

Cytotaxonomic studies in six species of *Vernonia* (Asteraceae: Vernonieae)

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Abstract — In this report, we describe a mitotic analysis (Giemsa technique) of six species of the genus *Vernonia sensu* Baker (Asteraceae, Vernonieae, section *Lepidaploa*) in order to assess the validity of maintaining this genus (*sensu* Baker) or dividing it into several lesser genera (*sensu* Robinson). Specimens of the seven species were collected in “cerrado” and “campo rupestre” areas in the states of São Paulo, Minas Gerais and Goiás. The chromosomal numbers ($2n=32$ and $2n=34$) and karyotypes revealed a predominance of metacentric chromosomes, with some sub-metacentrics. The chromosome sizes varied from 1.2 to 4.9 μm , the total chromatin length (TCL) ranged from 31.3 to 50.7, and the asymmetry index TF% ranged from 42.3 to 44.4. The intrachromosomal asymmetry index (A_1) varied from 0.15 to 0.19, while the interchromosomal asymmetry index (A_2) ranged from 0.14 to 0.21. A dispersion diagram showed that population 1 of *V. polyanthes* had the most asymmetrical karyotype. These results still do not allow a comprehensive and conclusive cytotaxonomic discussion of this group, mainly because of the lack of a characteristic or standard karyotype for most infrageneric groupings (sections and subsections) and also because most species have not yet been analyzed cytogenetically. Nevertheless, the available data indicate only a tenuous relationship between the chromosome numbers observed here and reported in the literature compared to the taxonomic reorganization of the genera *Lessingianthus*, *Vernonanthura* and *Chrysolaena* proposed by Robinson.

Key words: cytotaxonomy, karyotype, mitosis.

INTRODUCTION

Vernonia Schreb. (Asteraceae, Vernonieae) comprises more than 1.000 species distributed throughout the tropical regions of Asia, Africa and the Americas (DEMATTEIS 1998). In South America, there are about 350-400 species of *Vernonia*, with most species occurring in Brazil, Argentina, Paraguay and Bolivia (DEMATTEIS & FERNÁNDEZ 2000). According to JONES (1977), the greatest center of diversity of this genus is in southern Brazil. Most South American species of *Vernonia* belong to the section *Lepidaploa* (Cass.) DC. (BENTHAM 1873b; BAKER 1873), although some species also occur in the sections *Eremosia*, *Critoniopsis* (JONES 1973), *Leiboldia* (JONES 1979), *Hololepis*, *Trianthara* and *Stenocephalum* (BAKER 1873).

Taxonomically, *Vernonia* is one of the most complex genera of the family Asteraceae (DEMAT-

TEIS & FERNÁNDEZ 1998) because of its extreme diversity of biological forms, which range from small shrubs to large trees (STUTTS 1988). Although many studies have investigated the taxonomy of this genus (CABRERA 1944; JONES 1979a; 1981; 1982; STUTTS 1981; 1988; ROBINSON 1987a; b; c; 1988a; b; c; 1990a; b; 1992a; b; 1993a; b; 1994; 1996; 1999a; b), the conclusions regarding the relationships involved are contradictory. In a recent classification, ROBINSON (1987a; b; 1988a; 1990a; b; c; 1999a; b) dismembered some species of New World Vernonieae into 22 genera and restricted the genus *Vernonia* to North America. However, most of the modifications proposed were not accepted for other workers in this field. According to HIND (1993), elevation of the section *Lepidaploa* and its subsections to the generic level is premature and does not resolve the taxonomic problems of *Vernonia*.

Cytologically, *Vernonia sensu* Baker has been poorly studied, with the chromosome number having been investigated in less than 20% of the species, mainly North American and African. The chromosome numbers vary from $2n=18$ to $2n=160$ (DARLINGTON & WYLIE 1955; BOLKHO-

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VISIKIKH *et al.* 1969; MOORE 1973; 1974; 1977; GOLDBLATT 1981; 1984; 1985; 1988; GOLDBLATT & JOHNSON 1990; 1991; 1996; 1998). According to DEMATTEIS (2002), a variety of chromosome numbers have been reported for subsections of *Lepidaploa* (genus *Vernonia sensu* BAKER (1873)), including $x=10$ for *Oligocephalae*, $x=16$ for *Macrocephalae* and *Axilliflorae* and $x=17$ for *Paniculatae*.

The chromosome counts for New World species correspond to the basic numbers $x=10$, $x=14$, $x=15$, $x=16$, $x=17$, $x=19$ and $x=31$ (TURNER 1981; RUAS *et al.* 1991; DEMATTEIS 1998). However, the ancestral chromosome number in the Vernoniaeae is considered to be $x=9$ and $x=10$, as reported for African and southeast Asian species (JONES 1979a). According to JONES (1979a), polyploids are very frequent in the New World, with the most common chromosome number being $2n=34$.

In this report, we describe a cytotaxonomic analysis of four subsections of the section *Lepidaploa* of the genus *Vernonia* as part of an investigation into the validity of maintaining various species in diverse subsections (*sensu* Baker) or of allocating them to 22 genera, as suggested by ROBINSON (1987; 1988a; b; 1990; 1999a). The chromosome numbers were determined and the karyotypes were elaborated using conventional techniques in order to allow assessment of the relationship between the chromosomal characters and the taxonomic position of the species.

MATERIAL AND METHODS

Seven populations corresponding to six species of *Vernonia* (section *Lepidaploa*) were analyzed, with samples being collected in “cerrado” and “campo rupestre” areas. In the state of São

Paulo, samples were collected in the cities of Analândia, Campinas and Mogi Guaçu, while in the state of Minas Gerais they were collected in Diamantina and Ouro Branco, and in the state of Goiás they were collected in Chapadão do Céu (Table 1). Voucher specimens of all of the species studied were deposited in the herbarium at the Universidade Estadual de Campinas (UEC) (Table 1). The species were identified according to BENTHAM (1873) and BAKER (1873).

To determine the chromosome number and morphology in mitosis, the tips of recently germinated roots were collected and pretreated with 0.002 M 8-hydroxyquinoline (8Hq) for 5 h at 14–15°C. Subsequently, the root tips were fixed in Farmer solution (ethanol: acid acetic, 3:1, v/v) for 24 h and then transferred to 70% alcohol prior to storage at -20°C. Cytological preparations were obtained using the Giemsa technique (GUERRA 1983), and chromosome numbers were based on counts from an average of 20 cells per species or population.

To elaborate the karyotypes, the cells were drawn using a camera lucida and the chromosomes were measured using digital calipers. The chromosomal data were based on measurements in an average of 10 cells per species or population, with information on chromosome size, centromeric position and secondary constrictions being recorded. The nomenclature adopted for the chromosome morphology was that of GUERRA (1986).

