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# Karyotype characterization of two populations of *Vernonia geminata* (Asteraceae, Vernonieae) using banding and FISH techniques

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**ABSTRACT.** In order to extend our knowledge concerning karyotypes of the genus *Vernonia*, we applied various techniques of chromosome banding, including AgNOR and triple staining with the fluorochromes CMA/DA/DAPI (CDD), and of fluorescent *in situ* hybridization (FISH) for the 45S rDNA probe to specimens of two populations of *Vernonia geminata* collected from an open-pasture area, in southern Brazil. B chromosomes were observed in one of the populations. Both populations of *V. geminata* presented a pair of $CMA_3^+$ terminal bands and one pair of chromosomes with terminal AgNOR banding. The FISH evidenced, in one population, two pairs of small sites of 45S rDNA; these being two small terminal sites and two centromeric sites. In the other population, there was only one pair of small terminal sites and two sites in two B chromosomes, one in each chromosome. There was coincidence of localization between $CMA^+$ and NOR bands with one of the small terminal sites

 

of 45S rDNA of one chromosome of the normal complement, but not in B chromosomes.

**Key words:** AgNOR banding; CMA banding; Physical mapping; 45S rDNA site

## INTRODUCTION

*Vernonia* Schreb. (Asteraceae, Vernonieae) comprises small herbs to large trees (Stutts, 1988), occurring in tropical regions of Asia, Africa and Americas (Dematteis, 1998). It is one of the most complex genera of the family (Dematteis and Fernández, 1998), with more than 1000 species (Robinson, 1999). Recently, Robinson (1999) proposed that the traditional genera of Vernonieae must be regrouped, but their modifications were not accepted by most workers in this field. Studies involving genera of this tribe elaborated by Robinson (1999) resulted in the splitting of the genus *Vernonia* into 22 genera.

*Vernonia geminata* Kunth., which according to Robinson (1999) now belongs to the genus *Lepidaploa* (Cass.) Cass., occurs from Mexico to South America (Jorgensen and Ulloa, 1994), including the "cerrado" (Oliveira et al., 2007a) and disturbed areas in Brazil. It is an important honey species, where its pollen is detected in the honey and the larval food of *Tetragonisca angustula* Latreille, a bee species found in Chiapas, Mexico (Martínez-Hernández et al., 1994). Karyotypes of *V. geminata* and other species of *Vernonia* have been described by Oliveira et al. (2007a) by conventional staining. *V. geminata* has 2n = 20 chromosomes predominantly metacentric, varying from 2.0 to 4.6 µm. Oliveira et al. (2007a) reported the occurrence of B chromosomes (0-6) in one population of this species.

Karyotype analysis is essential for the cytogenetic characterization of species and to examine the variation between its individuals and/or populations (Guerra, 1988). The comparison of karyotypes of different species also allows the taxonomic and evolutionary analysis of a taxon, such as a genus. Many times, differences in karyotype asymmetry can indicate how these chromosomes have diversified in size and morphology within a group (Guerra, 1988).

Chromosome numbers of only 20% of *Vernonia* have been reported, varying from 2n = 18 to 160 (Oliveira et al., 2007a). The karyotypes determined with conventional techniques include only 5% of the species of *Vernonia*, and they do not discriminate very well species with the same chromosome number, due to small variation in chromosome size and centromeric position (Ruas et al., 1991; Dematteis, 1996, 1998; Dematteis and Fernández, 1998, 2000; Oliveira et al., 2007a,b). To date, information obtained by techniques that allow the linear differentiation of the chromosomes [chromosome banding and fluorescent *in situ* hybridization (FISH)] exists for only one species, *Vernonia condensata* Baker (2n = 40). Fluorochrome staining with chromomycin $A_3$/4',6-diamidino-2-phenylindole dihydrochloride ($CMA_3$/DAPI) has revealed five chromosome pairs bearing subterminal $CMA^+$/$DAPI^-$ heterochromatin (Salles-de-Melo et al., 2010).

