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Cytotaxonomy of *Lychnophora* Mart. (Asteraceae: Vernonieae: Lychnophorinae) species

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Abstract — Most species of the genus *Lychnophora* Mart. are endemic to the Brazilian "*campos rupestres*" of Minas Gerais, Bahia and Goiás, with high degree of endemism in many species. There is disagreement between different authors regarding delimitation of the species and, consequently, the amount of species (from 11 to 68). This interpretation difference leads to sinonimization and the transference of several species to closely related genera. The cytotaxonomic study of species of *Lychnophora* was made aiming at increase of knowledge of chromosome characteristics that could be useful to the understanding of the taxonomy of the group as a whole. Chromosome numbers of about eighteen species were determined, with 2n=34, 36 or 38. These chromosome numbers were distributed among species of four sections of *Lychnophora*, so they can't be used as distinctive characters for intergeneric and infrageneric levels below section level. However, chromosome numbers were very important for the differentiation of some species of *Lychnophora*, whose taxonomic limits have been questioned. Other karyotype characters were analysed in three species of the subtribe, like chromosome size and morphology, showing constancy of these characters. The chromosomes are small, with 1.0 to 2.58μm, and they are mainly metacentric, however some submetacentrics were observed.

Key words: Asteraceae, chromosome, cytotaxonomy, karyotype, *Lychnophora*, Lychnophorinae, Vernonieae.

INTRODUCTION

The genus *Lychnophora* was described by Martius (1822), who considered the presence of glomerular inflorescences and achenes with deciduous pappus as differentiating features. It occurs exclusively in Brazilian's "campos rupestres", with a great number of endemic species. *Lychnophora* shows some taxonomic problems related to the species circunscription and delimitation, with acceptance of 11 (Coile and Jones 1981), 34 (Robinson 1999), or 68 species (Semir 1991). Coile and Jones (1981) and Semir (1991) pointed out the possible occurrence of natural interspecific hybrids as one of the factors that complicated the genus' taxonomy. Also, there is some conflict in circumscription of the genera in subtribe Lychnophorinae (Coile and Jones 1981; MacLeish 1984; 1987; MacLeish and Schumaker 1984; Semir 1991; Robinson 1992; 1996a; b; 1999; Hind 1995 2000).

According to Semir (1991), the genus *Lychnophora* is divided in 6 sections: *Lychnophora, Lychnophoriopsis, Lychnophorioides, Lychnocephaliopsis, Sphaeranthus* and *Chronopappus*. The author classified these sections based on inflorescence morphology and presence or absence of sheath or petioles.

Before 1980, chromosome numbers for *Lychnophora sensu* Semir (1991) species were not known. Even in present day karyotype information, as chromosome length and morphology, is not available. The initial suggested chromosome number was n=17/2n=34, for *L. ericoides*, *L. tomentosa*, *L. heterotheca* (= *L. candelabrum)* and *L. diamantinana* (Coile and Jones 1981). Later, Jones (1982) cited 2n=36 for *Eremanthus reflexo-auriculatus (= L. reflexo-autriculata)* and Carr *et al.* (1999) reported an atypical chromosome number (2n=18 +1B) for *L. phylicifolia*.

Recently, Mansanares *et al.* (2002) carried out cytotaxonomic studies in *Lychnophora* Mart. *sensu* Semir (1991) and demonstrated how those approaches are important as a subsidy for the genus taxonomy. The observed data shows variation in chromosome numbers with 2n=34, 2n=36 and

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2n=38, but the most common number among studied species is 2n=34. According to MANSANARES *et al.* (2002), *L. ericoides* and *L. gardneri* (*sensu* SEMIR 1991) show different chromosome numbers (2n=34 and 2n=36, respectively), reinforcing the species segregation, in opposition to COILE and JONES (1981), who considered them only as *L. ericoides*.

