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Karyotype analysis in eight species of *Vernonia* (Vernonieae, Asteraceae) from South America

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Abstract — The somatic chromosome number and karyotypes of eight species of *Vernonia* were analyzed. The results include the first chromosome count for *V. hexantha* Sch.Bip. (2n=30), which is diploid with base number x=15. The karyotypes are analyzed for the first time for two cytotypes of *Vernonia cognata* Less. (2n=40= 28m + 12sm; 2n=80= 48m + 32sm), *V. hexantha* (2n=30= 24m+6sm), *V. membranacea* Gardner (2n=34= 24 + 10sm), *V. salzmannii* DC. (2n=20= 10m + 10sm) and *V. verbascifolia* Less. (2n=20= 12m + 8sm). All the studied species showed a predominance of metacentric chromosomes, with a lower proportion of submetacentric pairs. In *V. brevifolia* Less. 0 to 3 accessory or B chromosomes were found. The chromosomes varying in length from 1.25 to 3.98 µm. The differences in the symmetry of the karyotypes were small, for which it is possible to assume that the great diversification of the genus has been accompanied by very small changes in the structure of the chromosomes.

Key words: chromosome numbers, karyotypes, symmetry.

INTRODUCTION

Vernonia Schreb., is the largest genus of the tribe Vernonieae (Asteraceae) with about 1,700 species distributed in tropical and subtropical regions of Asia, Africa and America (Jones 1977). The genus is widely distributed in South America with 340-360 species that mainly occurs in north of Argentina, Brazil, Paraguay and Bolivia (Dematteis 2006).

The species of the genus present a great variability in habit and morphology, which leads to adopt different criteria of taxonomic delimitation. Several authors have attempted to resolve the complex taxonomy of *Vernonia* (Cabrera 1944; Keeley 1978; Jones 1979; 1981; Stutts 1988; Robinson 1988; 1992; 1993), however, nowadays they have not reached a satisfactory accord on this subject. Recently, Robinson (1999) confined *Vernonia* to North America and distributed all the South American species in 16 new genera. This new classification has not been adopted by several authors who have considered that the elevation of the different sections to generic level is premature and does not resolve the problem (Hind 1993; Keeley and Jansen 1994). For the delimitation of infrageneric categories, the authors have relied in morphological features as inflorescence pattern, number and shape of the florets, etc (Gleason 1906, 1923; Cabrera 1944; Jones 1979a, 1981; Stuts 1988).

The chromosomes are widely variable in number and morphology within the genus (Ruas *et al.* 1991; Dematteis and Fernandez 2000), which suggests that they can be useful for taxonomic and evolutionary studies (Keeley and Turner 1990; Dematteis and Robinson 1997; Robinson 1999). Previous chromosomes studies for the genus indicate some differences between the Old and New World species. The first present basic chromosome number x=9 and x=10, while the American species have a large variation of numbers that range between x=10 and x=19 (Keeley and Turner 1990; Ruas *et al.* 1990). At the present, the karyotypes of 60 entities belonging to the genus have been reported, from which only 40 are from South America (Gill 1978; Mathew

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and MATHEW 1982; RUAS *et al*. 1991; DEMATTEIS 1998; DEMATTEIS and ROBINSON 1997; DEMATTEIS and FERNANDEZ 2000). In most of the species, differences in karyotype formula, total chromosome length and asymmetry degree, have been observed, which allows a clear distinction among related taxa (DEMATTEIS 2002).

In this paper are analyzed the karyotypes of eight species of *Vernonia*, six from which are notified for the first time. The results are discussed in relation to the taxonomic position of the species and the evolution of the genus.

MATERIALS AND METHODS

The source of examined plants is presented in Table 1. Voucher specimens are deposited at the herbarium of the Instituto de Botanica del Nordeste (CTES).

Mitotic chromosome preparations were obtained from root-tips of germinating seeds. After a pretreatment of about 4-5 hours in bromonaftalene solution, the material was fixed in acetic acid - absolute alcohol (3:1) and then stained with the Feulgen's technique.

Nomenclature used for the karyotype description is that suggested for LEVAN *et al.* (1964). The chromosome morphology was determined using centromeric index (*ci*= short arm x 100/ total chromosomal length). Accordingly, the chromosomes were classified in metacentrics (m): 50-37.5, submetacentircs (sm): 37.5-25 and subtelocentrics (st): 25-12.5. The idiograms and chromosome measures were estimated from ten metaphases plates of 7-10 individuals per species.

