

## Karyotype studies in Brazilian species of *Lobelia* L., subgenus *Tupa* (Campanulaceae)

PAULO M. RUAS<sup>1</sup>, ANDRÉ L.L. VANZELA<sup>1,3</sup>, ANA ODETE S. VIEIRA<sup>2</sup>,  
CÉLIO BERNINI<sup>1</sup> and CLAUDETE F. RUAS<sup>1</sup>

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**ABSTRACT** - (Karyotype studies in Brazilian species of *Lobelia* L., subgenus *Tupa*, Campanulaceae). Karyotypes of 14 populations including eight species of the genus *Lobelia* were studied using root tip mitotic metaphases. All populations were tetraploid with  $2n = 28$  chromosomes. The chromosome base number  $x = 7$  was confirmed for the genus. Karyotype analysis showed that chromosome size varied from 1.05  $\mu\text{m}$  to 2.02  $\mu\text{m}$  with predominance of M and SM chromosome types. The karyotypes were similar among themselves with small intra- and interspecific variations on the size of haploid sets, symmetry indexes and centromere position of some chromosome pairs. These results showed that karyotypes of Brazilian lobelias of the subgenus *Tupa* were probably due to polyploidy associated with chromosomal rearrangements probably in small chromatin segments.

**RESUMO** - (Estudo cariotípico em espécies brasileiras de *Lobelia* L., subgênero *Tupa*, Campanulaceae). Cariótipos de 14 populações, incluindo oito espécies do gênero *Lobelia* foram estudados usando metafases mitóticas de raízes. Todas as populações mostraram-se tetraploides com  $2n = 28$  cromossomos. O número cromossômico básico  $x = 7$  foi confirmado para o gênero. A análise dos cariótipos mostrou que os tamanhos cromossômicos variaram de 1,05  $\mu\text{m}$  a 2,02  $\mu\text{m}$  com predominância de tipos cromossômicos M e SM. Os cariótipos foram similares entre si com pequenas variações intra e interespecíficas no tamanho do complemento haploide, índice de simetria e posição do centrômero em alguns pares cromossômicos. Esses resultados mostraram que os cariótipos das lobélias brasileiras do subgênero *Tupa* podem ter sido originados por poliploidia associada a rearranjos cromossômicos, possivelmente em pequenos segmentos de cromatina.

Key words - *Lobelia*, Campanulaceae, chromosomes, Feulgen, karyotype evolution

### Introduction

The genus *Lobelia* comprises 365 species distributed in temperate and tropical regions of America, Africa, Asia, Australia and Europe (Wimmer 1953). The African continent and Mexico seem to be the most probable genetic diversity centers (Lammers 1993). Species of *Lobelia* present different habit types, which vary from small delicate herbs to woody plants with rosette leaves (giant lobelias). The woody members are grouped in the subgenus *Tupa* in which almost all species are tetraploids with  $n = 14$  chromosomes (Vilmorin & Simonet 1927, Nevling 1966, Lammers & Hensold 1992, Lammers 1993).

Chromosome numbers have been established for 70% of the family, but only for 13% of Lobelioideae subfamily (Lammers & Hensold 1992, Lammers 1993). Approximately 75% of species present chromosome numbers that are multiples of 7, which is considered the chromosome base number of the genus (Thulin 1983, Lammers & Hensold 1992). According to Vieira (1988), Vieira & Shepherd (1996) and Lammers (1993), most of the herbaceous members of Lobelioideae show a polyploid series with  $n = 7, 14$  and 28 chromosomes.