The present study, aimed at enlarging the cytotaxonomic knowledge of *Lychnophora,* presents chromosome numbers for other species and karyotypes of some of them. The results were compared with the different taxonomic treatments for the genus by COILE and JONES (1981), SEMIR (1991) and ROBINSON (1999).

MATERIAL AND METHODS

Eighteen *Lychnophora* species were analysed (Table 1), all collected in "campos rupestres" of several areas of the State of Minas Gerais, as Diamantina, Montes Claros and Grão Mogol, and of the State of Bahia, in Rio de Contas. Voucher specimens were deposited at the herbarium of Campinas State University (UEC).

Lychnophora species were analysed and identified according to SEMIR's (1991) account, although some of the species (*L. angelae*, *L. cryptomerioides, L. grazielae, L. itacambirensis*, *L. mutica*, *L. nanuzae*, *L. prostrata* and *L. sobolifera*) have not yet been published and were only mentioned in Semir's doctorate dissertation.

The mitotic root-tips were pre-treated by 5 hours in 8-hydroxiquinoline 0,002M solution, at 15°C, fixed in Carnoy solution (acetic acid – ethanol (1:3) and then, stained according to HCl/ Giemsa technique (GUERRA 1983).

For the meiotic study, flower buds were also fixed in Farmer's solution and stored in ethanol 70 at 4°C. Squashes of pollen mother cells were made using the acetocarmine 1,2% technique (MEDINA and CONAGIN 1964).

The chromosome number counts were based on at least 20 metaphase plates from different individuals from each species, except *L. villosissima* (Population 01), for wich 59 metaphase mitotic and 61 meiotic cells were observed (Table 1).

Ideograms were prepared for three species: chromosome length was measured in at least 10 metaphase plates from each species/population, using the average measure for chromosome, of chromosome length and centromeric position. Chromosome nomenclatural morphology adopted here was suggested by GUERRA (1986). For karyotype characterization measures as TCL (total chromosome length), CI (centromeric in-

Table 1 — Species and populations of *Lychnophora* (subtribe Lychnophorinae) and respective localities and vouchers.

Species	Local	Population	Voucher
Lychnophora sect. Lychnophora			
L cryptomerioides Semir and Leitão Filho (inéd.)	MG, Diamantina	Pop01	Mansanares et al. 163
L. granmogolensis (A.P.Duarte) Semir and Leitão Filho (inéd.)	BA, Rio de Contas	Pop01	Moraes and Aona MDM452
L. itacambirensis *Semir and Leitão Filho (inéd.)*	MG, Itacambira	Pop01	Mansanares et al. 266
L. martiana Gard.	MG, Juramento	Pop01	Mansanares et al. 180
	MG, Montes Claros	Pop02	Semir and Duthil s/n°
L. mutica *Semir and Leitão Filho (inéd.)*	MG, Santana do Riacho	Pop01	Mansanares and Kinoshita 270
L.nanuzae *Semir and Leitão Filho (inéd.)*	MG, Diamantina	Pop01	Mansanares et al. 143
L. prostrata *Semir and Leitão Filho (inéd.)*	MG, Diamantina	*Pop01*	*Mansanares et al. 00/26*
L. ramosissima *Semir and Leitão Filho (inéd.)*	MG, Diamantina	*Pop01*	*Mansanares et al. 171*
L. rosmarinifolia *Mart.*	MG, Diamantina	*Pop01*	*Mansanares et al. 00/29*
	MG, Diamantina	*Pop02*	*Mansanares et al. 00/36*
L. salicifolia *Mart.*	MG, Juramento	*Pop01*	*Mansanares et al. 191*
L.sobolifera *Semir and Leitão Filho (inéd.)*	MG, Diamantina	*Pop01*	*Mansanares et al. 110*
L. villosissima Mart.	MG, Diamantina	*Pop01*	*Mansanares et al. 00/23*
	MG, Diamantina	*Pop02*	*Mansanares et al. 00/37*
	MG, Diamantina	*Pop03*	*Mansanares et al. 00/93*
L. uniflora Schultz-Bip.	BA, Rio de Contas		*Moraes and Aona MDM 508*
Lychnophora sect. Lychnophorioides			
L. angelae Semir and Leitão Filho (inéd.)	MG, Serra do Cipó	*Pop01*	*Mansanares and Verola 377*
L. leucodendron (Mattf.) Semir and Leitão Filho (inéd.)	BA, Rio de Contas	*Pop01*	*Moraes and Aona MDM 503*
	BA, Rio de Contas	*Pop02*	*Moraes and Aona MDM521*
L. sincephala Gard.	MG, Diamantina	*Pop01*	*Mansanares et al.232*
Lychnophora sect. Lychnocephaliopsis			
L. grazielae Semir and Leitão Filho (inéd.)	MG, Serra do Cipó	*Pop01*	*Mansanares and Verola376*
Lychnophora sect. Chronopappus			
L. markgravii G.M. Barroso	MG, Grão Mogol	Pop01	*Aona 701*