The karyological parameters, total length of karyotype (TKL), the mean chromosome length (ML), the average centromeric index (CI), and the ratio between the longest and the shortest chromosome pair (R) were evaluated. The karyotype asymmetry has been determined using the intrachromosomal (A_1) and interchromosomal (A_2) indexes suggested by ROMERO ZARCO (1986).

TABLE 1 — Studied material and somatic chromosome number of some species of *Vernonia*.

Species	2n	Location & Voucher specimens
V. brevifolia Less.	2n=2x=32+ 0-3Bs	Argentina. Corrientes. Dept. Mercedes. 11 km S of Mercedes. *Dematteis & Seo 2468* (CTES).
V. cognata Less.	2n=4x=40	Argentina. Misiones. Dept. San Ignacio. Teyú Cuaré. *Dematteis & al. 2594* (CTES).
V. cognata Less.	2n=8x=80	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Américo. *Dematteis & Krapovickas 1918* (CTES).
V. hexantha Sch.Bip. (*)	2n=2x=30	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Americo. *Dematteis & al. 2638* (CTES).
V. membranacea Gardner	2n=2x=34	Bolivia. Dept. La Paz. Province N Yungas. 16 km N of Yolosa. *Dematteis & al. 1155* (CTES).
V. membranacea Gardner	2n=2x=34	Bolivia. Dept. La Paz. Province Larecaja. 3 km N of La Aguada, between Guanay and Mapiri. *Dematteis & al. 1212* (CTES).
V. nudiflora Less.	2n=2x=34	Uruguay. Dept. Tacuarembó. Arroyo Tacuarembó Chico. *Dematteis & Schinini 1766* (CTES).
V. nudiflora Less.	2n=2x=34	Uruguay. Dept. Rivera. 10 km S of Rivera, on the way to Tacuarembó. *Dematteis & Schinini 1479* (CTES, SI).
V. saltensis Hieron.	2n=2x=32	Bolivia. Dept. Santa Cruz. Province Caballero. 5 km E of Saipina, on the way to Aiquile. *Dematteis & al. 2383* (CTES, SI).
V. salzmannii DC.	2n=2x=20	Bolivia. Dept. La Paz. Province Larecaja. 3 km N of La Aguada, between Guanay and Mapiri. *Dematteis & al. 1210* (CTES, SI, LPB).
V. verbascifolia Less.	2n=2x=20	Argentina. Misiones. Dept. General Manuel Belgrano. Campina de Américo. *Dematteis & al. 2608* (CTES).