Among banding techniques, staining with some fluorochromes determines the composition of bases of the heterochromatin (Schweizer, 1976). The fluorochrome DAPI preferentially stains AT-rich DNA, while $CMA_3$ preferentially stains GC-rich DNA. Guerra et al. (2000), in studying the pattern of heterochromatic bands in Rutaceae, subfamily Aurantioideae, observed that the genus most basal in the group showed smaller amounts of heterochro-

matin than did the more derived genera. Beyond fluorochrome banding, specific staining with silver nitrate or AgNOR can be used (Goodpasture and Bloom, 1975) to identify the nucleolus and chromosomes that have nucleolus organizer regions (NOR) that had been active in interphase. Using AgNOR staining, Moscone et al. (1995) showed possible trends in the karyotypic evolution of *Capsicum* L. (Solanaceae) with x = 12 chromosomes, and they proposed that the presence of bands, located in the first pair of metacentric chromosomes and in the 12th pair of subtelocentric chromosomes, is an ancestral condition for species belonging to the basal sub-group (A).

FISH is a good method to locate specific sites of nucleic acid (DNA or RNA) in the cytoplasm, organelles, chromosomes, or nucleus of biological material (Leitch et al., 1994). The most frequently used DNA sequences are ribosomal probes (45S rDNA and 5S rDNA) and telomeric probes (Weiss-Schneeweis et al., 2003). In some Asteraceae, such as *Artemisia*, FISH and chromosome banding techniques are of great taxonomic value. Torrel et al. (2003) did not support the taxonomic proposition of the separation of the subgenera *Artemisia* L. and *Seriphidium* (Besser ex W. Hook.) Poljakov as independent genera, due to similarity in the distribution pattern of 5S rDNA loci.

Aiming to extend our knowledge of karyotype diversity in *Vernonia*, we applied different chromosome banding techniques, including AgNOR and triple staining with the fluorochromes CMA, DA (distamycin) and DAPI (CDD), and the FISH technique for the 45S rDNA probe, in analyzing individuals of two populations of *V. geminata*.

## MATERIAL AND METHODS

We analyzed the same populations of *V. geminata* studied by Oliveira et al. (2007a), collected in disturbed area, in Analândia, São Paulo State, Brazil. The population 1 occurred in an urban area and the population 2 in an open-pasture area, about 15 km away. Fruits, floral and vegetative branches of both populations were collected. Vouchers (population 1, VM Oliveira 44; population 2, VM Oliveira 82) were deposited in the herbarium of the Universidade Estadual de Campinas (UEC).

Root tips of newly germinated plants were pretreated with 0.002 M 8-hydroxyquinoline (8Hq) for 5 h at 14°-15°C. Afterwards, the root tips were fixed in Farmer solution (ethanol:acetic acid, 3:1, v/v) for 24 h and then transferred to 70% alcohol and stored in a freezer. For the preparation of samples with enzymatic digestion, the root tips were washed in distilled water and were dried with paper. The root tips were softened in an enzymatic solution containing 4% cellulase and 40% pectinase, in a moist chamber at 37°C for 2 h. The squashing of the root tips between slides and coverslips was made in a drop of 45% acetic acid. The coverslips were removed in liquid nitrogen.

The slides were stored at room temperature for at least one day and then submitted to CDD and AgNOR banding. The CDD followed the protocol of Moscone et al. (1996). The slides were first stained with 0.5 mg/mL $CMA_3$ for 1 h, and afterwards washed with distilled water. The slides were stained with 10 mg/mL DA for 15 min and again washed with distilled water. Finally, they were stained with 2 mg/mL DAPI for 30 min. The slides were again washed with distilled water and mounted with a solution containing MacIlvaine buffer, pH 7.0, and distilled water (1:1, v/v) and $MgCl_2$. The slides were stored in a dark box, at 37°C for 3 days, before being examined with an epifluorescence microscope.

AgNOR banding followed the protocol of Moscone et al. (1995). The slides were washed in 0.01 M borate buffer for 10 min and then with distilled water. Each slide received a drop of 50% silver nitrate and a piece of meshed nylon. The slides were stored at 60°C for 1 h and then washed in distilled water for 5 min. The slides were mounted with Entellan.

