chamaedrys*, 24m + 10sm; (B) *V. loretensis*, 26m + 8sm; (C) *V. lucida*, 32m + 2sm; (D) *V. nudiflora*, 30m + 4sm; (E) *V. oligactoides*, 24m + 10sm. Scale 1 µm.

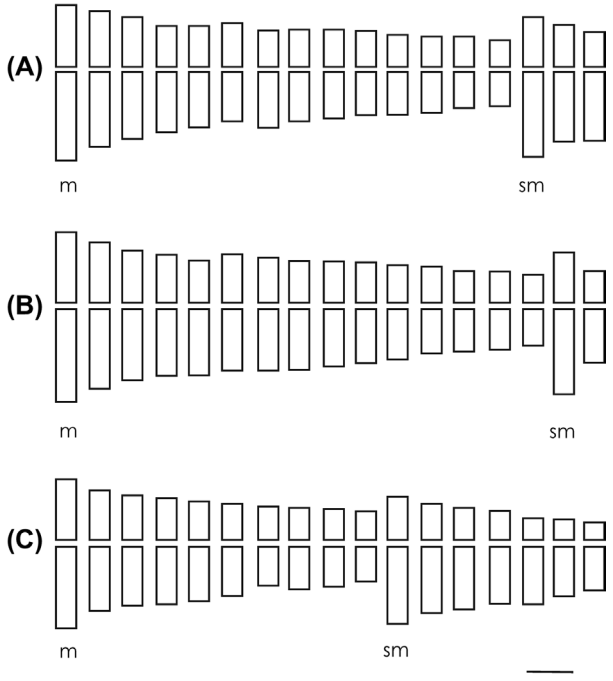

Figure 3. Idiograms of *Vernonanthura* species. (A) *V. pesudolinearifolia*, 28m + 6sm; (B) *V. squamulosa*, 30m + 4sm; (C) *V. tweediana*, 20m + 14sm. Scale 1 µm.

The karyotypes were determined for *Vernonanthura chamaedrys* (20m + 14sm), *V. loretensis* (26m + 8sm), *V. lucida* (32m + 2sm), *V. nudiflora* (30m + 4sm), *V. oligactoides* (24m + 10sm), *V. pseudolinearifolia* (28m + 6sm), *V. squamulosa* (30m + 4sm) and *V. tweediana* (20m + 14sm). For *V. lucida* (32m + 2sm), *V. oligactoides* (24m + 10sm) and *V. pseudolinearifolia* (28m + 6sm) they are presented for the first time. The ideograms of different species are shown in Figures 2 and 3.

All stages of meiosis were stable without chromosomal irregularities in two population of *V. nudiflora* and *V. chaquensis* while *V. tweediana* presented 0.62% of lagging chromosomes in anaphase II, 0.30% of micronucleus in telophase I and 1.78% of bridges in anaphase I.

The karyotype formula, total length of karyotype, average chromosome length, range of chromosome length, centromeric index and asymmetry indexes of each species are given in Table 2. In all analyzed species, the karyotypes are composed of m and sm chromosomes.

Total length of karyotype ranged from 31.67 µm to 40.48 µm, while the average chromosome length varied from 1.86 µm to 2.38 µm and the centromeric index varied from 38.61 to 42.99. *Vernonanthura oligactoides* had smaller variation of chromosome length within karyotype (1.42 µm), while in *V. squamulosa* it was larger (2.15 µm).

All taxa showed are relatively symmetrical in karyotype; A_1 ranged from 0.2437 in *V. nudiflora* to 0.3593 in *V. oligactoides* and A_2 varied from 0.1944 in *V. oligactoides* to 2.578 in *V. pseudolinearifolia*. The A_1 and A_2 indexes are plotted in a scatter diagram (Figure 4).

The UPGMA shows three clusters (Figure 5). Cluster A, composed of *V. nudiflora* and *V. lucida*, was characterized by the highest CI and the lowest A_1. Cluster B was formed by *V. squamulosa*, *V. pseudolinearifolia* and *V. loretensis*, the first two of which are grouped by ML, average CI and A_1 and A_2 indexes; within this cluster *V. loretensis* was separated by the greater phenetic distance. Cluster C was composed of *V. chamaedrys*, *V. tweediana* and *V. oligactoides*, which are characterized by a lower proportion of m chromosomes, higher proportion of sm chromosomes and a higher A_1. The PCA of the karyotypic parameters shows that the first two principal components account for the 77% of the total variation; these are projected in a two-dimensional graphic (Figure 6). Component one (61%) emphasizes R, S, ML and TKL, while component two (26%) accentuates variation in CI, proportions of m and sm chromosomes, and A_1.