(*) First count for the taxon

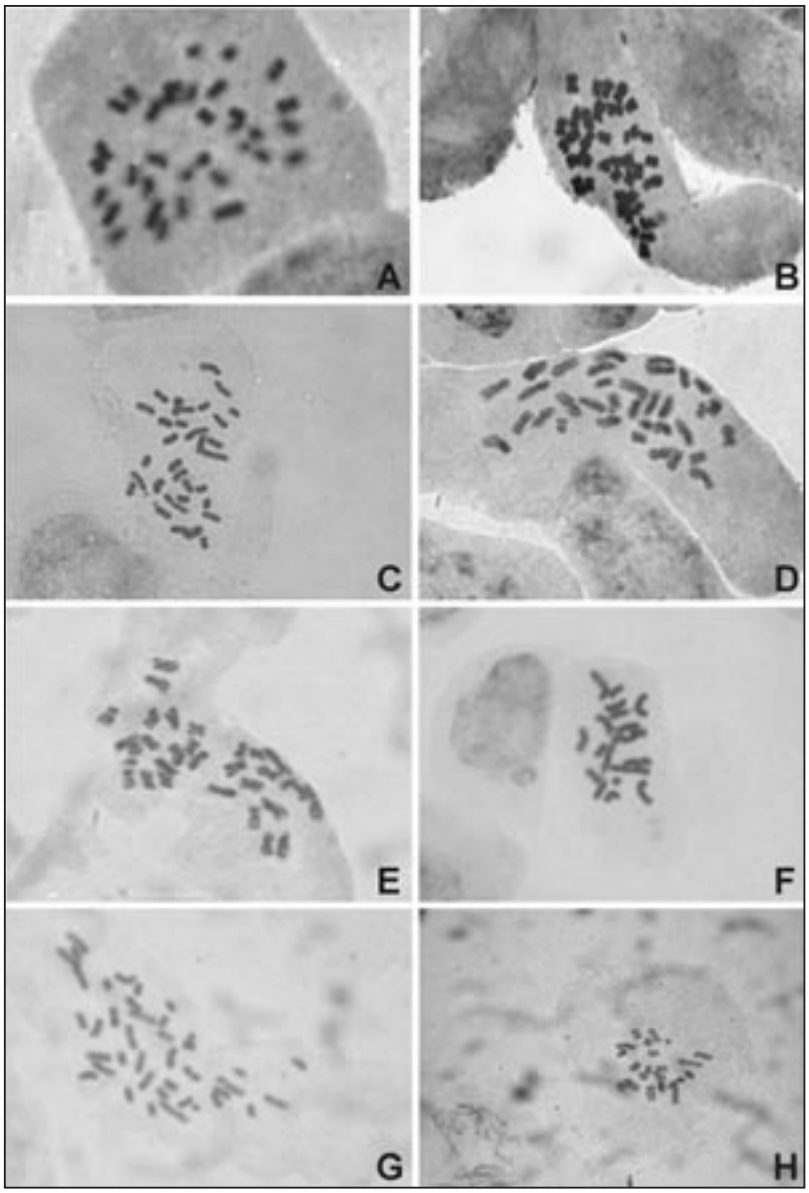

Fig. 1 — Somatic chromosomes of *Vernonia*. A: *V. nudiflora*, 2n=2x=34. B: *V. membranacea*, 2n=2x=34. C: *V. brevifolia*, 2n=2x=32. D: *V. brevifolia*, 2n=2x=32 + 2 Bs. E: *V. hexantha*, 2n=2x=30. F: *V. salzmannii*, 2n=2x=20. G: *V. cognata*, 2n=4x=40. H: *V. verbascifolia* 2n=2x=20.

RESULTS

The somatic chromosome number, karyotype formula, average chromosome length, total length of karyotype, centromeric index and asymmetry indexes of 8 species of *Vernonia* are indicated in Table 2. Four different basic numbers were found. *Vernonia membranacea* (2n=34) and *V. nudiflora* (2n=34) were diploid with x=17; *V. brevifolia* (2n=32) and *V. saltensis* (2n=32) were diploid with x=16; *V. hexantha* (2n=30) was diploid with basic number x=15 and the remaining species presented base x=10. Among them, *V. salzmannii* (2n=20) and *V. verbascifolia* (2n=20) were diploids, while *V. cognata* presented two different cytotypes, one tetraploid (2n=40) and the other ones octoploid (2n=80).

Vernonia brevifolia (2n=32) showed between 0 and 3 B chromosomes (Fig. 1). Among a total of 20 individuals examined, the 55% presented 0B, the 20% showed 1B, the 10% presented 2 Bs and the remaining 15% had 3Bs. The accessory chromo-