In Brazil the genus *Lobelia* is represented by 16 species, ten belonging to the subgenus *Tupa*. These species are distributed mainly in swampy areas of southeastern Brazil (Vieira 1988, Vieira & Shepherd 1998). Chromosome counts for five Brazilian species of the subgenus *Tupa* revealed a constant number of  $n = 14$  (Gadella *et al.* 1969, Vieira & Shepherd 1996). According to Lammers (1988, 1992, 1993) and Lammers & Hensold (1992) karyomorphological studies could be useful to elucidated taxonomy and evolutive mechanisms of this group, however, as up to date no karyotype data is available for the

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1. Universidade Estadual de Londrina, Centro de Ciências Biológicas, Departamento de Biologia Geral, Caixa Postal 6001, 86051-990 Londrina, PR, Brazil.
  2. Universidade Estadual de Londrina, Centro de Ciências Biológicas, Departamento de Biologia Animal e Vegetal, Caixa Postal 6001, 86051-990 Londrina, PR, Brazil.
  3. Corresponding author: avanzela@hotmail.com

subgenus *Tupa* or any member of the subfamily Lobelioideae. This work presents a comparative study applied to 14 populations of eight species of *Lobelia* subgenus *Tupa* native from Brazil. Results are discussed in relation to mechanisms of karyotype evolution.

## Material and methods

Samples of eight species of *Lobelia* were collected in 12 Brazilian localities (table 1, figure 1), and cultivated in a greenhouse. Voucher specimens are preserved in the Herbarium FUEL, Londrina, PR.

For cytogenetic analyses, root tips of five plants of each population were collected and pretreated in 2 mM 8-hydroxyquinoline at 9 °C for 4 hours, fixed in 3:1 Carnoy (ethanol-acetic acid, v/v) overnight at room temperature, transferred to 70% alcohol and stored at 4 °C. Chromosome preparations were done by conventional Feulgen method.

Somatic chromosome numbers were determined in at least ten metaphases of each taxa. The chromosome and karyotype features such as haploid set lengths, centromere positions and symmetry indexes (Huziwara 1962) were obtained by measuring five well spread metaphases. Chromosome types were classified as metacentric, submetacentric, acrocentric and telocentric. Photomicrographs were taken with Agfa Pan film and Kodak Kodabromide F-3 paper.

## Results and Discussion

All populations (14) belonging to the eight species of *Lobelia* exhibited  $2n = 28$  chromosomes (table 1, figure 2). Previous chromosome counts showed  $n = 14$  for Hawaiian Islands lobelias (Lammers 1988), the giant African species (Mabberley 1974, Knox & Kowal 1993), and also for the Brazilian species of the subgenus *Tupa* (Vieira & Shepherd

Table 1. Collection of analysed specimens and karyotype features of *Lobelia* species.