dex) and TF% (asymmetric index) were used (HUZIWARA 1968).

Cells with well spread chromosomes were observed through a common photomicroscope. Photographs were taken using Agfa Pan ISO 25 film.

RESULTS

Three different chromosome numbers were obtained in eighteen species of *Lychnophora* (*sensu* SEMIR 1991), 2n=34, 2n=36 and 2n=38 (Table 2, Figures 2 – 3). All these chromosome

Table 2 — Chromosome numbers in species of *Lychnophora,* as found in literature for Lycnhophorinae subtribe and present data (*: chromosome counts reported for the first time).

Species	n	2n	Reference
Lychnophora* sect. *Lychnophora			
L. cryptomerioides Semir and Leitão (inéd.)*	18	36	**Present work**
L. diamantinana Coile and Jones	17	-	Coile and Jones, 1981
	17	34	Mansanares *et al.* 2002
L. ericoides Mart.	17	-	Coile and Jones, 1981
	-	34	Mansanares *et al.* 2002
L. gardneri Sch. Bip.	-	36	Mansanares *et al.* 2002
L. granmogolensis (A.P.Duarte) Semir and Leitão (inéd.)		18+B	Carr *et al*., 1999
		34	**Present work**
L. itacambirensis Semir and Leitão (inéd.)*	18	36	**Present work**
L. martiana Gard.*	-	36	**Present work**
L. mutica Semir and Leitão (inéd.)	ca.19	-	Mansanares *et al.* 2002
	-	38	**Present work**
L nanuzae Semir and Leitão (inéd.)*	-	36	**Present work**
L. passerina (Mart. ex DC.) Gardner	17	34	Mansanares *et al.* 2002
L. pinaster Mart.	17	-	Mansanares *et al.* 2002
L. pohlii Sch. Bip.	18	-	Mansanares *et al.* 2002
L. prostrata Semir and Leitão (inéd.)	17	-	Mansanares *et al.* 2002
	-	34	**Present work**
L. pseudovillosissima Semir and Leitão (inéd.)	-	38	Mansanares *et al.* 2002
L. ramosissima Mart.*	18	36	**Present work**
L. rosmarinifolia Mart.*	-	36	**Present work**
L. rupestris Semir and Leitão (inéd.)	17	34	Mansanares *et al.* 2002
L. salicifolia Mart.*	18	36	Mansanares *et al.* 2002
	-	36	**Present work**
L. sobolifera Semir and Leitão Filho (inéd.)*	18	-	**Present work**
L staavioides Mart.	34	34	Mansanares *et al.* 2002
L. villosissima Mart.*	18	36	**Present work**
L. uniflora Schultz-Bip.*	-	36	**Present work**
Lychnophora* sect. *Lychnophorioides			
L. angelae Semir and Leitão Filho (inéd.)*	17	-	**Present work**
L. leucodendron (Mattf.) Semir and Leitão (inéd.)*	-	34	**Present work**
L. sincephala (Sch. Bip.) Sch. Bip.*	-	34	**Present work**
Lychnophora* sect. *Chronopappus			
L. markgravii G.M. Barroso*		38	**Present work**
Lychnophora* sect. *Lychnocephaliopsis			
L. cipoensis Semir and Leitão (inéd.)	19	38	Mansanares *et al.* 2002
L. grazielae Semir and Leitão Filho (inéd.)*	19	-	**Present work**
L. joliana Semir and Leitão (inéd.)	18	36	Mansanares *et al.* 2002
L. mello-barretoi G.M.Barroso	19	38	Mansanares *et al.* 2002
L. sellowii Sch. Bip.		38	Mansanares *et al.* 2002
L. tomentosa (Mart. ex DC.)Sch.Bip.	17	-	Coile and Jones, 1981
	19	38	Mansanares *et al.* 2002
Lychnophora* sect. *Lychnophoriopsis			
L. heterotheca (Sch.Bip.) Coile and Jones *(=L. candelabrum* Sch. Bip.)	17		Coile and Jones, 1981
L. candelabrum Sch. Bip.	-	36	Mansanares *et al.* 2002
Eremanthus			
E. eleagnus Sch.Bip.	15	-	Turner *et al.* 1979
E. reflexo auriculatus Barroso (*L reflexoauriculata*)		36	Jones 1982
Minasia			
M. alpestris	17	-	Dematteis 1998