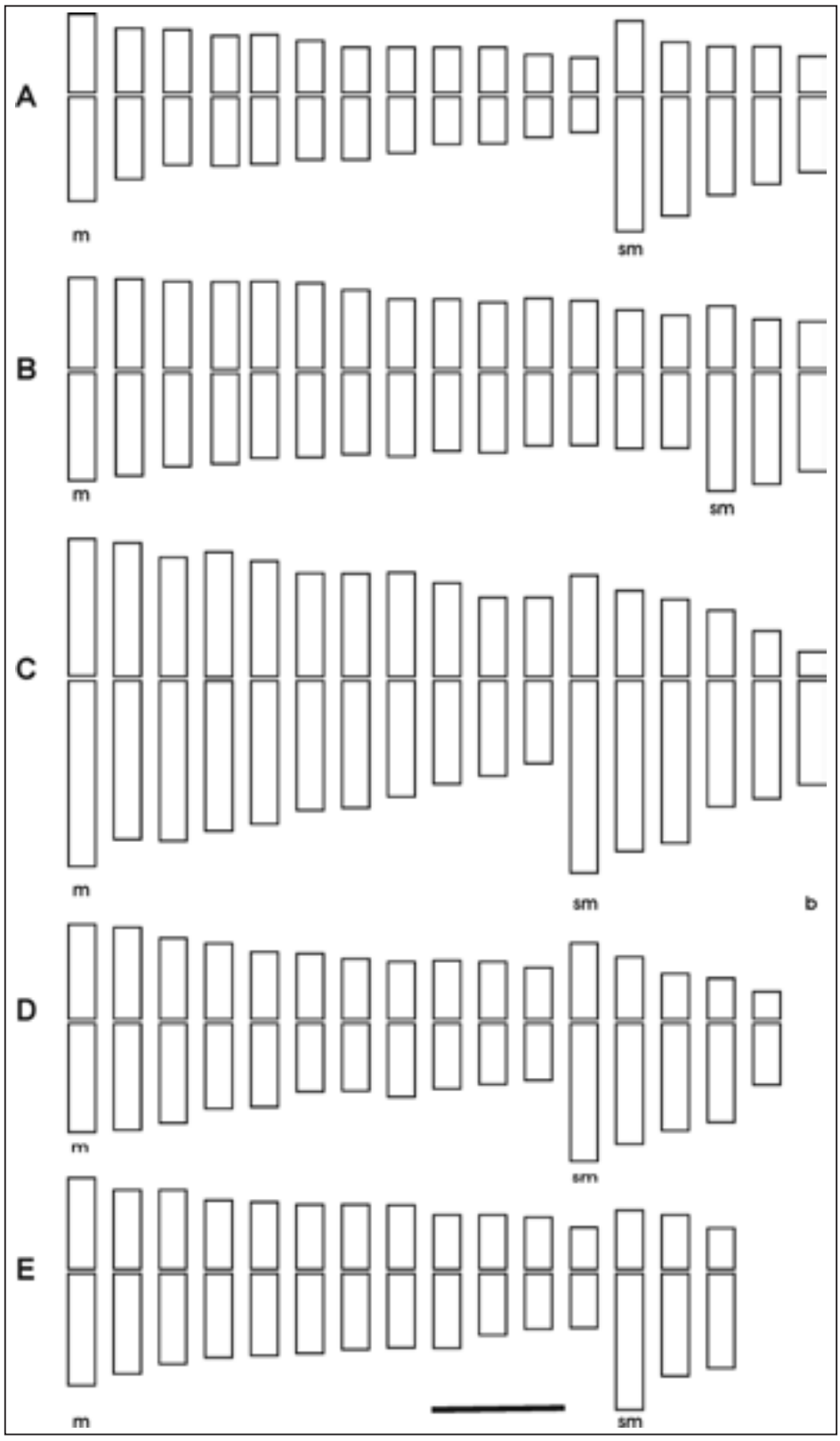

Fig. 2 — Idiograms of some of species *Vernonia*. A: *V. membranacea*, 2n=34= 24m + 10sm. B: *V. nudiflora*, 2n=34= 28m + 6sm. C: *V. brevifolia*, 2n=32 + 0-3B= 22m + 10sm. D: *V. saltensis*, 2n=32= 22m +10sm. E: *V. hexantha*, 2n=30= 24m + 6sm. Bar: 1.5 µm.