Other parameters used to characterize the karyotype included the total chromatin length (TCL) and the centromeric index (CI), as defined by GUERRA (1988), and the asymmetry index (TF%; HUZIWARA 1962). The intrachromosomal (A_1) and interchromosomal (A_2) asymmetry indexes were calculated according to ZARCO (1986).

Table 1 — *Vernonia* species analyzed in this study, with their respective locations, habitats and voucher specimens. C - cerrado, CR - campo rupestre. Subsections according to BAKER (1873).

Species	Locations	Habitats	Voucher
Subseção <i>Macrocephalae</i> Benth.			
<i>V. buddleiaefolia</i> Mart. ex DC.	GO, Chapadão do Céu	C	MA Batalha 2757
<i>V. tomentella</i> Mart. ex DC.	MG, Diamantina	CR	ME Mansanares 413
Subseção <i>Oligocephalae</i> Benth.			
<i>V. simplex</i> Less.	MG, Ouro Branco	CR	IR Costa 555
Subseção <i>Paniculatae</i> Benth.			
<i>V. polyanthes</i> Less. – pop. 1	SP, Campinas	C	VM Oliveira 55
<i>V. polyanthes</i> Less. – pop. 2	SP, Mogi Guaçu	C	VM Oliveira 11
<i>V. polyanthes</i> Less. – pop. 3	SP, Analândia	C	VM Oliveira 79
<i>V. shwenkiaefolia</i> Mart. in DC.	MG, Diamantina	CR	ME Mansanares 387
Subseção <i>Scorpioideae</i> Benth.			
<i>V. tweediana</i> Baker	SP, Campinas	C	VM Oliveira 30

In preparing the ideograms, the chromosomes were arranged according to their morphology and size, based on the previous studies of DEMATTEIS (1998) and DEMATTEIS & FERNÁNDEZ (1998) in which they described the karyotypes of species of the tribe Vernonieae. Arranging the chromosomes in this manner allowed better visualization of their relationships in the ideogram (DEMATTEIS 1998).

All observations were made using an Olympus model BX51 microscope, with suitable preparations being photographed with Agfa Pan ISO 25 film.

RESULTS

Two chromosome numbers, $2n=32$ and $2n=34$, were seen in the six species of *Vernonia* (Table 2, Figure 3). There were few differences among the karyotypic formulas and ideograms for *V. buddleiaefolia*, *V. tweediana*, *V. simplex* and three populations of *V. polyanthes* (Table 2, Figure 1). All of the specimens had metacentric and submetacentric chromosomes, with the former type predominating. A pair of chromosomes with a secondary constriction was seen in *V. polyanthes* pop. 1 and 2 and in *V. buddleiaefolia* (Figure 1).

The chromosome size varied from 1.2 to 4.9 μm (Table 2), the TCL varied from 31.3 to 50.7 μm and the karyotype symmetry TF% varied from 42.3 to 44.4 (Table 2). The intrachromosomal asymmetry index (A_1) varied from 0.14 to 0.22, and the interchromosomal asymmetry index (A_2) varied from 0.15 to 0.19 (Table 2). The dispersion diagram showed that *V. polyanthes* pop. 1 had the most asymmetrical karyotype (Figure 2).

DISCUSSION

Numerous reports, including those of DARLINGTON & WYLIE (1955), BOLKHOVSKIKH *et al.* (1969), MOORE (1973; 1974; 1977), GOLDBLATT (1981; 1984; 1985; 1988) and GOLDBLATT & JOHNSON (1990; 1991; 1996; 1998), have shown that the chromosomal number in *Vernonia* varies from $2n=18$ to $2n=120$, with the most frequent being $2n=32$ and $2n=34$. The chromosome number of $2n=32$ seen here in a population of *V. polyanthes* (pop. 1) did not agree with previous reports since RUAS *et al.* (1991) mentioned $2n=34$ whereas COLEMAN (1968) reported $n=18$. However, for populations 2 and 3, the count of $2n=34$ coincided with that reported by RUAS *et al.* (1991). The chromosome number of $2n=34$ for *V. simplex* seen here agreed with that reported by JONES (1979a). The chromosome numbers of $2n=32$ for *V. buddleiaefolia* and $2n=34$ for *V. shwenkiaefolia* have not previously been reported.

Among subsections of *Lepidaploa*, basic chromosome numbers have been reported for *Oligocephalae* Benth. ($x=10$), *Macrocephalae* Benth. ($x=16$), *Axilliflorae* Benth. ($x=16$) and *Paniculatae* Benth. ($x=17$) (DEMATTEIS 2002). A cytotoxic discussion of the subsection *Axilliflorae* has been provided by OLIVEIRA *et al.* (2007).

JONES (1979b), GALIANO & HUNZIKER (1987) and ROBINSON (1999a) indicated that the subsection *Oligocephalae*, which is restricted to the New World, had a basic chromosome number of $x=10$. DEMATTEIS (2002) reported a basic number of $x=10$ for four species of *Oligocephalae* Benth., namely, *V. flexuosa* Sims ($n=20$ and $2n=40$), *V. platensis* (Spreng.) Less. ($n=10, 20, 30$ and $2n=20, 40, 50, 60$), *V. pronpiqua* Hieron. var. *pronpiqua*

Table 2 — Chromosomal number, variation in chromosomal size, total chromatin length (TCL), symmetry index (TF%), intrachromosomal asymmetry index (A_1) and interchromosomal asymmetry index (A_2) for the *Vernonia* species investigated in this study. * = first documentation. Subsections according to BAKER (1873).

Species	$2n$	Variation in chromosomal size (μm)	TCL	TF%	A_1	A_2
Subseção <i>Macrocephalae</i> Benth.						
<i>V. buddleiaefolia</i> *	32	2,0 - 4,9	50,7	43,6	0,22	0,19
<i>V. tomentella</i> *	32	-	-	-	-	-
Subseção <i>Oligocephalae</i> Benth.						
<i>V. simplex</i>	34	1,8-3,8	44,3	44,2	0,20	0,19
Subseção <i>Paniculatae</i> Benth.						
<i>V. shwenkiaefolia</i> *	34	-	-	-	-	-
Subseção <i>Scorpioideae</i> Benth.						
<i>V. polyanthes</i> – pop. 1	32	1,4 – 3,4	40,7	44,4	0,14	0,15
<i>V. polyanthes</i> – pop. 2	34	1,2 – 3,1	31,3	42,5	0,20	0,19
<i>V. polyanthes</i> – pop. 3	34	1,4 – 3,3	32,0	42,3	0,21	0,19
<i>V. tweediana</i>	34	1,2 - 3,0	33,3	44,0	0,20	0,18