| Species - Populations                                   | 2n | Localities – Coordinates                         | Voucher   | Chromosome size* |             | Haploid set length* | Symetry* TF% |
|---|----|--|-----------|------------------|-------------|---------------------|--------------|
|   |    |  |           | largest          | smallest    |                     |              |
| <i>L. brasiliensis</i> A.O.S.<br>Vieira & G.J. Shepherd | 28 | Brasília, DF<br>15°46' S – 47°55' W              | FUEL11115 | 1.90 ± 0.08      | 0.90 ± 0.05 | 20.1 ± 0.90         | 41.4 ± 1.10  |
| <i>L. exaltata</i> Pohl                                 | 28 | Carmo de Minas, MG<br>22°07' S – 45°07' W        | FUEL9292  | 2.00 ± 0.21      | 1.10 ± 0.09 | 22.6 ± 1.90         | 41.8 ± 2.20  |
| <i>L. exaltata</i> Pohl                                 | 28 | São Bernardo do Campo, SP<br>23°41' S – 46°33' W | FUEL9296  | 2.30 ± 0.18      | 1.10 ± 0.13 | 24.1 ± 1.90         | 38.2 ± 1.60  |
| <i>L. fistulosa</i> Vell.                               | 28 | Águas de Lindóia, SP<br>22°28' S – 46°47' W      | FUEL9291  | 2.10 ± 0.24      | 1.30 ± 0.14 | 23.0 ± 2.20         | 40.7 ± 0.60  |
| <i>L. fistulosa</i> Vell.                               | 28 | Carmo de Minas, MG<br>22°07' S – 45°07' W        | FUEL9293  | 2.00 ± 0.09      | 1.20 ± 0.06 | 22.2 ± 1.00         | 36.8 ± 1.10  |
| <i>L. hassleri</i> Zahlb.                               | 28 | Curitiba, PR<br>25°25' S – 49°16' W              | FUEL5955  | 1.70 ± 0.11      | 0.90 ± 0.06 | 20.4 ± 1.80         | 40.1 ± 0.70  |
| <i>L. imperialis</i> E. Wimm.<br>var. <i>imperialis</i> | 28 | Jaboticatubas, MG<br>19°30' S – 43°44' W         | FUEL24001 | 1.90 ± 0.16      | 1.00 ± 0.10 | 20.2 ± 1.50         | 38.4 ± 0.90  |
| <i>L. imperialis</i> var.<br><i>kanitzii</i> E. Wimm.   | 28 | Diamantina, MG<br>18°14' S – 43°36' W            | FUEL20557 | 1.90 ± 0.20      | 1.00 ± 0.12 | 20.6 ± 2.40         | 40.5 ± 0.80  |
| <i>L. langeana</i> Dusen                                | 28 | Serra do Mar, PR<br>25°35' S – 49°24' W          | FUEL8112  | 2.10 ± 0.06      | 1.00 ± 0.07 | 21.9 ± 0.80         | 43.2 ± 0.80  |
| <i>L. organensis</i> Gard.                              | 28 | Teresópolis, RJ<br>22°24' S – 42°57' W           | FUEL24002 | 2.20 ± 0.09      | 1.10 ± 0.11 | 23.1 ± 0.80         | 41.2 ± 1.20  |
| <i>L. thapsoidea</i> Schott                             | 28 | Nova Friburgo, RJ<br>22°16' S – 42°31' W         | FUEL9748  | 2.20 ± 0.15      | 1.10 ± 0.05 | 23.8 ± 1.30         | 40.6 ± 1.30  |
| <i>L. thapsoidea</i> Schott                             | 28 | Petrópolis, RJ<br>22°30' S – 43°10' W            | FUEL9746  | 1.90 ± 0.13      | 1.00 ± 0.07 | 20.9 ± 0.80         | 43.0 ± 1.50  |
| <i>L. thapsoidea</i> Schott                             | 28 | Teresópolis, RJ<br>22°24' S – 42°57' W           | FUEL9745  | 1.90 ± 0.11      | 1.00 ± 0.05 | 20.0 ± 1.30         | 41.1 ± 1.20  |
| <i>L. thapsoidea</i> Schott                             | 28 | Vale da Revolta, RJ<br>22°24' S – 42°57' W       | FUEL9759  | 1.80 ± 0.14      | 0.90 ± 0.07 | 19.7 ± 1.20         | 43.5 ± 1.10  |

\* Values of largest and smallest chromosome size (µm), haploid set length (µm) and symetry index (%) are accomplished by standard deviation.