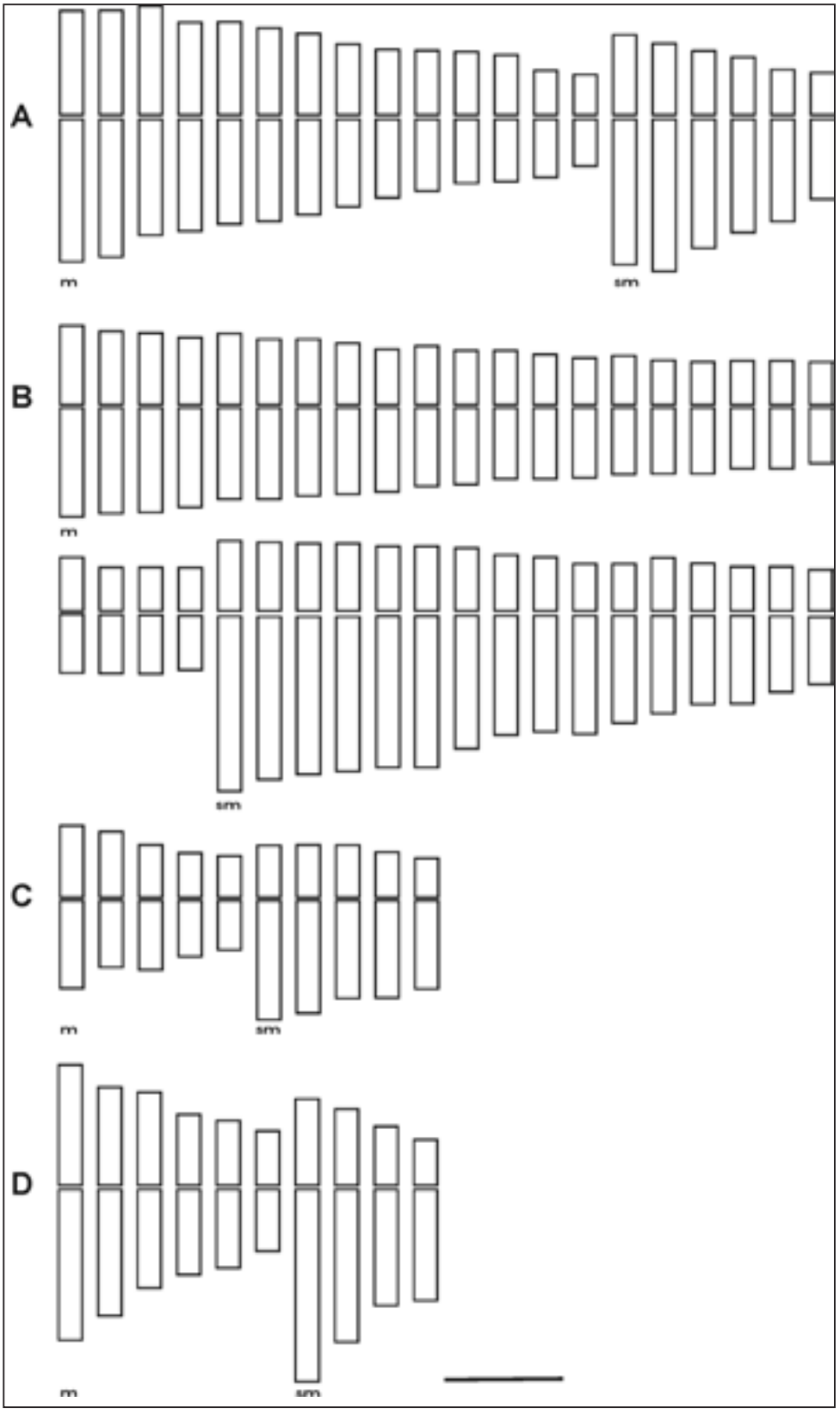

Fig. 3 — Idiograms of some of species *Vernonia*. A: *V. cognata*, 2n=40= 28m + 12sm. B: *V. cognata*, 2n=80= 48m + 32sm. C: *V. salzmannii*, 2n=20 = 10m + 10 sm. D: *V. verbascifolia*, 2n=20= 12m + 8sm. Bar: 1.5 µm.

TABLE 2 — Chromosome number, karyotype formula, average chromosomic length (ML), range (R), total chromosome length (TKL), centromeric index (CI), and intra (A_1) and interchromosomal (A_2) indexes in 8 species of *Vernonia*.

Species	2n	Karyotype Formula	X (µm)	Range (µm)	TKL (µm)	CI	A_1	A_2
V. brevifolia	2n=32	22m+10sm+B	2.52	1.50-3.24	323.22 ± 0.10	40.75 ± 0.635	0.280	0.201
V. cognata	2n=40	28m + 12sm	2.43	1.15-3.48	340.67 ± 0.13	41.69 ± 0.381	0.273	0.276
V. cognata	2n=80	48m + 32sm	2.68	1.65-3.09	214.46 ± 0.32	39.85 ± 0.311	0.319	0.302
V. hexantha	2n=30	24m + 6sm	1.60	1.08-2.09	192.91 ± 0.07	43.10 ± 0.296	0.225	0.182
V. membranacea	2n=34	24m + 10sm	2.04	1.25-2.83	69.36 ± 0.40	42.58 ± 0.336	0.235	0.384
V. nudiflora	2n=34	28m + 6sm	1.81	1.57-2.24	61.54 ± 0.08	44.01 ± 0.271	0.195	0.220
V. saltensis	2n=32	22m +10sm	1.73	1.32-2.35	55.36 ± 0.04	42.42 ± 0.154	0.247	0,231
V. salzmannii	2n=20	10m + 10sm	1.99	1.36-2.61	39.85 ± 1.41	39.02 ± 0.11	0.347	0.206
V. verbascifolia	2n=20	12m + 8sm	2.70	1.62-3.98	162.44 ± 0.21	40.10 ± 0.278	0.306	0.276