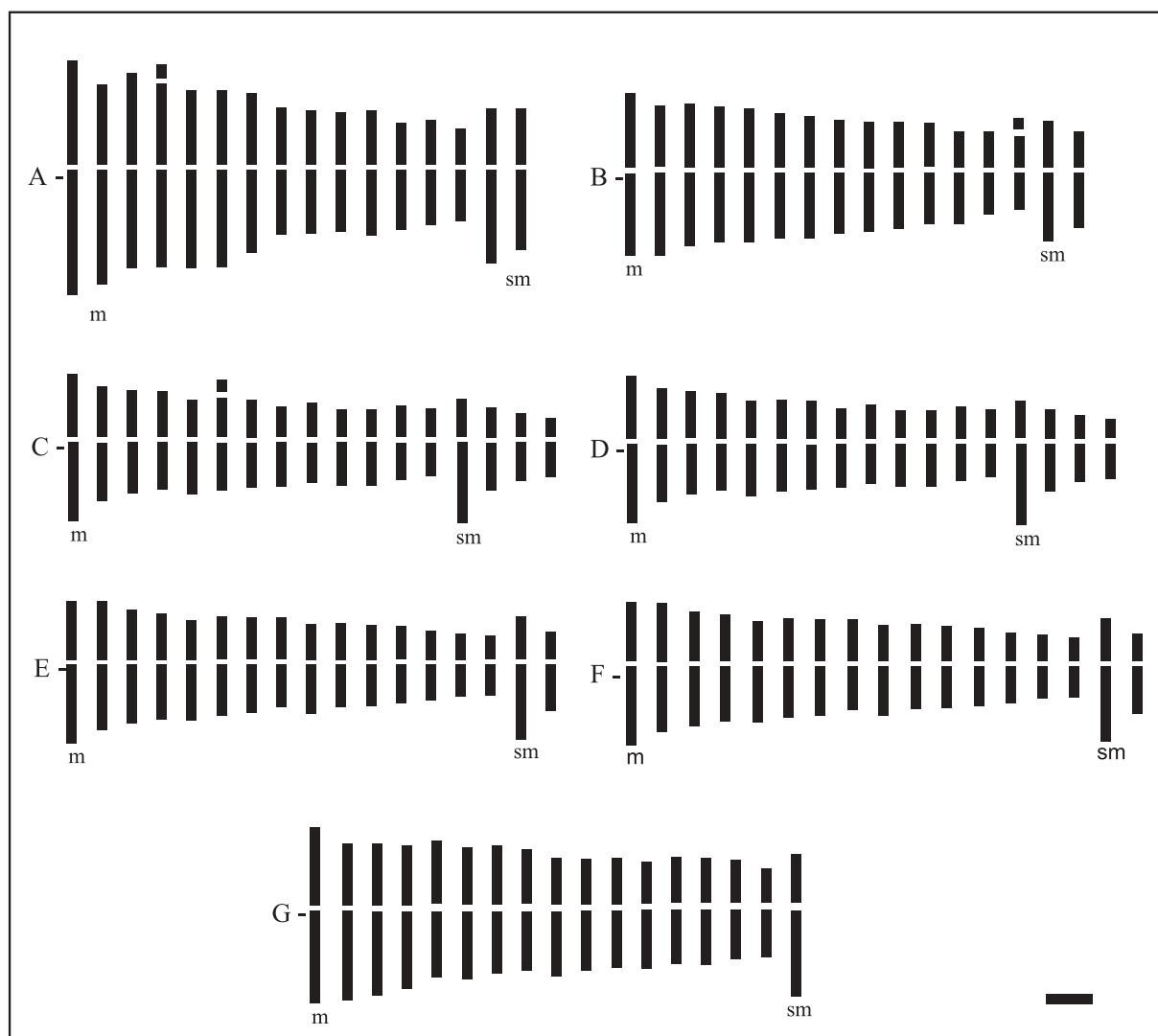


Fig. 1 — Ideograms of species of *Vernonia*. A - *V. buddleiaefolia* ($2n=32$), B - *V. polyanthes* pop.1 ($2n=32$), C - *V. polyanthes* pop.2 ($2n=34$), D - *V. polyanthes* pop.3 ($2n=34$), E - *V. shwenkiaefolia* ($2n=34$), F - *V. simplex* ($2n=34$), G - *V. tweediana* ($2n=34$). m=metacentric, sm=submetacentric. Scale bar = $1\mu\text{m}$

($n=10$ and $2n=20$), *V. pronpiqua* Hieron. var. *canescens* (Chodat) Dematteis ($n=10$ and $2n=20$) and *V. sceptrum* Chodat ($2n=80$). BAKER (1873) included *V. platensis* (Speng.) Less. and *V. flexuosa* Sims in the subsection *Scorpioideae*. However, the subsection *Oligocephalae* also contains species with a basic chromosome number other than 10, such as *V. mollissima* Hook. & Arn., in which $n=\text{ca.}54$ (BERNADELLO 1986). In the present work, *V. simplex*, the only species of this subsection that was studied, had a chromosome number of $2n=34$. RUAS *et al.* (1991) previously reported $2n=40$ for this species, in contrast to JONES (1982), who reported $n=17$, in agreement with our findings.

Chromosome numbers that are multiples of 10 have also been described for species not belong-

ing to the subsection *Oligocephalae* Benth. Recently, OLIVEIRA *et al.* (2007) observed chromosome numbers that are multiples of 10 in two species of the *Axilliflorae*, *V. geminata* ($2n=20$) and *V. adamantium* ($2n=40$), that also showed karyotypic differentiation (larger chromosomes, some of which were submetacentric) compared to other species of this group. DEMATTEIS (1998) reported $2n=20$ for two Argentinian species, *V. lithospermifolia* Hieron. and *V. verbascifolia* Less., belonging to the subsection *Macrocephalae*. According to DEMATTEIS (2002), these two species could be transferred to the *Oligocephalae* because the morphology of their pollen is similar to that of species in the latter group and also because they have chromosome numbers that are multiples of 10.