The FISH was done according to Cuadrado and Jouve (1994), with the modifications of Fregonezi et al. (2004). The p*Ta*71 probe (18S-5.8S-26rDNA) was labeled by nick translation (Bionick Gibco kit) with biotin-14dATP. Before hybridization, the spreads were pretreated with 1% RNase (1 RNase: 99 2X SSC), pH 7.0, at 37°C for 1 h, washed in 2X SSC, fixed in 4% paraformaldehyde, and again washed in 2X SSC. The slides were dehydrated in a 70 to 100% ethanol series and air-dried. Each slide was treated with labeling solution [100% formamide, 50% polyethylene glycol, 20X SSC, labeled probe (45S rDNA; 100-200 ng), salmon sperm and 10% SDS], denatured at 70°C and chilled on ice for 5 min. Chromosomes were denatured and hybridized using a thermal cycler for 10 min at 90°C, 10 min at 50°C, 10 min at 38°C, and overnight at 37°C in a moist chamber. After hybridization, the slides were washed in 2X SSC, 0.1X SSC, 20% formamide, 0.1X SSC, 2X SSC and 4X SSC/0.2% Tween 20 for 5 min at 42°C each. The signals were detected with avidin-FITC and the chromosomes were counterstained using propidium iodide.

The analysis of the slides was carried out with a light microscope (AgNOR banding) and an epifluorescence microscope (FISH and CDD banding). Photomicrographs of AgNOR banding were taken using black and white film, ISO 25. Photomicrographs of FISH were taken using black and white film, ISO 100, and Kodak ProImage color film, ISO 100, was used for FISH. In each slide, we examined at least 10 cells with adequate scattering and chromosome contraction. Oliveira et al. (2007a) previously reported ideograms of the same populations studied here. These authors used the conventional Giemsa staining and the classification and nomenclature of chromosomes suggested by Guerra (1988). In the present study, we used this ideogram to indicate the chromosome localization of CMA, DAPI and NOR bands, as well as small 45S rDNA sites.

## RESULTS

The two populations of *V. geminata* analyzed showed 2n = 20 and both possessed a pair of $CMA^+$ bands in the terminal region of the short arm of the fourth pair of submetacentric chromosomes (Figures 1 and 2). Neither showed bands with DAPI (neutral DAPI).

AgNOR banding demonstrated one pair of bands in both populations in the terminal region of the short arm of the fourth pair of submetacentric chromosome (Figure 1).

FISH showed in population 1 two pairs of 45S rDNA sites, one small pair next to the centromeric region, in the short arm of the sixth pair of metacentric chromosomes, and another larger pair in the terminal region of the short arm of the fourth pair of submetacentric chromosomes. In population 2, we observed only one pair of small 45S rDNA sites, also situated in the terminal region of the short arm of the fourth pair of submetacentric chromosomes (Figures 1 and 2).

In some individuals of population 2, besides the 20 chromosomes of the normal karyotype, there were B chromosomes, varying from 0 to 6 in different cells of the same individual (2n = 20 + 0-6 B). In these B chromosomes, we could not see any CMA, DAPI or AgNOR bands, but one 45S rDNA site was observed in two different chromosomes.

 