Table 2. Karyotype formula, total karyotype length (TKL), mean chromosome length (ML), average centromeric index (CI), and asymmetry indexes (A_1 and A_2) of eight species of *Vernonanthura*.

Species	Karyotype formula	TKL	ML	Range	CI	A_1	A_2
V. chamaedrys	20m+14sm	33.83 ± 0.12	1.99	1.21–3.12	41.64 ± 0.79	0.28	0.25
V. loretensis	26m+8sm	40.48 ± 0.13	2.38	1.39–3.43	40.94 ± 1.12	0.29	0.23
V. lucida	32m+2sm	33.94 ± 0.11	1.99	1.26–2.99	42.99 ± 0.49	0.24	0.23
V. nudiflora	30m+4sm	33.53 ± 0.11	1.97	1.35–2.99	42.94 ± 0.56	0.24	0.24
V. oligactoides	24m+10sm	31.67 ± 0.08	1.86	1.37–2.79	38.89 ± 1.04	0.35	0.19
V. pseudolinearifolia	28m+6sm	35.85 ± 0.13	2.10	1.32–3,24	42.06 ± 0.72	0.27	0.25
V. squamulosa	30m+4sm	37.99 ± 0.13	2.23	1.37–3.52	42.12 ± 0.57	0.27	0.25
V. tweediana	20m+14sm	33.23 ± 0.11	1.95	1.32–3.07	38.61 ± 1.45	0.35	0.23

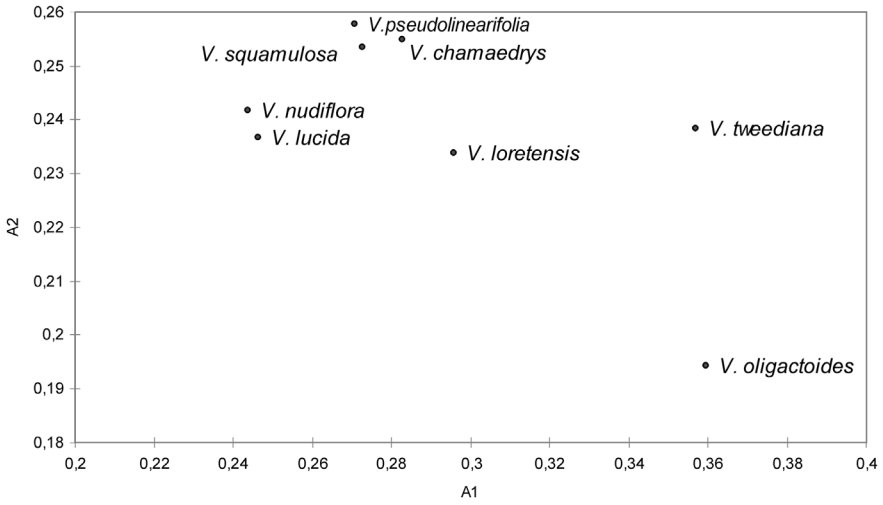

Figure 4. Dispersion diagram representing the relations between the A1 and A2 karyotype asymmetry indexes.

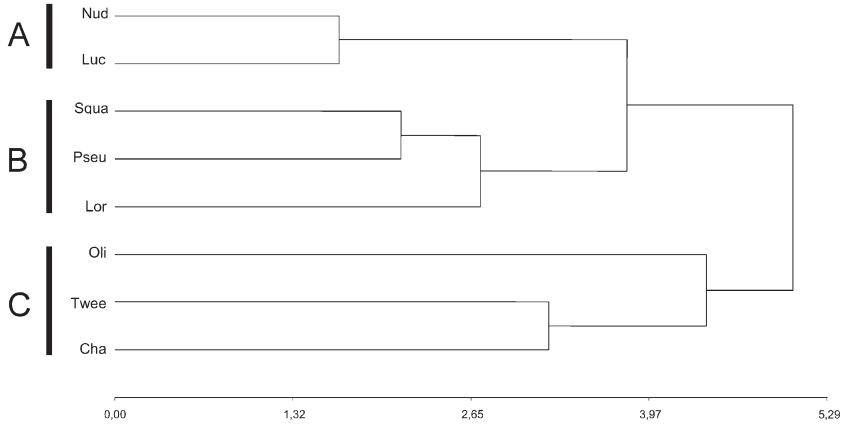

Figure 5. Dendrogram showing the phenetic relationships among the studied species of. *Vernonanthura*, constructed using the matrix of karyotype similarities with UPGMA. Cophenetic correlation coefficient r = 0.757. Abbreviations: Nud, *V. nudiflora*; Luc, *V. lucida*; Squa, *V. squamulosa*; Pseu, *V. pseudolinearifolia*; Lor, *V. loretensis*; Oli, *V. oligactoides*; Twee, *V. tweediana*; Cha, *V. chamaedrys*.