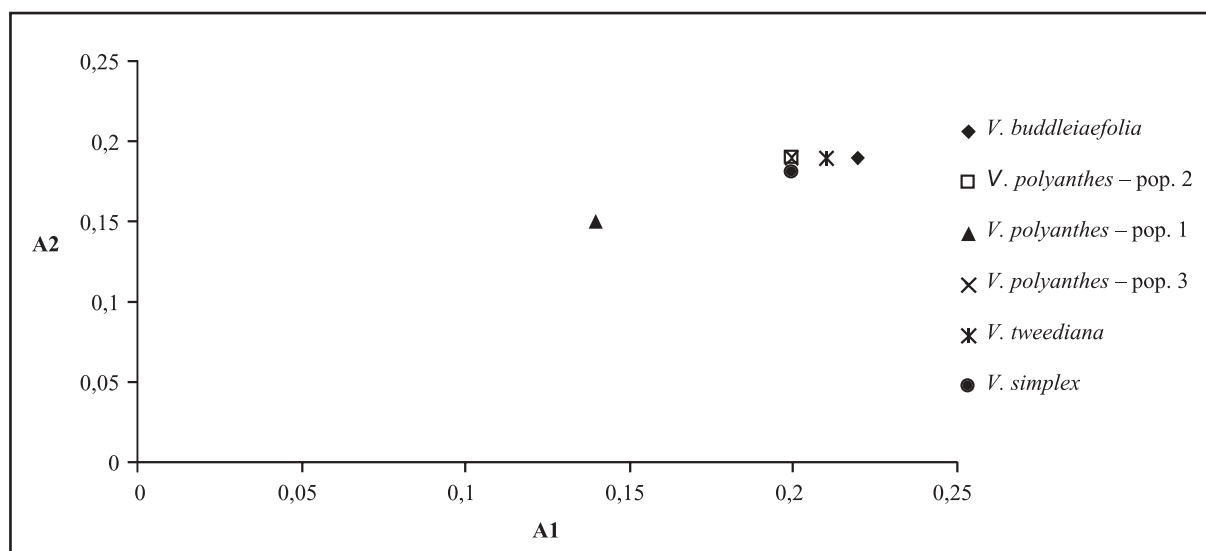


Fig. 2 — Dispersion diagram representing the karyotype asymmetry of species of *Vernonia*. A1 = intrachromosomal asymmetry index and A2 = interchromosomal asymmetry index.

In the taxonomic arrangement proposed by ROBINSON (1999a), *V. lithospermifolia* Hieron. and *V. verbascifolia* Less., with $2n=20$ (DEMATTEIS 1998a), as well as *V. simplex*, with $2n=40$ (RUAS *et al.* 1991), were included in the genus *Lessingianthus*, that previously included the only species of the *Oligocephalae* with a chromosome numbers that was not a multiple of 10, i.e., *V. mollissima* (ca. 54, BERNADELLO 1986) and a population of *V. simplex* ($n=17$, RUAS *et al.* 1991). According to ROBINSON (1999a), *V. pronpiqua* Hieron. var. *pronpiqua* ($n=10$ and $2n=20$), *V. pronpiqua* Hieron. var. *canescens* (Chodat) Dematteis ($n=10$ and $2n=20$) and *V. sceptrum* Chodat ($2n=80$) were included in the genus *Chrysolaena*, which was separated from *Vernonia* (ROBINSON 1988). The species studied by OLIVEIRA *et al.* (2007) that had multiples of 10 (*V. geminata* and *V. adamantium*) were grouped by ROBINSON (1999a) into a third genus, *Lepidaploa*.

According to DEMATTEIS (2002), most of the species in the subsections *Macrocephalae* and *Axilliflorae* have a chromosome number that is a multiple of 16. In addition, the *Macrocephalae* is characterized by a high frequency of polyploids. DEMATTEIS (2002) studied ten species of this subsection, nine of which had a basic chromosome number that was a multiple of 16: five were diploids ($2n=32$), three were tetraploids ($2n=64$) and one was an octaploid ($2n=128$). The two species of the *Macrocephalae* examined here had a basic chromosome number of $x=16$: *V. buddleiaefolia*

($2n=2x=32$) and *V. tomentella* ($2n=2x=32$). JONES (1979b) reported $n=17$ and 34 for *V. bardanoides* Less. and DEMATTEIS (2002) recorded $2n=30$ for *V. rugulosa* Sch. Bip. ex Baker, numbers opponents for the subsection *Macrocephalae*. All of the species of this subsection were included in the genus *Lessingianthus* by ROBINSON (1999a).

The subsection *Paniculatae* Benth. comprises shrubs and small trees. According to JONES (1979b; 1982) and STUTTS (1988), all of the South American and most of the North American species of this group have a basic chromosome number of $x=17$. *Vernonia westiniana*, found in the state of São Paulo, has a chromosome number of $n=17$ (JONES 1979b). All of the species belonging to this subsection have been included by ROBINSON (1999a) in the genus *Vernonanthura* and have a chromosome number that is a multiple of 17, except for *V. nudiflora* Less. that, in addition to $n=17$ (JONES 1974; BERNADELLO 1986) and $2n=34$ (STUTTS *et al.* 1988; RUAS *et al.* 1991), also has $n=16$ and $2n=32$ (COVAS & HUNZIKER 1954; HUNTER 1964). Only one species of this subsection, *V. shwenKiaefolia*, was studied here and had a chromosome number of $2n=34$ (Table 2), in agreement with the basic chromosome number for this subsection. *Vernonia polyanthes* was placed in the *Paniculatae* by DEMATTEIS (2002), whereas BAKER (1973) placed it in the subsection *Scorpioideae*.

The subsection *Scorpioideae sensu* Baker has a variety of multiple chromosome numbers (10, 16

and 17), with most of the species in this subsection being included by ROBINSON (1999a) in the genus *Chrysolaena*. *Vernonanthura* includes *V. mariana* Mart., *V. polyanthes* Less. and *V. tweediana* Baker, whereas *V. argyrotrichia* Sch. Bip. ex Baker is included in *Lepidaploa* (ROBINSON 1999a). The three populations of *V. polyanthes* studied here are part of the subsection *Paniculate* described by DEMATTEIS (2002), as mentioned previously. Two populations had $2n=34$ while one had $2n=32$. RUAS *et al.* (1991) also observed $2n=34$ for this species, whereas COLEMAN (1968; 1970) reported $n=18$ for a population in the city of São Paulo (SP).

One of the main problems in cytotaxonomy is the reporting of wrong chromosome numbers, frequently because of the small size of plant chromosomes (GUERRA 1988). Variation in the chromosome number of a species can also arise from incorrect botanical identifications, and can create serious problems in interpretation of the data. Errors in the identification of a given specimen often reflect the taxonomic complexity of this group because of its diversity and/or the existence of hybrids and polyploids that can generate individuals or species with intermediate morphological characters (STACE 1989). Since the chromosomal analyses of large families and genera are often done by various independent groups, there is need for a critical analysis of the data reported (FAVARGER 1978; GUERRA 2000; MANSANARES *et al.* 2002).