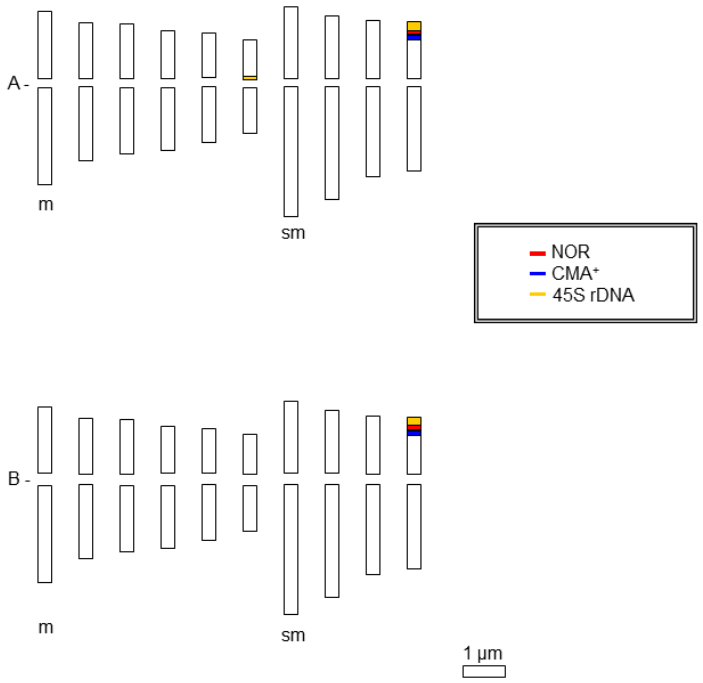


**Figure 1.** Ideograms of *Vernonia geminata* indicating the number and position of CMA$^+$, NOR bands and number of 45S rDNA sites. **A.** Population 1. **B.** Population 2. m = metacentric; sm = submetacentric.

## DISCUSSION

According to Ruas et al. (1991), Dematteis (1996, 1998), Dematteis and Fernández (1998, 2000) and Oliveira et al. (2007a,b), despite the occurrence of variation in chromosome number among species of *Vernonia*, the karyotypes with conventional techniques do not discriminate well the species already studied, due to small variation in chromosome size and centromeric position. Among the species of *Vernonia* that belong to the group *Axilliflorae* Benth., studied by Oliveira et al. (2007a), *V. geminata* has the most differentiated karyotype, showing chromosomes with greater variation in length (2.0 to 4.6 µm) and greater proportion of submetacentric chromosomes, in relation to the metacentrics (6m + 4sm), where it is the only one with 2n = 20 chromosomes (pattern of the group is x = 16). Dysploidy can play a prominent role in the evolution of the species studied, as is the case for many species of Asteraceae. In species of *Hypochaeris* L., small structural alterations, such as translocations, and inversions involving loss and gain of chromosome fragments, sometimes containing the 45S rDNA region, are suggested to explain the dysploidy found in its karyotype evolution (Weiss-Scheneeweiss et al., 2003).

There was coincidence between the AgNOR, CMA$^+$ and the small 45S rDNA sites, only in one chromosome near the terminal region, in the short arm of the fourth submetacentric chromosome, in both populations (Figures 1 and 2). CMA$^+$ bands are not part of the 45S rDNA sequence, but are arranged contiguously (Appels et al., 1998). Garnatje et al. (2004)