numbers were obtained in the section *Lychnophora*, but in the other analysed sections in this study 2n=36 was not found. Of the twenty-five species that constitute the section *Lychnophorioides* (Semir 1991) only three were analysed, *L. angelae, L. leucodendron* and *L. sincephala,* for the time being appearing constant (2n=34). One species of the section *Lychnocephaliopsis* and one of *Chronopappus* presented 2n=38.

There were some differences in chromosome length and morphology among *Lycnhophora markgravii, L. rosmarinifolia* and *L. uniflora* (Figure 1, Table 3). Chromosome length varied from 1.10μm to 2,58μm, (Table 3) and the total chromosome length (TCL) was relatively similar between *L. rosmarinifolia* and *L. markgravii*, it was larger in *L. uniflora,* although this species showed an equal or smaller chromosome number than the other species here analysed.

All analysed species showed metacentric and submetacentric chromosomes, although in different proportions according to karyological formula (Table 3). Similarities in karyological symmetry were observed in TF% values, all about 42 (Table 3).

In *Lychnophora villosissima* (population 01) chromosome numbers of 2n=36, 2n=37 and 2n=38 were observed (Figure 2-E). These numbers occurred in cells from the same root-tip: three root-tips had only 2n=36 chromosomes, one had only 2n=37 and one only 2n=38 while most root-tips showed all three chromosome numbers in the same root-tip.

DISCUSSION

Among the eighteen species of *Lychnophora* studied in this work, fourteen had their chromo-