Of the three populations of *V. polyanthes* studied here, population 2 from Mogi Guaçu ($2n=34$) was probably a hybrid of *V. polyanthes* with *V. rubriramea* since it showed morphometric characteristics of these two species (SEMIR, personal communication). According to STUTTS (1988), hybridization is common in *Vernonia* and many hybrid species occupy perturbed environments. Hybrids have been observed between *V. marginata* and *V. baldwini* ssp *interior*, as well as between *V. fasciculata* and *V. baldwini* ssp *interior*, *V. gigantea*, *V. arkansa* and *V. missurica* (JONES 1972).

Another source of divergent chromosome numbers is the existence of chromosomal races or cytotypes, a common finding among vegetables (FAVARGER 1978; MORAWETZ 1984; MANSANARES *et al.* 2002). FAVARGER (1978) stated that determining the true basic chromosome number of a taxon required a knowledge of the chromosomal variation with the taxon based on an analysis of many populations. MORAWETZ (1984) considered that the karyological stability

of tropical plants was much less than generally thought. An example of this is *Duguetia furfuraceae* (Annonaceae), a species widely distributed in the Brazilian "cerrados" that has three chromosomal races of morphologically indistinguishable individuals.

Based on a study of the chromosomes of some species of *Vernonia*, DEMATTEIS (1998) suggested the existence of two cytotypes in *V. saltensis* Hieron, with $2n=64$; BERNADELLO (1986) reported $n=16$ for this taxon. As pointed out by RUAS *et al.* (1991), variation in the chromosome number of a given species is common in *Vernonia*. For example, three chromosome numbers, $2n=16$ (HUNTER 1964; COVAS & HUNZIKER 1954), $2n=32$ (COVAS & HUNZIKER 1954) and $2n=34$ have been reported in *V. nudiflora*, (JONES 1974). Displod cytotypes can occur in *V. polyanthes*, with populations possessing $2n=32$ (present study), $2n=34$ (RUAS *et al.* 1991 and present study) and $2n=36$ (COLEMAN 1968).

According to JONES (1979b) and RUAS *et al.* (1991), the Vernoneae of the New World, in contrast to those of the Old World, shows marked diversity in chromosome number and a high ratio of polyploid species. JONES (1979b) considered $x=17$ as the basic chromosome number for species of New World *Vernonia*, with $x=16$ being uncommon or doubtful. However, other reports (RUAS *et al.* 1991; DEMATTEIS 1998; DEMATTEIS & FERNÁNDEZ 2000; OLIVEIRA *et al.* 2007) and the present study have shown that chromosome numbers that are multiples of 16 are relatively frequent in South American species of *Vernonia*.

The chromosomal analysis described here showed that the karyotypic formula and chromatin total length (TCL) were the main differences among the species, whereas the chromosomal form and the asymmetry index TF% were quite similar (Table 2). The variation in chromosome size seen here (1.2 to 4.9 μm) was greater than that reported by DEMATTEIS & FERNÁNDEZ (2000) (from 1.43 μm for *V. lilacina* to 2.08 μm for *V. aurea*). The pretreatment used by these authors to prepare the chromosomes was the same as that used here. The range of variation was also greater than that reported by OLIVEIRA *et al.* (2007) for species of the subsection *Axilliflorae* of *Lepidaploa*, except for *V. geminata* and *V. adamantium*, the larger chromosomes of which reached 3.3 and 4.63 μm , respectively. Nevertheless, as shown in the ideograms (Figure 1) and pictures (Figure 3), the range of variation was similar among the species, except for *V. bud-*