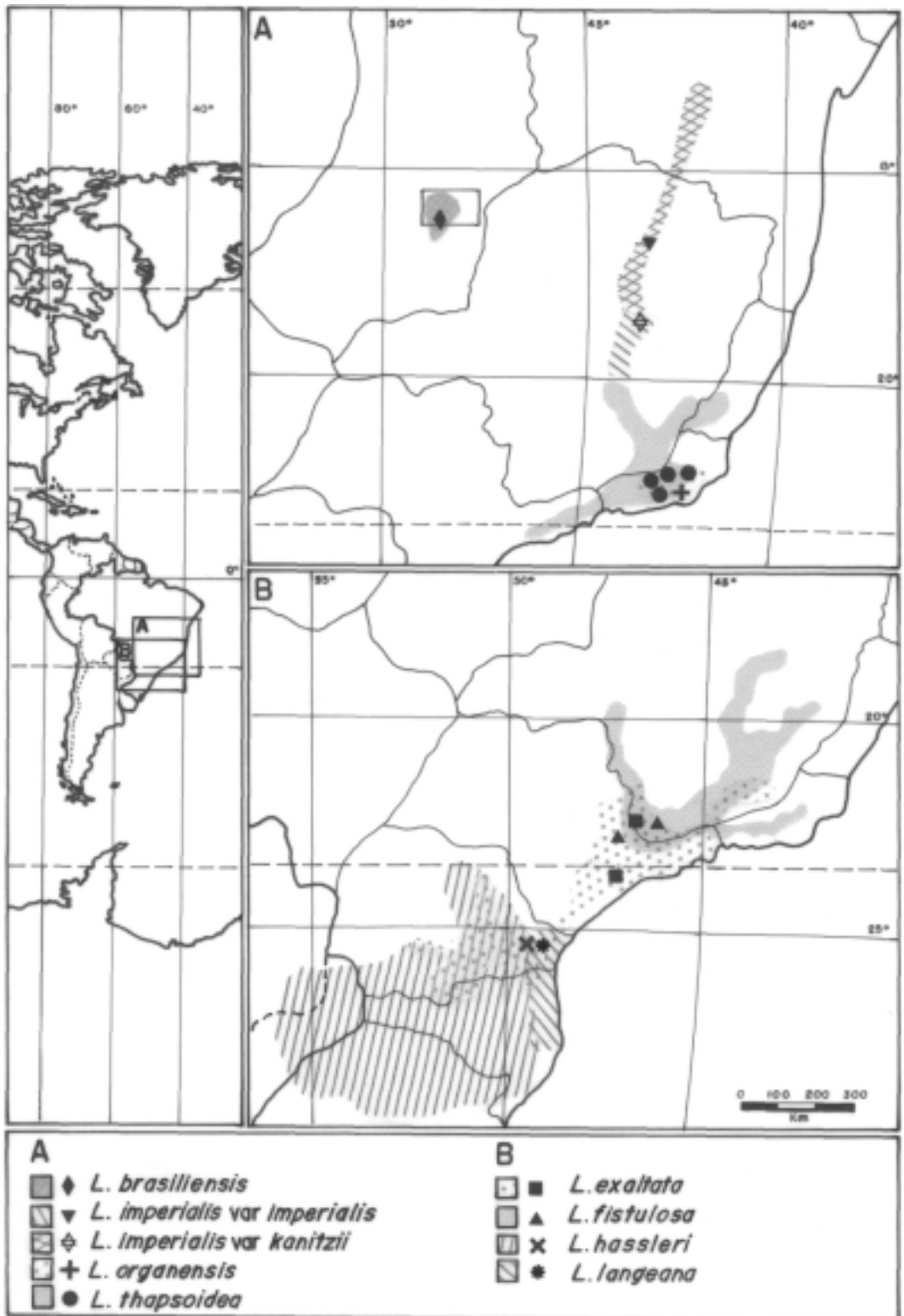


Figure 1. Geographic distribution of *Lobelia* species. Dark spots inside the distribution area represent collection sites.



Figure 2. Karyotypes of *Lobelia* species. A. *L. brasiliensis*; B. *L. exaltata*, Carmo de Minas; C. *L. exaltata*, São Bernardo do Campo; D. *L. fistulosa*, Águas de Lindóia; E. *L. fistulosa*, Carmo de Minas; F. *L. hassleri*; G. *L. imperialis* var. *imperialis*; H. *L. imperialis* var. *kanitzii*; I. *L. langeana*; J. *L. organensis*; K. *L. thapsoidea*, Nova Friburgo; L. *L. thapsoidea*, Petrópolis; M. *L. thapsoidea*, Terezópolis; N. *L. thapsoidea*, Vale da Revolta. Bar = 2.0 μm.