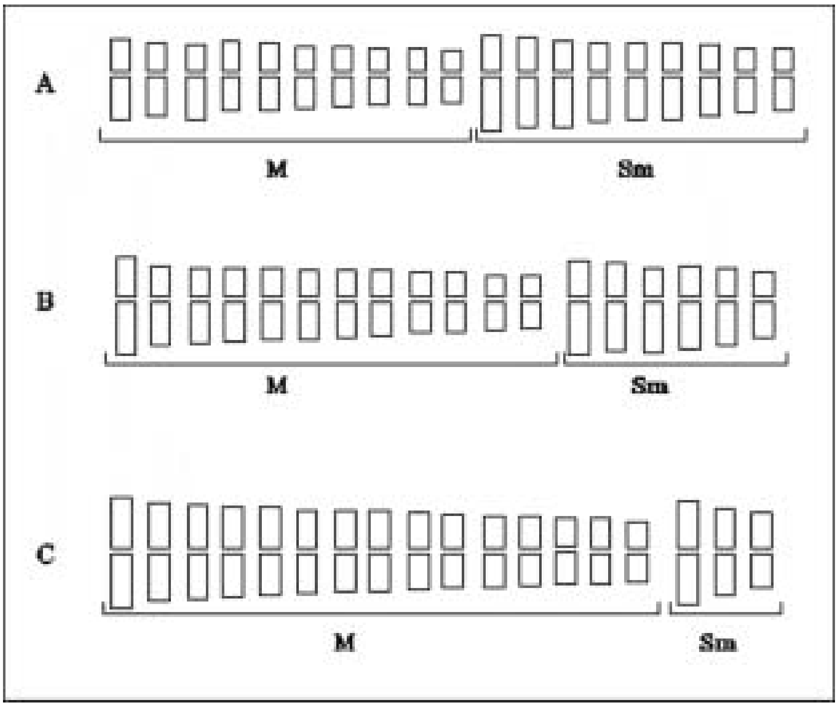

Fig. 1 — Ideograms of *Lychnophora* species **A:** *L. markgravii*, 2n=38, 10m+9sm. **B:** *L. rosmarinifolia (Pop01)*, 2n=36, 15m+3sm. **C:** *L. uniflora*, 2n=36, 12m+6sm. (m = metacentric; sm = submetacentric).

Table 3 — Chromosome numbers, karyotypical formula, chromosome length variation into species, TCL (total chromosome length), TF% (karyotipical symmetry) of *Lychnophora* species.

Species	2n	Karyotipical Formula	Length Variation (μm)	TCL (μm)	TF %
L. markgravii	38	20m+18sm	1.14-2.23	61.50	41.26
L. rosmarinifolia	36	30m+6sm	1.10-2.10	54.88	42.34
L. uniflora	36	24m+12sm	1.30-2.58	68.69	41.35