± standard error

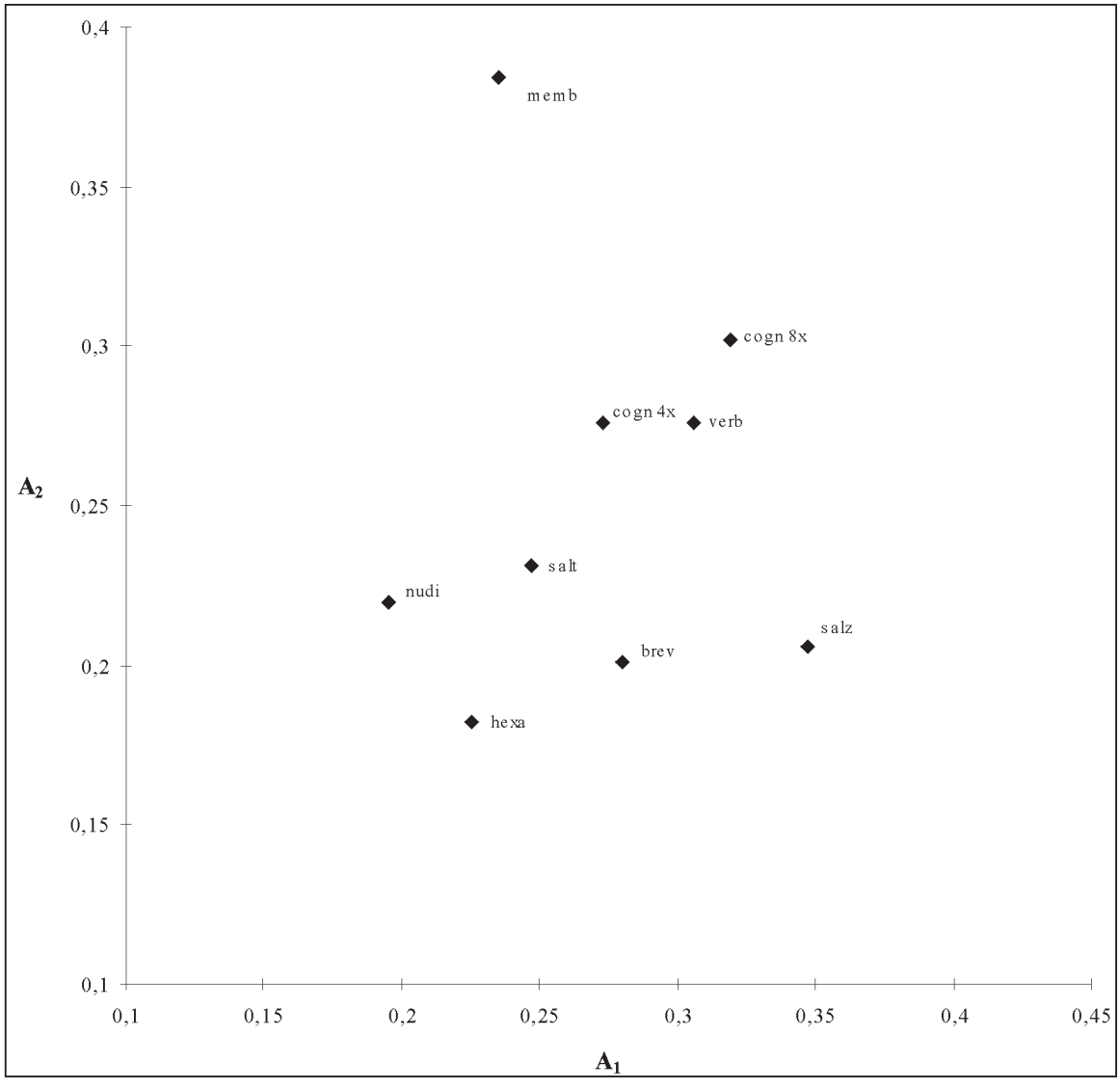

Fig. 4 — Dispersion diagram representing the karyotype asymmetry.

some was characterized as a subtelocentric element with an average chromosome length of 1.41 µm.