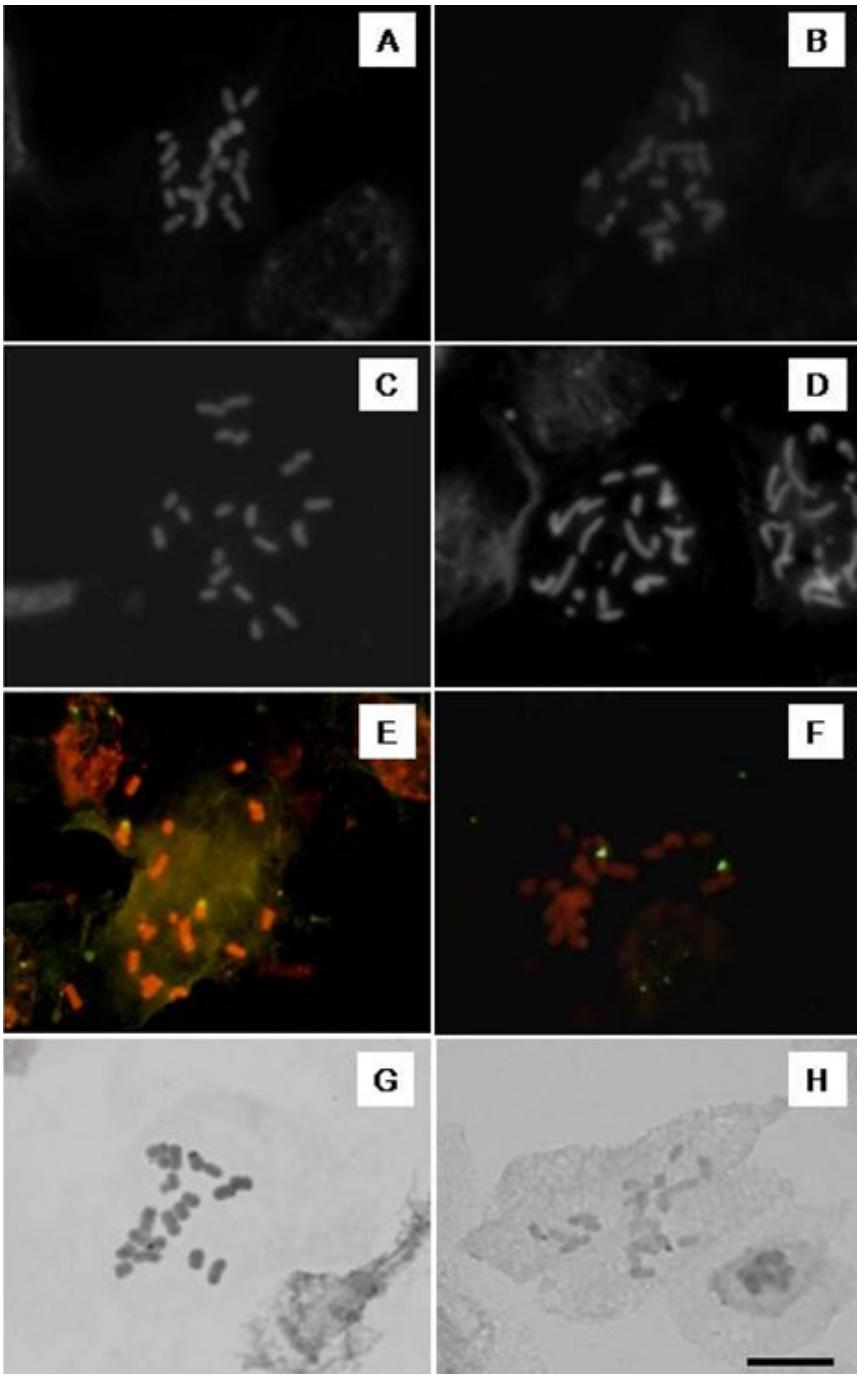


**Figure 2.** Metaphases of *Vernonia geminata* population 1 (**A**, **C**, **E**, and **G**) and population 2 (**B**, **D**, **F**, and **H**). A to D = CCD banding (A and B = $CMA_3$/C and D = DAPI); E and F = FISH; G and H = AgNOR banding. Bar = 10 µm.

 

studied seven species of *Xeranthemum* L. and related genera belonging to the tribe Carduceae (Asteraceae), and observed that the $CMA^+$ bands and 45S rDNA sites, most of the time, share the same location, suggesting that repeat ribosomal sequences could be interspaced in heterochromatin.

The similar localization of AgNOR and CMA bands and 45S rDNA sites observed on the fourth submetacentric chromosome was not observed on the sixth metacentric chromosome of population 1. In this chromosome, only one 45S rDNA site was observed in the centromeric region of the short arm (Figures 1A and 2). Different numbers of 45S rDNA sites and $CMA^+$ bands were previously observed in *Cestrum* L. (Fregonezi et al., 2004), *Helianthus* L. (Vanzela et al., 2002) and *Crotalaria* L. (Mondim et al., 2007). According to Mondim et al. (2007), in *Crotalaria*, $CMA^+$ bands could not be visualized due to the reduced size of its chromosomes.

In general, there is coincidence in the location of AgNOR and $CMA^+$ bands (Berger and Greilhuber, 1993). In accordance with Torrel et al. (2003), in *Artemisia*, small sites of rDNA are GC-rich and basically linked to the NORs. This exact pattern also occurs in other plants, such as *Hypochaeris* (Cerbah et al., 1995) and *Dendranthema* (DC.) Des Moul. (Kondo et al., 1996).