1996). The chromosome number  $n = 7$  was reported for two other species of subgenus *Tupa*, the Peruvian *L. decurrens* Cav (Diers 1961) and the West Indian *L. portoricensis* (Vatke) Urban (Nevling 1966), while  $n = 21$  was found in Chilean lobelias (Stace & James 1996). Chromosome numbers  $n = 14$  and  $21$  should be considered as polyploid ( $2n = 4x = 28$  and  $2n = 6x = 42$ ) derivatives from ancestral diploid species with  $n = 7$  (Nevling 1966, Lammers & Hensold 1992, Lammers 1993). This hypothesis was followed by Raven (1975) and Lammers (1988) who suggested that low diploid numbers are a primitive characteristic in this subfamily. This hypothesis also follows the general tendency of most of the angiosperms in which 80% of the species are polyploid (see Leitch & Bennett 1997). Moreover, Mabberley (1974) and Stace & James (1996) proposed that high chromosome numbers  $n = 21$  and  $n = 14$  should represent the most primitive number for the genus. This statement is supported by the absence of multivalents in meiosis, as observed in four Chilean species (Lammers & Hensold 1992), and by a cpDNA phylogenetic tree (Knox *et al.* 1993). These authors showed that certain herbaceous taxa of the subgenus *Lobelia* section *Lobelia* derived from ancestral stocks including the old woody giants of subgenus *Tupa* and other woody lobeliads. Carlquist (1969, 1992) also pointed out that woodiness is an apomorphic character in the subfamily Lobelioideae. Therefore, if  $n = 21$  is the diploid primitive number, the woodiness with  $n = 7$  and  $n = 14$  could be apomorphic derived by diploid reduction ( $n = 21 \rightarrow n = 14 \rightarrow n = 7$ ). In the present study, all karyotypes were very similar with metacentric and submetacentric chromosomes. Satellites were observed in chromosome 8 for all taxa. These results are not consistent with the mechanism of diploid chromosome reduction discussed before.

In general, only few inter- and intraspecific variations were found in the karyotypes (see populations of *L. thapsoidea* from Nova Friburgo and Vale da Revolta, table 1). Variations in chromosome morphology, as observed in some chromosome pairs, were probably due to the occurrence of chromosome rearrangements involving the centromere (figure 2: pair 2 of B and D, pair 4 of I and K, pair 6 of J and K and pair 14 of B and D). The range of the chromosome pairs varied from 1.7  $\mu\text{m}$  (*L. hassleri* Zahlb.) to 2.3  $\mu\text{m}$  (*L. exaltata* Pohl) and from 0.9  $\mu\text{m}$  (*L. brasiliensis* A.O.S. Vieira & G.J. Shepherd, *L. hassleri*, and *L. exaltata*) to 1.3  $\mu\text{m}$

(*L. fistulosa* Vell.). The total size of the haploid complement varied from 19.7  $\mu\text{m}$  in *L. thapsoidea* Schott to 24.1  $\mu\text{m}$  in *L. exaltata* while the karyotype symmetry index ranged from 36.8% in *L. fistulosa* to 43.5% in *L. thapsoidea* (table 1, figure 2). All these variations could be related to the accumulation of minor chromosome rearrangements, as small amplifications, deletions or inversions. The occurrence of chromosomes rearrangements was reported in recent study using fluorescence *in situ* hybridization (FISH) by Vanzela *et al.* (1999). These authors showed differences in the distribution and number of 5S rDNA sites between *L. brasiliensis* and *L. imperialis* var. *kanitzii* E. Wimm., which result from amplification and interruption of continuous arrays by insertion of unrelated DNA sequences in 5S rDNA repeated units.

The maintenance of karyotype variability of Brazilian *Lobelia* species can be also explained by some ecological features. These species are distributed in isolated populations (figure 1), most of them adapted to swamps and small river banks at relatively high altitudes (Vieira 1988, Vieira & Shepherd 1998). The isolation of these populations and the occurrence of self-pollinated seeds, as observed in Japanese *Lobelia* species (Mariko & Kachi 1995), could also explain the accumulation of karyotype differences.

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