Discussion

All the analyzed species were diploid with basic number $x = 17$, which agrees with previous records (Hunter 1964; Jones 1974, 1979, 1982; Galiano and Hunziker 1987; Stutts 1988; Dematteis 1996, 1997, 2002; Dematteis and Fernández 1998; Carr et al. 1999; Watanabe et al. 2007; Salles de Melo et al. 2010; Vega and Dematteis 2012). The genus *Vernonanthura* has the basic number $x = 17$ and almost all its species are diploid with $2n = 34$, excepting *V. pinguis* (Griseb.) H. Rob., which is tetraploid with $2n = 68$ (Dematteis and Fernández 1998; Dematteis 2002).

Jones (1979) proposed that all the New World Vernonieae have the basic chromosome number $x = 17$. However more recent studies (Ruas et al. 1991; Dematteis and Fernández 1998, 2000; Angulo and Dematteis 2012), indicate that $x = 16$ is the more frequent basic number in South America. Most species with $x = 16$ belong to *Lessingianthus* H. Rob., one of largest genera of Vernonieae in the New World, with more than 130

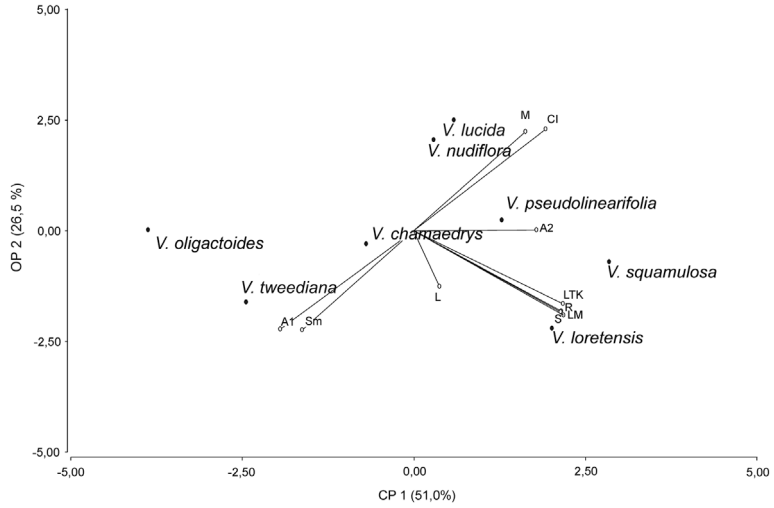

Figure 6. Diagram of principal component analysis of *Vernonanthura* species. Abbreviations: total length of karyotype (TKL), mean chromosome length (ML), size of the smallest (S) and size longest (L) chromosomes, average centromeric index (CI) and ratio between the longest and shortest chromosome pair (R), number of metacentric (m) and submetacentric chromosome (sm).

taxa widely distributed in southeastern Brazil, Paraguay, Uruguay, Bolivia and Argentina (Robinson 1999). It is cytologically characterized by the basic number $x = 16$ and possesses the greatest proportion of polyploid entities (Dematteis 2002).

Chromosome numbers are useful to distinguish the different South American genera of the tribe Vernonieae. Species belonging to *Chrysolaena* presents basic number $x = 10$, a base commonly found in Old World members of the tribe (Dematteis 2009). *Lessingianthus* presents a basic number $x = 16$, while *Vernonanthura* present the basic number $x = 17$. *Lepidaploa* is the only heterogeneous group showing four basic numbers, $x = 14, 15, 16$ and 17 (Dematteis 2002).

Vernonanthura is a cytologically homogeneous genus; almost all species are diploid with $2n = 34$, excepting *V. pinguis* which is the only tetraploid taxon with $2n = 68$ (Dematteis and Fernández 1998; Dematteis 2002). *Vernonia* sen. str. has only two South American species, *V. incana* is diploid with $2n = 34$ (Dematteis and Fernández 1998; Dematteis 2002), while *V. echioides* has two cytotypes, one diploid with $2n = 34$ and the other tetraploid with $2n = 68$ (Vega and Dematteis 2012). The low variation in ploidy levels seems to be characteristic of these two genera because other South American members of Vernonieae are highly variable. In *Chrysolaena* there are species such as *C. platensis* (Spreng.) H. Rob. with cytotypes $2x$, $4x$, $6x$, $8x$; *C. flexuosa* (Sims) H. Rob. has 2x, 4x while that *C. cognata* (Less.) Dematt. has $2x$, $4x$, $5x$, $6x$ and $8x$ (Dematteis 2002; Via do Pico and Dematteis 2012). In *Lessingianthus* it has been observed that *L. rubricaulis* and L. *sellowii* have $2x$ and $4x$ cytotypes (Angulo and Dematteis 2012).