In the present study, we did not observe agreement between the number of 45S rDNA sites and AgNOR bands. AgNOR banding only detects the active NORs that were transcribed during interphase proceeding cell division, while FISH shows the active and inactive regions (Murray et al., 1992).

In *Vigna unguiculata* (L.) Walp. (Galasso et al., 1995) and *Lathyrus sativus* L. (Murray et al., 1992), among the multiple small sites of 45S rDNA, only the largest was impregnated with silver ions, which was interpreted as inactivation of some NORs. Shi et al. (1996) also reported that in *Glycine max* (L.) Merr. and *Phaseolus vulgaris* L., not all signals of 5S and 45S rDNA were associated with nucleoli during interphase.

The absence of small sites of 45S rDNA and NOR/$CMA^+$ bands on the sixth metacentric chromosome of population 2 (Figure 1B) can indicate deletion of this sequence, present in population 1 (Figure 1A). However, it can be related to the reduced size of this sequence, as a result of small structural alterations, hindering the visualization of the small sites/bands. In *Helianthus*, small variations in the chromosomes had been detected, indicating in some cases, the loss or gain of rDNA regions, and these structural alterations could have an important role in the speciation of this genus (Vanzela et al., 2002). The results of the present study are still premature to conclude which mechanisms are involved in *Vernonia.* The FISH technique is more qualitative than quantitative, but an association between the size of the FISH sites and the number of repeated sequences of rDNA can be demonstrated, and thus, more intense signals represent small sites with a larger number of repeated sequences of less intense signals (Fregonezi et al., 2004).

Lima-de Faria (1976) suggested that 45S rDNA sites in short arm of chromosomes are a general trend within the Asteraceae. Studies involving FISH of 45S rDNA in species of this family have also shown this trend (Vanzela et al., 2002; Torrel et al., 2003; Garnatje et al., 2004; Fregonezi et al., 2004).

In population 2, the occurrence of up to six chromosomes (2n = 20 + 0 - 6B) was previously observed by Oliveira et al. (2007a) in the same population. These extra-chromosomes occur in some species of *Vernonia* (Dematteis, 1997, 1998) and other Asteraceae, such as *Mikania* Willd. (Maffei et al., 1999), *Crepis capillaris* (L.) Wallr. (Whitehouse et al., 1981) and *Calycadenia pauciflora* A. Gray (Carr and Carr, 1982). The observation of 45S rDNA sites

in two B chromosomes of *V. geminata* (Figure 2F) was not coincident with fluorescent and AgNOR bands. Thus, these sites must be inactive NOR (Murray et al., 1992). The presence of 45S rDNA sites in B chromosomes was previously reported in other Asteraceae such as *C. capillaris* (Jamilena et al., 1994) and *Brachycome dichromosomatica* C.R. Carter (Marschner et al., 2007).

There is no published data based on the FISH technique in other species of *Vernonia*, and fluorochrome staining with CMA/DAPI was reported only for *V. condensata* (Salles-de-Melo et al., 2010), which revealed five chromosome pairs bearing subterminal $CMA^+/DAPI^-$ heterochromatin. We observed here in two populations of *V. geminata* only one chromosome pair bearing terminal $CMA^+/DAPI^-$ heterochromatin. However, *V. geminata* and *V. condensata* have different chromosome numbers and belong to different sections, *Lepidaploa* (Cass.) DC. and *Orbisvestus* S.B. Jones, respectively (Baker, 1873).

There appears to be substantial intraspecific variation in *V. geminata*, in view of the different possible numbers of 45S rDNA sites in B chromosomes and next to the centromeric region, in the short arm of the sixth pair of metacentric chromosomes. According to Vanzela et al. (2002), loss or gain of rDNA regions plays an important role in speciation in the genus *Helianthus*. Complementary chromosome studies in *V. geminata* and other species of *Vernonia* would contribute to our knowledge of the evolution and taxonomy of this group of plants.

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## REFERENCES

Appels R, Moris R, Gill S and May C (1998). Chromosome Biology. Kluwer, Boston.