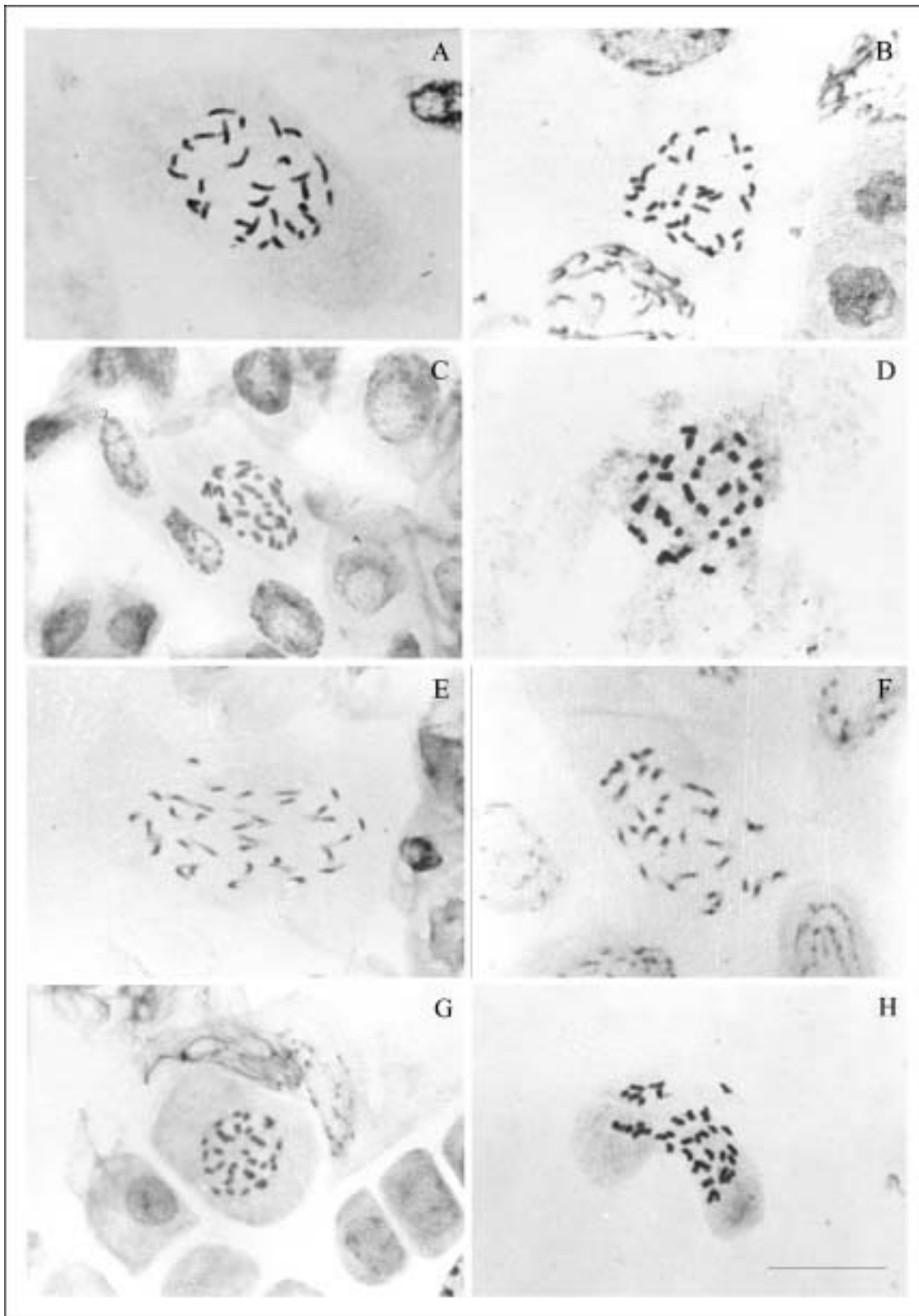


Fig. 3 — Mitotic chromosomes of *Vernonia*. A - *V. buddleaefolia* ($2n=32$), B - *V. polyanthes* pop.1 ($2n=32$), C - *V. polyanthes* pop.2 ($2n=34$), D - *V. polyanthes* pop.3 ($2n=34$), E - *V. shwenkiaefolia* ($2n=34$), F - *V. simplex* ($2n=34$), G - *V. tomentella* ($2n=32$), H - *V. tweediana* ($2n=34$). Scale bar = 10 μ m.

dleiaefolia, which has larger chromosomes than the others (2.0 to 4.9 μm).

The TCL varied considerably among species studied (from 31.3 to 50.7). *Vernonia buddleiaefolia*, which had $2n=32$ chromosomes, had the greatest TCL (50.7) because of the presence of large chromosomes (2.0 – 4.9 μm), whereas *V. polyanthes* pop. 2 (with $2n=34$) had the smallest TCL (31.3 μm), probably because of its small chromosomes (1.2 – 3.1 μm).

All of the species analyzed here had metacentric and submetacentric chromosomes, but in different ratios (Table 3). The submetacentric chromosomes had a minor size (C and D), except for *V. simplex* and *V. buddleiaefolia*, for which short, bigger chromosomes (B) were also observed (Table 3). The predominance of metacentric chromosomes agreed with other reports for the Vernoniaeae (RUAS *et al.* 1991; DEMATTEIS 1996; 1998; DEMATTEIS & FERNÁNDEZ 1998; 2000). DEMATTEIS & FERNÁNDEZ (2000) reported the presence of metacentric and submetacentric chromosomes in nine South American species of *Vernonia*, with *V. lilacina* and *V. polyphylla* showing two pairs of subtelocentric chromosomes.

Table 3 — Karyotype formula for the *Vernonia* species investigated here. Centromeric position: m=metacentric, sm=submetacentric. Chromosomal size: A - entre 4,6 e 3,6 μm ; B - entre 3,5 e 2,6 μm ; C - entre 2,5 e 1,6 μm ; D - entre 1,5 e 0,9 μm .

Species	Karyotype formula
<i>V. buddleiaefolia</i>	6m(A)+3m(B)+5m(C)+1sm(B)+1sm(C)
<i>V. polyanthes</i> – pop. 1	6m(B)+8m(C)+1sm(C)+1sm(D)
<i>V. polyanthes</i> – pop. 2	1m(B)+8m(C)+4m(D)+3sm(C)+1sm(D)
<i>V. polyanthes</i> – pop. 3	1m(B)+8m(C)+4m(D)+3sm(C)+1sm(D)
<i>V. simplex</i>	1m(A)+6m(B)+9m(C)+1sm(B)
<i>V. tweediana</i>	2m(B)+3m(C)+10m(D)+2sm(C)
<i>V. tomentella</i>	-

The interchromosomal (A_2) and intrachromosomal (A_1) asymmetry indexes, which are preferred when there are no marked differences in chromosomal size and morphology, were very similar among the species analyzed (Table 2, Figure 2). The A_1 indexes were very similar (0.14 – 0.22), whereas the A_2 indexes showed even less variation (0.15 – 0.19). The species with the most asymmetrical karyotype was *V. polyanthes* pop.1, which had A_1 and A_2 indexes of 0.14 and 0.15, respectively (Figure 2). The results presented here are more homogenous than those reported by DEMATTEIS (2000), who observed that the most asymmetrical species was *V. polyphylla*, with A_1 and A_2 indexes of 0.285 and 0.210, respectively.

For species of *Axilliflorae* studied by OLIVEIRA *et al.* (2007), the range of variation in A_1 (0.13 – 0.22) and A_2 (0.14 – 0.19) was similar to that of the species studied here, except for *V. geminata*, which had a more asymmetrical karyotype ($A_1=0.25$ and $A_2=0.21$).

According to RUAS *et al.* (1991), DEMATTEIS (1996; 1997) and DEMATTEIS & FERNÁNDEZ (1998), more primitive species ($x=10$, $x=17$) have larger chromosomes than more derived species ($x=14$ and 15). Species with a basic chromosome number of $x=17$ had a chromosomal size that varied from 1.9 to 2 μm , whereas species with $x=14$ or $x=15$ had a chromosomal size that varied from 1.3 to 1.4 μm (DEMATTEIS & FERNÁNDEZ 1998). Our results did not agree with those of DEMATTEIS & FERNÁNDEZ (1998). *Vernonia tweediana* ($2n=34$) had the smallest chromosomal size (1.2 – 3.0 μm) while *V. buddleiaefolia* ($2n=32$) had the largest (2.0 – 4.9 μm).

The difference among the karyotypes of the three populations of *V. polyanthes* analyzed here (Table 2) deserves further investigation. As already mentioned, *V. polyanthes* shows some differences in its chromosome number, size and morphology, including the localization of secondary constrictions. Populations 2 and 3 ($2n=34$) had similar karyotypes and ideograms, while it was not possible to locate the secondary constriction in population 3 using conventional staining (Figure 1). Population 1 ($2n=32$) differed from populations 2 and 3 in its chromosome number ($2n=34$) and karyotypic formula (Figure 1, Tables 2 and 3). The chromosomes were larger, with the main difference being the ratio of submetacentric chromosomes of intermediate size (type C) (Table 3): only one pair of this type was seen in this population, while in populations 2 and 3 three pairs were observed. OLIVEIRA *et al.* (2007) have provided possible explanations for the karyotypic variation among populations of *V. remotiflora*.