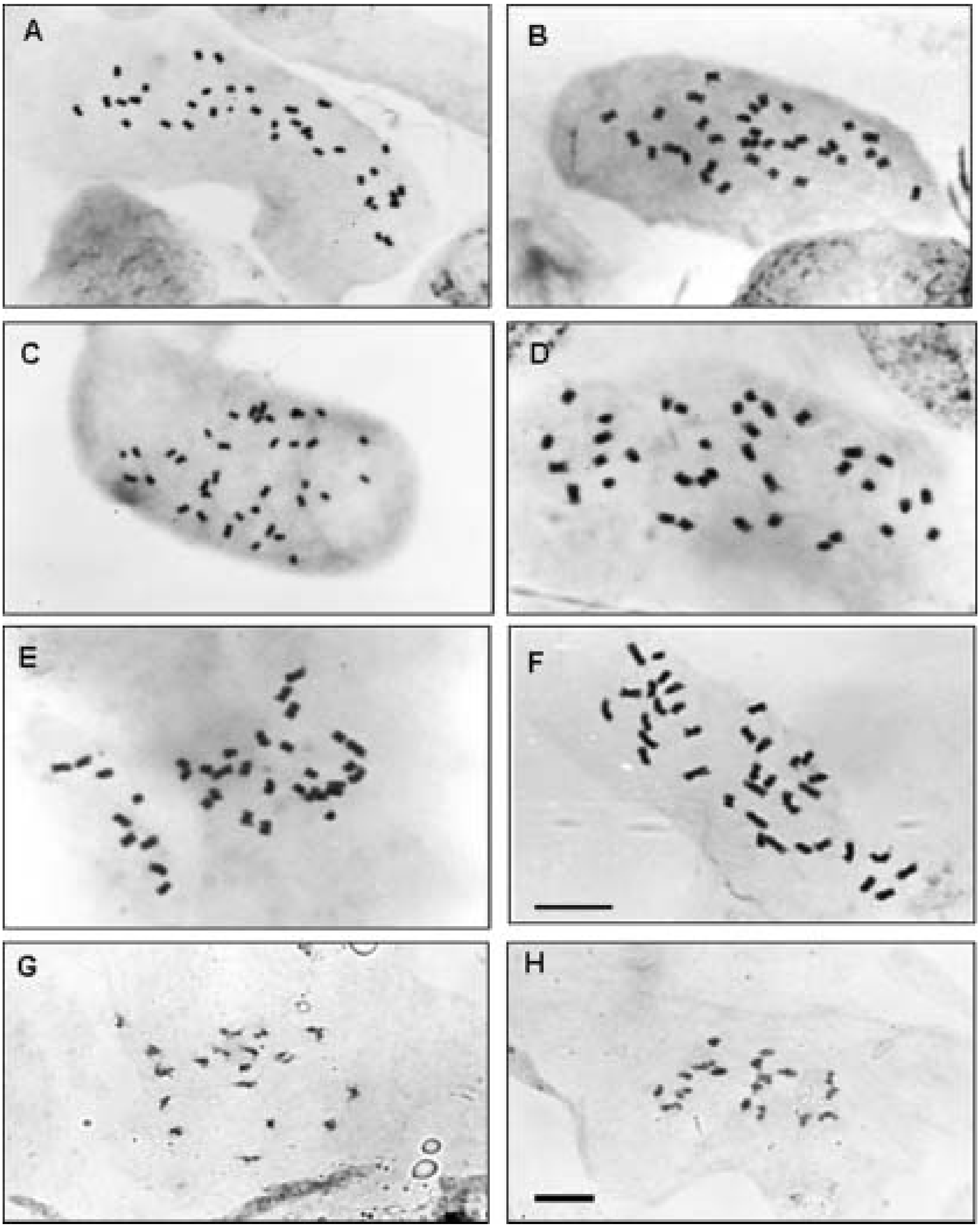

Fig. 2 — Mitotic metaphasis of *Lychnophora* sect. *Lychnophora* species: **A** – *L. cryptomerioides,* 2n=36 **B** – *L. granmogolense*, 2n=34; **C** – *L. mutica*, 2n=38; **D** – *L. rosmarinifolia,* 2n=36; **E** – *L. villosissima,* 2n=36; **F** - *L. uniflora,* 2n=36. Meiotic plates of *Lychnophora* sect. *Lychnophora* species: **G** – *L. ramosissima*, n=18; **H** – *L. sobolifera*, n=18. Bar = 10μm.

some number reported for the first time (Table 2, Figures 2 and 3). The chromosome numbers obtained (2n=34, 2n=36 and 2n=38) coincided with other genera of subtribe Lychnophorinae, as *Eremanthus* and *Minasia* (Turner *et al*. 1979; Jones 1982; Dematteis 1998) and another *Lychnophora* species (Coile and Jones 1981; Mansanares *et al.* 2002).

Of currently species with cytological reports, eleven show n=17 (2n=34), thirteen n=18 (2n=36)