Baker JG (1973). Compositae I. Vernonieae. In: Flora Brasiliensis (Martius CF and Eichler AG, eds.). Monachii, Lipsiae, 1-180.

Berger C and Greilhuber J (1993). Cold-sensitive chromosome regions and heterochromatin in *Cestrum* (Solanaceae): *C. strigillatum*, *C. fasciculatum*, and *C. elegans*. *Plant Syst. Evol.* 185: 133-151.

Carr GD and Carr RL (1982). Micro and nucleolar-organizing B-chromosomes in *Calycadenia* (Asteraceae). *Cytologia* 47: 79-87.

Cerbah M, Coulaud J, Godelle B and Siljak-Yakovlev S (1995). Genome size, fluorochrome banding, and karyotype evolution in some *Hypochoeris* species. *Genome* 38: 689-695.

Cuadrado A and Jouve N (1994). Mapping and organization of highly-repeated DNA sequences by means of simultaneous and sequential FISH and C-banding in 6x-triticale. *Chromosome Res.* 2: 331-338.

Dematteis M (1996). Estudios cromosómicos en especies argentinas de *Vernonia* (Asteraceae). *Bonplantia* 9: 103-110.

Dematteis M (1997). Números cromosómicos y cariotipos en algunas espécies de *Vernonia* (Asteraceae). *Bol. Soc. Argent. Bot.* 33: 85-90.

Dematteis M (1998). Chromosome studies of some *Vernonia* species (Asteraceae). *Genet. Mol. Biol.* 21: 381-385.

Dematteis M and Fernández A (1998). Karyotypes of seven South American species of *Vernonia* (Asteraceae). *Cytologia* 63: 323-328.

Dematteis M and Fernández A (2000). Chromosome studies on nine South American species of *Vernonia* (Vernonieae, Asteraceae). *Caryologia* 53: 55-61.

Fregonezi JN, Torezan JMD and Vanzela ALL (2004). A karyotypic study of three southern Brazilian Asteraceae species using fluorescence *in situ* hybridization with a 45S rDNA probe and C-CMA3 banding. *Genet. Mol. Biol.* 27: 223-227.

Galasso I, Schmidt T, Pignone D and Heslop-Harrison JS (1995). The molecular cytogenetics of *Vigna unguiculata* (L.)

Walp: the physical organization and characterization of 18S-5.8S-25S rRNA genes, 5S rRNA genes, telomere-like sequences, and a family of centromeric repetitive DNA sequences. *Theor. Appl. Genet.* 91: 928-935.

Garnatje T, Valles J, Vilatersana R, Garcia-Jacas N, et al. (2004). Molecular cytogenetics of *Xeranthemum* L. and related genera (Asteraceae, Cardueae). *Plant Biol.* 6: 140-146.

Goodpasture C and Bloom SE (1975). Visualization of nucleolar organizer regions im mammalian chromosomes using silver staining. *Chromosoma* 53: 37-50.

Guerra M (1988). Introdução à Citogenética Geral. Editora Guanabara, Rio de Janeiro.

Guerra M, Dos Santos KG, Barros E Silva AE and Ehrendorfer F (2000). Heterochromatin banding patterns in Rutaceae-Aurantioideae - a case of parallel chromosomal evolution. *Am. J. Bot.* 87: 735-747.

Jamilena M, Ruiz RC and Ruiz RM (1994). A molecular analysis of the origin of the *Crepis capillaris* B chromosome. *J. Cell Sci.* 107 (Pt 3): 703-708.

Jorgensen PM and Ulloa CU (1994). Seed plants of the high Andes of Ecuador: a checklist. *AAU Reports* 34: 1-443.

Kondo K, Honda Y and Tanaka R (1996). Chromosome marking in *Dendranthema japonica* var. *wakasaense* and its closely related species by fluorescence *in situ* hybridization using rDNA probe. *Kromosomo* II-81: 2785-2791.

Leitch AR, Schwarzacher T, Jackson D and Leitch IJ (1994). *In Situ* Hybridization. BIOS Scientific Publishers Ltd., Oxford.