Another type of numerical variation of chromosomes is the occurrence of accessory or B chromosomes, which are relatively frequent in the Asteraceae family (Jones 1995), including in species of *Chrysolaena* (Via do Pico and Dematteis 2012), *Lessingianthus* (Angulo and Dematteis 2009a, 2012), *Mikania* (Ruas et al. 2000), *Crepis* (Jamilena et al. 1994), and *Haplopappus* (Jackson and Newmark 1960). So far, the presence of these accessory chromosomes has not been documented in *Vernonanthura* and *Vernonia* (Stutts 1988; Dematteis and Fernández 1998; Dematteis 2002).

In Asteraceae dysploidy occurs in 214 genera, 21.9% of the 978 genera with counts reported (Semple and Watanabe 2009) and probably played an important role in the speciation mechanism of Vernonieae (Stebbins 1971; Dematteis 1996, 1998, 2002; Mansanares et al. 2002). This process was shown by Oliveira et al. (2007) in the genus *Vernonanthura*, where *V. polyanthes* present populations $2n = 32$, $2n = 34$ and $2n = 36$ in specimens from Brazil (Coleman 1968; Ruas et al. 1991; Oliveira et al. 2007).

According to Guerra (1988) dysploidy and polyploidy are types of numerical chromosome variations that reflect phylogeny and karyotype evolution in plants. On the other hand variations such as aneuploidy and the presence of B chromosomes have clear implications beyond the species level. In *Vernonia* and *Vernonanthura* there are few records of these phenomena.

One of the most studied species of the genus has been *Vernonanthura nudiflora* and almost all the reports indicate $2n = 34$ (Jones 1974; Bernardello 1986; Stutts 1988; Ruas et al. 1991; Dematteis and Fernández 1998; Angulo and Dematteis 2009a; Vega and Dematteis 2012). However, these results disagree with a prior analysis carried out by Covas and Hunziker (1954) on a population of this species from western Argentina that recorded $n = 16II$ ($2n = 32$). Although this chromosome number report could be attributed to an incorrect species identification, it is most likely due to the small size of species chromosomes, which in some cases contributes to the disparity of reported chromosome numbers (Guerra

1988) or to the occurrence of dysploidy in *V. polyanthes* (Oliveira et al. 2007).

Chromosomes of *Vernonanthura* species in general show a small size according to the classification suggested by Lima de Faria (1980), with an average of 2.05 µm, which is consistent with previous studies (Ruas et al. 1991; Dematteis and Fernández 1998; Oliveira et al. 2007). The chromosome size is a feature that distinguishes genera and species of South American Vernonieae. *Chrysolaena* has an average size of chromosomes that varies between 2.4 and 2.5 µm (Dematteis 1997, 2009), while *Lessingianthus*, a related genus, has an average size of 1.75 µm (Angulo and Dematteis 2009a, 2009b). It can thus be assumed that during the evolution of the South American Vernonieae changes may have occurred in the chromosome size. These variations may be due to reciprocal translocations, thereby resulting in a decrease in the size of the chromosomes (Metel et al. 2004). The karyological information available indicates that the most primitive species ($x = 10$) have comparatively larger chromosomes than more advanced taxa (Ruas et al. 1991; Dematteis 1997; Dematteis and Fernández 1998). Species with base number $x = 17$ have an average size of 1.9–2.0 µm (Dematteis and Fernández,1998), while species with $x = 14$ and $x = 15$ show an average length of 1.3–1.4 µm (Dematteis 1996; Dematteis and Fernández 1998).

All the analyzed species showed similar chromosomal features, although some differences in karyotype formula, chromosome size and karyotype asymmetry can be observed. The interchromosomal and intrachromosomal asymmetry index showed some differences among all species, except *Vernonanthura chamaedrys*, *V. pesudolinearifolia* and *V. squamulosa*. These species have similar asymmetry index, but they present different karyotype formulae (*V. chamaedrys*, 24m + 10sm; *V. pseudolinearifolia*, 28m + 6sm and *V. squamulosa*, 30m + 4sm). Species with lower values of A_1 (*V. lucida*; *V. nudiflora*; *V. pseudolinearifolia* and *V. squamulosa*) tend to have a greater number of metacentric chromosomes. In regard to A_2, all species have low values, suggesting that there is little variation in the size of the chromosomes within each species.