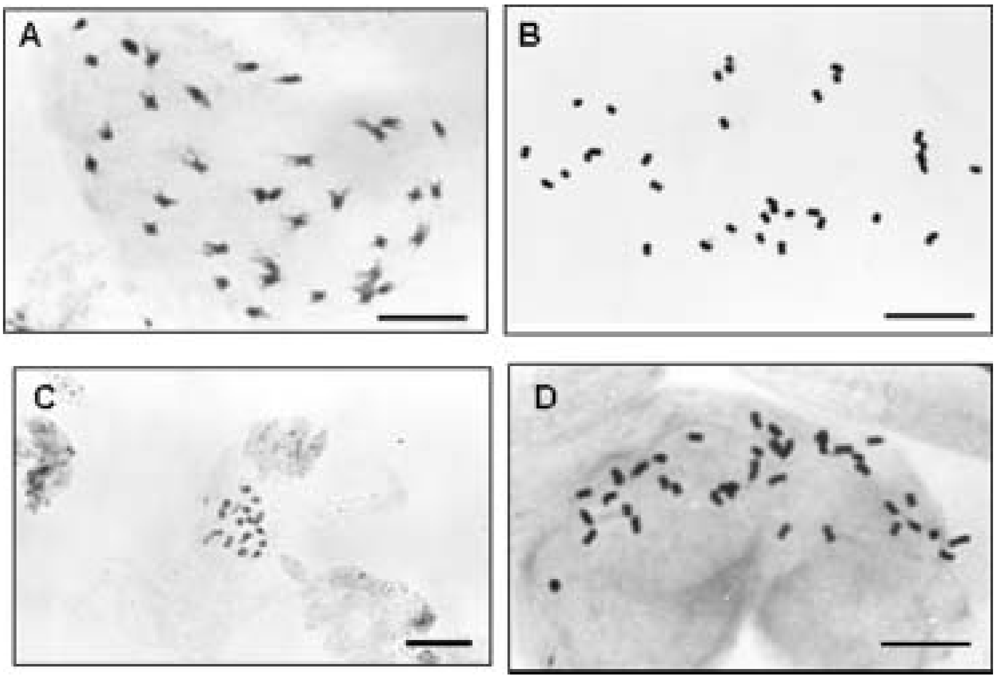

Fig. 3 — Mitotic metaphasis of *Lychnophora* sect. *Lychnophorioides* species: **A** – *L. leucodendron*, 2n=34; **B** – *L. syncephala*, 2n=34. Meiotic plates of *Lychnophora* sect. *Lychnophorioides* species: **C** – *L. angelae*, n=17. Mitotic metaphasis of *Lychnophora* sect. *Chronopappus* species: **D** - *L. markgravii*, 2n=38. Bar = 10μm.

and eight n=19 (2n=38) (Coile and Jones 1981; Jones 1982; Carr *et al.* 1999; Mansanares *et al.* 2002).

In *Lychnophora candelabrum,* Coile and Jones (1981) reported n=17. In the present work 2n=36 (n=18) was observed, coinciding with the previous report by Mansanares *et al.* (2002) where the differences in both counts were discussed.

The chromosome number obtained for *L. granmogolensis* in this study was 2n=34, in agreement with other counts for the genus (Mansanares *et al.* 2002). According to Mansanares *et al.* (2002), the chromosome number reported by Carr *et al.* (1999) of 2n=18+B as *L. phylicifolia* is atypical for the whole group. In addition, *L. phylicifolia* was considered as an incorrect identification of *L. granmogolensis*.

The species *Lychnophora mutica* (2n=38), *L. prostrata* (2n=34) and *L. salicifolia* (2n=36) were analysed by Mansanares *et al.* (2002), chromosome number counts agree with those found in the present work.

Karyotypic differences among three analysed species, *L. markgravii*, *L. rosmarinifolia* and *L. uniflora,* were related to number, length and chromosome morphology. The individual chromosome length and total chromosome length (TCL) were different among the three species. *Lychnophora uniflora* (*Lychnophora* section) with 2n=36 shows a bigger TCL value (68.68μm) in comparison to *L. markgravii* that shows 2n=38 and TCL=61.50μm.

Although all species present metacentric and submetacentric chromosomes, there is a predominance of metacentric ones, in agreement to literature data for the tribe Vernonieae (Ruas *et al.* 1991; Dematteis 1996; 1998; 2002; Dematteis and Fernández 1998; 2000).

Although chromosome number observed for *L. villosissima* from populations 01 and 02 was constant (2n=36), individuals from population 01 showed some variation of the number (2n=36, 2n=37 and 2n-38). This is the first report for chromosome numerical variation in a species not related with polyploidy for *Lychnophora.* The characteristic diploid number of the species is 2n=36, whereas 2n=37 and 2n=38 could indicate aneuploidy/disploidy.

Aneuploidy and disploidy occur by loss or gain of one or few chromosomes. In disploidy, the genotipic DNA quantities is not alterated, as occurs in aneuploidy. Aneuploidy/disploidy arises from meiotic or mitotic deviations, or radiation and chemical treatment response (Stebbins 1971; Malallah *et al.* 2001), and probably played an important role in the speciation mechanism of Vernonieae (Stebbins 1971; Dematteis 1996; 1998; 2002; Mansanares *et al.* 2002).