The results obtained here for several species of *Vernonia* are still insufficient for a conclusive general cytotaxonomic discussion of this group, primarily because of the lack of a characteristic or standard karyotype for the species in each of the different taxonomic groups, regardless of whether these are defined according to BAKER (1873) or ROBINSON (1999a). In addition, the number of species analyzed is still small. Nevertheless, there is apparently only a very tenuous relationship between the chromosome numbers observed here and reported in the literature compared with the taxonomic alterations suggested by ROBINSON

(1999a) for the genera *Lessingianthus*, *Vernonanthura* and *Chrysolaena* that were originally part of *Vernonia sensu* BAKER (1873). ROBINSON (1999a) included some species belonging to the subsections *Scorpioideae* and *Oligocephalae sensu* BAKER (1873) in the genus *Chrysolaena*, most of which have a basic chromosome number of $x=10$. Most of the species belonging to the subsection *Macrocephalae sensu* BAKER (1873), and which were transferred to the genus *Lessingianthus* by ROBINSON (1999a), have a basic chromosome number of $x=16$. Likewise, most of the species belonging to the subsection *Paniculatae sensu* BAKER (1873) and transferred to the genus *Vernonanthura* by ROBINSON (1999a) have a basic chromosome number of $x=17$.

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REFERENCES

- BAKER J.G., 1873 — *Compositae I. Vernoniae*. In C.F. Martius and A.G. Eichler (Eds), *Flora brasiliensis*, 6: 180.
- BENTHAM G., 1873a — *Compositae*. In G. Bentham and J.D. Hooker (Eds), *Genera Plantarum*, 2: 163-533.
- 1873b — *Notes of the classification history, and geographical distribution of the Compositae*. *Botanical Journal of Linnean Society*, 13: 355-577.
- BERNARDELLO L.M., 1986 — *Números cromosômicos en Asteraceae de Córdoba*. *Darwiniana*, 27: 169-178.
- BOLKHOVISKIKH Z., V. GRIF, T. MATVEJEVA, H.V. ZAKHARYEVA, 1969 — *Chromosome number of flowering plants*. V.L. Komarov Botanical Institute, Academy of Sciences of the USSR, Moscow.
- CABRERA A.L., 1944 — *Vernoniae argentinas (Compositae)*. *Darwiniana*, 6: 265-379.
- COLEMAN J.R., 1968 — *Chromosome numbers in Brazilian Compositae*. *Rhodora*, 70: 228-379.
- 1970 — *Additional chromosome numbers in Brazilian Compositae*. *Rhodora*, 72: 789-794.
- COVAS G. and J. HUNZIKER, 1954 — *Estudios cariológicos en Antófitas IV parte*. *Revista Investigación Agrícola*, 8: 249-253.
- DARLINGTON C. and A.P. WYLIE, 1955 — *Chromosome Atlas of Flowering Plants*. George Allen & Unwin LTD, Ruskin House Museum Street, London.
- DEMATTEIS M., 1996 — *Estúdios cromosômicos en especies Argentinas de Vernonia (Asteraceae)*. *Bonplandia*, 9(1-2): 103-110.
- 1997 — *Números cromosômicos y cariotipos de algunas especies de Vernonia (Asteraceae)*. *Boletín de la Sociedad Argentina de Botánica*, (1-2): 85-90.
- 1998 — *Chromosome studies of some Vernonia species. (Asteraceae)*. *Genetics and Molecular Biology*, 21: 381-385.
- 2002 — *Cytotaxonomic analysis of South American species of Vernonia (Vernoniae: Asteraceae)*. *Botanical Journal of Linnean Society*, 139: 401-108.
- and A. FERNÁNDEZ, 1998 — *Karyotypes of seven South American species of Vernonia (Asteraceae)*. *Cytologia*, 63: 323-328.
- and A. FERNÁNDEZ, 2000 — *Chromosomes studies on nine South American species of Vernonia (Vernoniae, Asteraceae)*. *Caryologia*, 53(1): 61.
- FAVARGER C., 1978 — *Philosophie des comptages de chromosomes*. *Taxon*, 27(5-6): 441-448.
- GALLIANO N.G. and J.H. HUNZIKER, 1987 — *Estudios cariológicos em Compositae. IV. Vernoniae y Eupatorieae*. *Darwiniana*, 28: 1-8.
- GILL L.S., 1978 — *Karyotype of Vernonia aemulans Vatke (Compositae)*. *Adansonia*, 18: 375-376.
- GOLDBLATT P., 1981 — *Index to plant chromosome numbers 1975-1978*. Monographs in systematic Botany from the Missouri Botanical Garden. V5. Saint Louis, Missouri Botanical Garden.
- 1984 — *Index to plant chromosome numbers 1979-1981*. Monographs in Systematic Botany from the Missouri Botanical Garden. V8. Saint Louis, Missouri Botanical Garden.
- 1985 — *Index to plant chromosome numbers 1982-1983*. Monographs in Systematic Botany from the Missouri Botanical Garden. V13. Saint Louis, Missouri Botanical Garden.
- 1988 — *Index to plant chromosome numbers 1984-1985*. Monographs in Systematic Botany from the Missouri Botanical Garden. V23. Saint Louis, Missouri Botanical Garden.
- and D.E. JOHNSON, 1990 — *Index to plant chromosome numbers 1986-1987*. Monographs in Systematic Botany from the Missouri Botanical Garden. V30. Saint Louis, Missouri Botanical Garden.
- and D.E. JOHNSON, 1991 — *Index to plant chromosome numbers 1988-1989*. Monographs in Systematic Botany from the Missouri Botanical Garden. V40. Saint Louis, Missouri Botanical Garden.
- and D.E. JOHNSON, 1996 — *Index to plant chromosome numbers 1992-1993*. Monographs in Systematic Botany from the Missouri Botanical Garden. V58. Saint Louis, Missouri Botanical Garden.
- and D.E. JOHNSON, 1998 — *Index to plant chromosome numbers 1994-1995*. Monographs in Systematic Botany from the Missouri Botanical Garden. V69. Saint Louis, Missouri Botanical Garden.
- GUERRA M., 1983 — *O uso de Giemsa em citogenética vegetal - comparação entre a coloração simples e o bandamento*. *Ciência e cultura*, 35: 190-193.
- 1986 — *Reviewing the chromosome nomenclature of Levan et al.* *Revista Brasileira de Genética*, 4: 741-743.