Vernonanthura chamaedrys and *V. tweediana* have the same karyotype formula (20m + 24sm) and similar total karyotype length (*V. chamaedrys*, 33.83 µm and *V. tweediana*, 33.23 µm) and average chromosome length (*V. chamaedrys*, 1.99 µm and *V. tweediana*, 1.95 µm). However, they are different in chromosome size (*V. chamaedrys*, 1.91 µm and *V. tweediana*, 1.75 µm), centromeric index (*V. chamaedrys*, 41.64 µm and *V. tweediana*, 38.61 µm) and intrachromosomal index (*V. chamaedrys*, 0.2826 and *V. tweediana*, 0.3568). Both species are easily recognized in the field because *V. tweediana* is a shrub around 220 cm tall with lanceolate leaves, while *V. chamaedrys* is a subshrub around 80–150 cm with ovate-oblong leaves.

The same karyotype formula was observed in *Vernonanthura nudiflora* and *V. squamulosa* (30m + 4sm), but they differ in other karyotype characteristics: *V. squamulosa* is longer in the total length of karyotype (37.99 µm) than *V. nudiflora* (33.53 µm), and consequently *V. squamulosa* has the longest average chromosome length. Additionally, they are easily recognized by their morphology and geographical distribution. *Vernonanthura nudiflora* has linear leaves, pedunculate heads and involucre 7–10 mm long, while *V. squamulosa* has ovate-lanceolate leaves, shortly pedunculate heads and involucre 9–10 mm long (Cristóbal and Dematteis 2003).

The karyotypes of *V. lucida* (32m + 2sm), *V. oligactoides* (24m + 10sm) and *V. pseudolinearifolia* (28m + 6sm) are presented for first time. The chromosome number of *V. pseudolinearifolia* was reported in the first cytological study of this species (Vega and Dematteis 2012).

The chromosome complement of *V. chamaedrys* was analyzed by Dematteis (1996) and Dematteis and Fernández (1998). In both cases, the karyotype formula was 22m +12sm, which does not coincide with the results obtained here, where this species presented a karyotype formula of 24m + 10sm. A previous analysis in *V. loretensis* showed a karyotype formula of 20m + 14sm (Dematteis 1996); meanwhile the results obtained in this work show that this species presents 26m + 8sm. *Vernonanthura nudiflora* is one of the most cytologically studied species; and different karyotype formula have been observed: 28m + 6sm (Ruas et al. 1991; Angulo and Dematteis 2009a) and 24m + 10sm (Dematteis 1997; Dematteis and Fernández 1998).The results obtained here (30m + 4sm) show a slight difference to those obtained by Ruas et al. (1991) and Angulo and Dematteis (2009b). The karyotype formula of *V. squamulosa* (30m + 4sm) does not match the previous analysis by Dematteis and Fernández (1998), who proposed a karyotype formula of 20m + 12sm + 2 m-sm.

The UPGMA analysis demonstrated certain congruence between karyotype analysis and classification based on morphological characters. Cluster A contains *V. nudiflora* and *V. lucida*, which have been traditionally grouped under *Vernonia* section *Lepidaploa* subsect. *Nudiflorae* (Cabrera 1944). On the other hand, cluster B is composed of quite different species and the group does not reflect any previous classification. In cluster C, *V. oligactoides* and *V. chamaedrys* are morphologically similar and both were included under *Vernonia* section *Lepidaploa* subsection *Chamaedrys* by Cabrera and Klein (1980) and Stutts (1988).

PCA allowed observation of the main differences between *Vernonanthura* species with respect to different karyotype parameters. Three groups were recognized: *V. oligactoides*, *V. chamaedrys*, and *V. tweediana* were grouped in the lower left quadrant, characterized by a high values of A_1 and a high proportion of sm

chromosomes; *V. loretensis* and *V. squamulosa* were grouped in the lower right quadrant with high R, ML, S and TKL; and *V. lucida*, *V. nudiflora* and *V. pseudolinearifolia*, with high values CI and a high proportion of m chromosomes, were located toward the positive side of APC1 and ACP2. In this analysis, *V. pseudolinearifolia* is closer to this group and not to group B as in UPGMA analysis.

The results obtained in this work show that there are differences between the karyotypes of *Vernonanthura* species that may be utilized in taxonomic and evolutionary studies.

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