Lima-de Faria A (1976). The chromosome field I. Prediction of the localization of ribosomal cistrons. *Hereditas* 83: 1-22.

Maffei EMD, Marin-Morales MA, Ruas PM and Ruas CF (1999). Chromosomal polymorphism in 12 populations of *Mikania micrantha* (Compositae). *Genet. Mol. Biol.* 22: 433-444.

Marschner S, Meister A, Blattner FR and Houben A (2007). Evolution and function of B chromosome 45S rDNA sequences in *Brachycome dichromosomatica*. *Genome* 50: 638-644.

Martínez-Hernández E, Cuadriello-Aguilar JI, Ramírez Arriaga E, Medina Camacho M, et al. (1994). Foraging of *Nannotrigona testaceicornis*, *Tetragona jaty*, *Scaptotrigona pachysoma* and *Plebeia* sp. at Tacana región, Chiapas, México. *Grana* 33: 205-217.

Mondin M, Santos-Serejo JA and Aguiar-Perecin MLR (2007). Karyotype characterization of *Crotalaria juncea* (L.) by chromosome banding and physical mapping of 18S-5.8S-26S and 5S rRNA gene sites. *Genet. Mol. Biol.* 30: 65-72.

Moscone EA, Loidl J, Ehrendorfer F and Hunziker AT (1995). Analysis of active nucleolus organizing regions in *Capsicum* (Solanaceae) by silver staining. *Am. J. Bot.* 82: 276-287.

Moscone EA, Lambrou M and Ehrendorfer F (1996). Fluorescent chromosome banding in the cultivated species of *Capsicum* (Solanaceae). *Plant Syst. Evol.* 202: 37-63.

Murray BG, Bennett MD and Hanmet RW (1992). Secondary constrictions and NORs of *Lathyrus* investigated by silver staining and *in situ* hybridization. *Heredity* 68: 473-478.

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

Robinson H (1999). Generic and subtribal classification of American Vernonieae. *Smith. Contr. Bot.* 89: 1-116.

Ruas PM, Ruas CF, Vieira AOS, Matzenbacher N, et al. (1991). Cytogenetics of genus *Vernonia* Schreber (Compositae). *Cytologia* 56: 239-247.

Sales-de-Mello MRC, de Lucena RM, Semir J, de Carvalho R, et al. (2010). Karyological features and cytotaxonomy of the tribe Vernonieae (Asteraceae). *Plant Syst. Evol.* 285: 189-199.

Schweizer D (1976). Reverse fluorescent chromosome banding with chromomycin and DAPI. *Chromosoma* 58: 307-324.

Shi L, Zhu T and Kein P (1996). Ribosomal RNA genes in soybean and common bean: chromosomal organization, expression, and evolution. *Theor. Appl. Genet.* 93: 136-141.

Stutts JG (1988). Taxonomic revision of *Vernonieae* Subsect. *Chamaedrys* (Compositae: Vernonieae). *Rhodora* 90: 37-99.

Torrell M, Cerbah M, Siljak-Yakovlev S and Vallès J (2003). Molecular cytogenetics of the genus *Artemisia* (Asteraceae, Anthemideae): fluorochrome banding and fluorescence *in situ* hybridization. I. Subgenus *Seriphidium* and related taxa. *Plant Syst. Evol.* 239: 141-153.

Vanzela ALL, Ruas CF, Oliveira MF and Ruas PM (2002). Characterization of diploid, tetraploid and hexaploid *Helianthus* species by chromosome banding and FISH with 45S eDNA probe. *Genetica* 114: 105-111.

Weiss-Schneeweiss H, Stuessy T, Siljak-Yakovlev S, Baeza C, et al. (2003). Systematic and evolutionary implications of karyotypes of *Hypochaeris* (Asteraceae, Lactuceae) from South America. *Plant Syst. Evol.* 241: 171-184.

Whitehouse C, Edgar LA, Jones GH and Parker JS (1981). The population cytogenetics of *Crepis capillaris* I. Chiasma variation. *Heredity* 47: 95-103.