All the karyotypes are presented for the first time with the exception of that belonging to *V. brevifolia, V. saltensis* and *V. nudiflora*. The karyotype data is presented in Table 2 and their respective idiograms are shown in Figs. 2 and 3. In all the analyzed species, the karyotypes are composed of metacentric and submetacentric chromosomes that differ in their proportion among species with the same somatic chromosome number. The mean chromosome length ranged between 1.60 µm in *V. hexantha* (24m + 6sm) and 2.70 µm in *V. verbascifolia* (12m + 8sm). Also, in the first species the smallest chromosome pair was found with an average length of 1.08 µm, while in the latter the largest chromosome pair showed 3.98 µm long.

The species with basic number x=10 showed, in general, more asymmetric karyotypes than the remaining entities studied. *Vernonia salzmannii* (10m + 10sm) presented an average centromeric index of 39.02, the tetraploid cytotype of *V. cognata* (28m + 12sm) showed an average of 41.69, while in the octoploid cytotype (48m + 32sm), a value of 39.85 was found. *Vernonia verbascifolia* presented a mean centromeric index of 40.10, and showed the higher values of asymmetry indexes A_1 and A_2. The most symmetric chromosome complement was observed in *V. nudiflora* (28m + 6sm), having a mean centromeric index of 44.01, which agrees with the presence of a majority of metacentric chromosomes.

The relationship between the intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indexes of the 8 species is represented in Fig. 4. The dispersion diagram shows grouped species with basic number x= 15 and x=16, which present comparatively lower values of A_1 and A_2 indexes. On the right area of the diagram, the entities with basic number x=10 appear with higher values of A_1 index. At the top, is one of the species with basic number x=17, which presents the highest A_2 index.

DISCUSSION

The results obtained in the 8 analyzed species showed that the chromosomes were small to medium in size, varying between 1.08 and 3.98 µm, which coincides with previous reports for the genus (GILL 1978; MATHEW and MATHEW 1982; RUAS *et al*. 1991; DEMATTEIS 1998).

First karyotype analysis are presented for *Vernonia membranacea* (24m + 10sm), *V. salzmannii* (10m + 10sm), *V. hexantha* (24m + 6sm), *V. verbascifolia* (12m + 8sm), and the two cytotypes of *V. cognata,* a tetraploid (28m + 12sm) and an octoploid (48m + 32sm).

Vernonia nudiflora has been studied by several authors and almost all the reports shows n= 17 or 2n=34 (JONES 1974; BERNARDELLO 1986; STUTTS 1988; RUAS *et al*. 1991; DEMATTEIS 1997, 2002). Only one exception was obtained by COVAS and HUNZIKER (1954), in material of western Argentina, with a chromosome number of n=16. In our study, the karyotype formula for this species (28m + 6sm) differs from a previous analysis that shows 24m + 10sm (DEMATTEIS 1997).

The recount realized in *V. saltensis* agrees with a study realized in a population of Cordoba, Argentina (BERNARDELLO 1986), but differ from the 2n=64 reported by DEMATTEIS (1998) for material from Jujuy (Argentina). These records confirm the occurrence of two different cytotypes for this species, one diploid with 2n=32 and other ones tetraploid having 2n=64. The karyotype of the diploid cytotype is described here for the first time. DEMATTEIS (1998) reported the karyotype formula for the tetraploid as composed of 2n=64= 48m + 16sm.

The occurrence of B chromosomes has been informed previously for several species of *Vernonia* (JONES 1979; GALIANO and HUNZIKER 1978; DEMATTEIS 1997, 1998). A previous study in *V. brevifolia* agree with the karyotype formula and the number of accessory chromosomes found in our analysis (DEMATTEIS 1998).

All the karyotypes are composed of metacentric chromosomes accompanied by a lower proportion of submetacentric chromosomes, which results agree with previous reports for the tribe Vernonieae (RUAS *et al*. 1991; DEMATTEIS 1998; DEMATTEIS and FERNANDEZ 1998, 2000). Because of the prevalence of metacentric chromosomes, the karyotypes almost always present a high degree of symmetry. The entities with basic number x=10 showed the highest level of asymmetry, while the species with x=17 were less asymmetric than the previous ones. Due to the slight difference in the symmetry of the karyotypes, it is possible to assume that the great diversification of the genus has been accompanied by very small changes in the structure of the chromosomes. This become obvious when comparing the karyotypes of the species with the same basic number, where the karyotypes formula differ very little from one species to another.

The cytological knowledge of *Vernonia* is still limited and additional chromosome counts are

necessary. More karyotype studies should provide essential information in understanding the systematic and evolution of the genus.

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