Semir (1991) proposed six sections in genus *Lychnophora.* Three of them (*Lychnophora, Lychnophoriopsis and Lychnocephaliopsis*) were analysed by Mansanares *et al.* (2002). Here two more sections were studied (*Lychnophorioides* and *Chronopappus*) (Table 2).

Chromosome numbers had been registered for twenty-two, among twenty-five species of section *Lychnophora*. This section grouped most of the species with 2n=34. *Lychnophora granmogolensis* and *L. prostrata* were here confirmed. Besides, other chromosome numbers were obtained for the section, as 2n=36, observed in most of species and previously reported for only three species, *L. gardneri, L. pohlii* and *L. salicifolia*, and to nine more in this study, *L. cryptomerioides, L. itacambirensis, L. martiana, L. nanuzae, L. ramosissima, L. rosmarinifolia, L. sobolifera, L. villosissima* and *L. uniflora*. The number 2n=38 (n=19) was earlier reported for two species, *L. pseudovillosissima* and *L. mutica* (Mansanares *et al*. 2002).

Two chromosome numbers (2n=36 and 2n=38) were reported for five species of section *Lychnocephaliopsis* (Mansanares *et al.* 2002). For *L. grazielae* 2n=38 was observed, coinciding with the number of most other species previously reported by Mansanares *et al.* (2002).

Section *Chronopappus* is constituted by two species, and only *L. markgravii* was here analysed. Karyotypic data obtained in this study is interesting because *L. markgravii* is more distinct in several features of their karyotype than the other two species, which are representatives of section *Lychnophora*. According to Semir (1991), species of section *Chronopappus* differ from others by several leaf characters and inflorescence.

The three different chromosome numbers observed occur distributed among the species, in at least four of six sections of *Lychnophora sensu* Semir (1991), and for the time being, is an important cytotaxonomic character to be used in distinction of species but not in the segregation in distinct genera as suggested by Robinson (1983; 1999), MacLeish (1984) and Hind (2000).

By on the one hand, the chromosome numerical variations do not allow to characterize or distinguish sections of *Lychnophora*, on the other hand, Mansanares *et al.* (2002) suggested that some species could be differentiated through comparison of this character, for example among the species *L. ericoides* and *L. gardnerii*, which could be separated by their chromosome number (2n=34 and 2n=36, respectively). Other species, one studied in present work, *Lychnophora passerina* and *L. ramosissima,* which were considered by Coile and Jones (1981) as *Haplostephium passerina,* can also be differentiated by their chromosome numbers, in agreement to taxonomic position established by Semir (1991). This author considered these two species in *Lychnophora* based on morphological and taxonomic considerations. The chromosome number here observed for *L. ramosissima* (2n=36) is distinct of the one obtained for *L. passerina* (2n=34) by Mansanares *et al.* (2002).

Based on data presented on this and on literature related, we suggest to subtribe Lychnophorinae four basic chromosome numbers x = 15, 17, 18 and 19, and the genus *Lychnophora* was represented by x = 17, 18 and 19 (Mansanares *et al.* 2002).

The mechanism that gives rise to chromosome variations in the genus *Lychnophora* may be aneuploidy or disploidy, in agreement with Mansanares *et al.* (2002) and other authors who studied the genera of tribe Vernonieae (Ruas *et al.* 1991; Dematteis 1996; 1998; 2002; Dematteis and Fernández 1998; 2000). The karyotypical evolution model in Vernonieae species suggest that chromosome data from Old World species of Vernonieae are based on x=9 and 10, while Neotropical species on x=17 (Jones 1974; 1979; Mathew and Mathew 1976; Turner *et al.* 1979; Ruas *et al.* 1991; Dematteis 1996; 1998; 2002; Dematteis and Fernández 1998; 2000).

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