- 1988 — *Introdução à citogenética Geral*. Editora Guanabara. Rio de Janeiro.
- 2000 — *Chromosome number variation and evolution in Monocots*. In K.L. Wilson and D.A. Morrison, (Eds), *Monocots: Systematic and Evolution*, Melbourne, CSIRO, p. 127-136.
- HIND D.J.N., 1993 — *Notes of Compositae of Bahia, Brazil*. I. Kew Bulltein, 48: 245-277.
- HUNTER G.E., 1964 — *Chromosome numbers in Vernonia: section Lepidaploa: subsection Paniculatae v. rae*. Southwestern Naturalist, 9: 239-244.
- HUZIWARA Y., 1962 — *Karyotype analysis in some genera of Compositae*. VIII. Further studies on the chromosomes of *Aster*. American Journal of Botany, 49: 116-119.
- JONES S.B., 1972 — *Hibridization of acaulis and V. novaboracensis (Compositae) in the piedmont of North Carolina*. Castanea, 37: 244.
- 1973 — *Revision of Vernonia section Eremosis (Compositae) in North America*. Brittonia, 25(2): 86-115.
- 1977 — *Vernonieae - systematic review*. In V.H. Heywood, J.B. Harbone and B.L. Turner (Eds), *The biology and chemistry of the Compositae I*, Vol. I, American Press. London, p. 503-521.
- 1979a — *Synopsis and pollen morphology of Vernonia (Compositae: Vernonieae) in the New World*. Rhodora, 81: 425-447.
- 1979b — *Chromosome numbers of Vernonieae (Compositae)*. Bulletin of the Torrey Botanical Club, 106: 79-86.
- 1981 — *Synoptic classification and pollen morphology of Vernonia (Compositae: Vernonieae) in the Old World*. Rhodora, 83: 59-75.
- 1982 — In A. Lové (Eds), *IOPB chromosome number report LXXIV*. Taxon, 31: 126-127.
- LOMBELLO R.A. and E.R. FORNI-MARTINS, 1998 — *Chromosomal studies and evolution in Sapindaceae*. Caryologia, 51 (1): 81-93.
- MANSANARES M.E., E.R. FORNI-MARTINS and J. SEMIR, 2002 — *Chromosome numbers in the genus Lychnophora Mart. (Lychnophorinae, Vernonieae, Asteraceae)*. Caryologia, 55(4): 367-374.
- MATHEW A. and P.M. MATHEW, 1982 — *Studies on South Indian Compositae II. Cytology of the genus Vernonia Schreb.* Cytologia, 47: 163-169.
- MOORE R.J.(Eds), 1973 — *Index to plant chromosome numbers 1967-1971*. Regnum veget., 90: 1-539.
- 1974 — *Index to plant chromosome numbers 1972*. Regnum veget., 91: 1-108.
- 1977 — *Index to plant chromosome numbers 1973 - 1974*. Regnum veget., 96: 1-257.
- MORAWETZ W., 1984 — *Kariological races and ecology of the brazilian Duguetia furfuracea as compared with Xylopia aromatica (Annonaceae)*. Flora, 175: 195-209.
- OLIVEIRA V.M., E.R. FORNI-MARTINS and J. SEMIR, 2007 — *Cytotaxonomy of species of Vernonia (Asteraceae: Vernonieae), section Lepidaploa, subsection Aixilliflorae*. Botanical Journal of the Linnean Society (accepted).
- ROBINSON H., 1987a — *Studies of the Lepidaploa complex (Vernonieae: Asteraceae), III: two new genera, Cyrtucymura and Eirmocephala*. Proceedings of the Biological Society of Washington, 100: 844-855.
- 1987b — *Studies of the Lepidaploa complex (Vernonieae: Asteraceae), the genus Stenocephalum Sch. Bip.* Proceedings of the Biological Society of Washington, 100: 578-583.
- 1987c — *Studies of the Lepidaploa complex (Vernonieae: Asteraceae), a new genus Echinocoryne*. Proceedings of the Biological Society of Washington, 100: 584-589.
- 1988a — *A new combination of Vernonia libertadenensis. S.B. Jones, with notes and descriptions of additional Andean species of Baccharis*. Phytologia, 65: 34-46.
- 1988b — *Studies in the Lepidaploa complex (Vernonieae: Asteraceae) IV. The new genus Lessinglanthus*. Proceedings of the Biological Society of Washington, 100 (4): 929-951.
- 1988c — *Studies in the Lepidaploa complex (Vernonieae: Asteraceae) V. The new genus Chrysolaena*. Proceedings of the Biological Society of Washington, 100 (4): 952-958.
- 1990a — *Studies in the Lepidaploa complex (Vernonieae: Asteraceae), VII: the genus Lepidaploa*. Proceedings of the Biological Society of Washington, 103: 248-253.
- 1990b — *Six combinations in Baccharoides Moench and Cyathillium Blume*. Proceedings of the Biological Society of Washington, 103: 248-253.
- 1992a — *A new genus Vernonanthuria (Vernonieae: Asteraceae)*. Phytologia, 72: 65-76.
- 1992b — *Mesanthopora, a new genus of Vernonieae (Asteraceae) from Paraguay*. Novon, 2: 169-172.
- 1993a — *Three new genera of Vernonieae from South America, Dasyandantha, Dasyandanthina and Quechualia (Asteraceae)*. Proceedings of the Biological Society of Washington, 106 (4): 775-785.
- 1993b — *A review of the genus Critoniopsis in Central and South America (Vernonieae: Asteraceae)*. Proceedings of the Biological Society of Washington, 106 (3): 606-627.
- 1994 — *Cololobus, Pseudopiptocarpha and Trepadonia, three new genera from South America (Vernonieae: Asteraceae)*. Proceedings of the Biological Society of Washington, 107 (3): 557-568.
- 1996a — *The status of generic and subtribal revisions in the Vernonieae*. In D.J.N. Hind and H.J. Beentje (Eds), *Compositae: Systematics*. Kew, Whitestable Litho Printers Ltd., London, n. 1, p. 511-529.
- 1999a — *Generic and subtribal classification of American Vernonieae*. Smithsonian Contributions to Botany, 89: 1-116.
- 1999b — *Two new subtribes, Stokessinae and Paourinae, of the Vernonieae (Asteraceae)*. Proceedings of the Biological Society of Washington, 112(1): 216-219.
- RUAS P.M., C.F. RUAS, O.S. VIEIRA, N.I. MATZENBACHER and N.S. MARTINS, 1991 — *Cytogenetics of*

- genus *Vernonia* Schreber (Compositae). *Cytologia*, 56: 239-247.
- STACE C.A., 1989 — *Plant Taxonomy and Biosystematics*. Cambridge University Press, New York.
- STUTTS J.G., 1981 — *Taxonomic revision of Pollalesta H.B.K. (Compositae: Vernonieae)*. *Rhodora*, 83: 385-419.
- 1988 — *Taxonomic revision of Vernonieae Subsect. Chamaedrys (Compositae: Vernonieae)*. *Rhodora*, 90: 37-99.
- TURNER B.L., 1981 — *New species and combinations in Vernonia sections Leiboldia and Lepidonia (Asteraceae), with a revisional conspectus of the groups*. *Brittonia*, 33: 401-412.
- ZARCO C.R., 1986 — *A new method for estimating karyotype asymetry*. *Taxon*, 35(3): 526-530.

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