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# KARYOLOGICAL STUDY IN THE CHILEAN RHATANY *Krameria cistoidea* HOOK. & ARN. (KRAMERIACEAE)

# ESTUDIO CARIOLÓGICO EN EL PACUL CHILENO *Krameria cistoidea* HOOK. & ARN. (KRAMERIACEAE)

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## ABSTRACT

The karyotype of the plant species *Krameria cistoidea* Hook. & Arn. was studied by assessing chromosome characters such as morphology, size, and C-banding pattern. The karyotype of *K. cistoidea* was composed only by metacentric chromosomes in the two populations studied. The haploid set length was 51.9 ± 2.3 µm and the mean chromosome size was 8.68 ± 0.78 µm. Some similarities in chromosome morphology and size can be observed among *K. cistoidea* and *K. triandra*, in addition to the chromosome number 2n = 12 which is conserved within the genus. *K. cistoidea* exhibited a symmetric banding pattern with large C-bands in the telomeres of the short and long arms of all chromosomes, except the short arm of pair 1. The relative length of the C-bands was 23.5% of the total haploid set length. These cytological results on *K. cistoidea* are the first data on quantitative karyotype morphology and C-banding patterns in the genus *Krameria*.

Key words: *Krameria*, karyotype, C-banding.

## RESUMEN

El cariotipo de la especie vegatal *Krameria cistoidea* Hook. & Arn., 2n=12, se estudió en individuos de dos poblaciones considerando las variables de tamaño, morfología y patrón de bandas C. La longitud del set haploide fue de 51,9 ± 2,3 µm con un tamaño cromosómico promedio de 8,68 ± 0,78 µm. Se encontraron algunas similitudes de morfología y tamaños cromosómicos entre el cariotipo de *K. cistoidea* y el descrito para *K. triandra*, ambas con 2n=12 guarismo conservado dentro del género. Los cromosomas de *K. cistoidea* muestran un patrón simétrico de grandes bandas C en los telómeros de todos ellos, excepto en el brazo corto del par 1 y con una longitud relativa de los segmentos con bandas C de un 23,5% del set haploide. Estos resultados son los primeros datos cuantitativos relativos al cariotipo y patrón de bandas C en el género *Krameria*.

Palabras clave: *Krameria*, cariotipo, bandas-C.

## INTRODUCTION

*Krameria cistoidea* (Krameriaceae) Hook. & Arn., is a plant species endemic to Chile with a center of distribution located between Huasco (28º S) and Limari rivers basins (30º S) in the coastal and pre-Andean slopes of a semiarid zone (Squeo *et al.*, 2001). Along its geographical range *K. cistoidea* shares the habitat with *K. lappacea*. At present, almost 16 *Krameria* species constitute the monogeneric family *Krameriaceae* which is distributed across the Americas, but only two species are present in Chile. The taxonomic classification of *Krameria* has been principally based upon morphology, anatomy, pollen ultra-structure, wood anatomy and DNA sequences (Heusser, 1971; Robertson, 1973; Simpson and Skvarla, 1981; Soltis *et al.*, 2000; Simpson *et al.*, 2004; Carlquist, 2005). Nevertheless, since its description by Loefling in 1758, the genus *Krameria* has presented a problem to taxonomists as to its placement within the dicotyledons (Robertson, 1973; Simpson and Skvarla, 1981). Currently, *Krameria* is considered within the Zygophyllales order together with other genera belonging to the Zygophyllaceae family (Soltis *et al.*, 2000; Simpson *et al.*, 2004). Historical reports have described that the roots of *K. cistoidea* have had a variety of uses such as medicinal herb, for liqueur production, and as an important source of dye (Muñoz, 1985). However, despite of its extraction and habitat degradation by anthropic and natural effects, information on its conservation is scarce, but this species does not meet the criteria to be considered vulnerable (Benoit, 1989; Squeo *et al.*, 2001). *Krameria* species show a haploid chromosome number n=6 (Turner, 1958; Lewis *et al.*, 1962), which later was corroborated with the count of the diploid number 2n=12 described in *K. triandra* (Teppner, 1984). Recently, the chromosome number 2n=12 was also found in *K. cistoidea*, which was complemented with data on DNA C-value (1C=9.3 pg) (Palma Rojas *et al.*, 2017), thus supplying new cytological data for the genus. However, despite these advances, the karyotype morphology has not been described for species of the genus, and there are no reports on specific chromosome markers, for example, C-banding patterns, which show the location of constitutive heterochromatin. It is remarkable that the Chilean *taxa* of *Krameria* form part of the most southern species along the geographical range of the genus in America (Simpson *et al.*, 2004). In this sense karyotype studies including heterochromatin location may be fundamental to understand patterns on genetic variation, genome evolution and speciation in these plants (Stebbins, 1971; Guerra, 2000; Levin, 2002; Jara Seguel *et al.*, 2010; Jara Arancio *et al.*, 2012; Jara Seguel and Urrutia, 2012), and may contribute significantly to establish the cytological relationships among North American and South American *Krameria* species, supplying also additional evidence for its taxonomic status within the Zygophyllales. For this reason, we describe the karyotype morphology and the distribution of constitutive heterochromatin in *K. cistoidea*, the most representative species of the genus present in the Chilean flora.

## MATERIALS AND METHODS

Plants of *Krameria cistoidea* Hook. & Arn. were collected from two naturally growing populations, in Punta Colorada (28º 30´ S; 70º 48´ W, altitude 585 m above sea level), and Cuesta Buenos Aires (30º 2´ S; 70º 49´ W, altitude 380 m above sea level), both spaced at a distance of approximately 170 km in Central Chile. Voucher specimens for both populations were deposited at the Herbarium of the Universidad de La Serena, La Serena, Chile (Herbario ULS). Roots of germinated seeds were pre-treated with 8-Hydroxiquinoline 2 mM at 7º C for 3 h, fixed in ethanol-glacial acetic acid (3:1 v/v) at 4º C for 24 h, and stored in ethanol 70% (v/v) at 4º C until use. To determine chromosome morphology, the roots were stained with Feulgen reaction and chromosome preparations were made by squashing the root tips. For Giemsa C-banding, the fixed roots were washed in distilled water and treated with a solution of pectinase-cellulase (Fluka; 2:1 w/w) at 7.5% (w/v) in 0.2 M citrate buffer pH 4.2 at 37º C for 30 minutes. The procedure used to obtain C-bands was based on the technique described by Summer (1972). In photomicrographs of ten metaphase plates (Feulgen preparations) obtained from ten plants, the short and long arms were measured and the total relative length of each chromosome pair (expressed as percentage of the total haploid set length) was calculated. Additionally, total haploid set length (THL in µm), and mean chromosome size (in µm) were estimated. The karyotype was constructed according to decreasing chromosome length and chromosome morphology, using the nomenclature by Levan (Levan *et al.*,1964; Spotorno, 1985). The C-bands were classified according to their chromosome location as centromeric, pericentromeric, interstitial or telomeric. Relative length values of C-bands (RLC) were calculated by using the follow equation described by Linde-Larsen *et al.* (1980): RLC= (C-band length of the haploid set / Total haploid set length) x 100.

## RESULTS

The Feulgen stained karyotype of *K. cistoidea* is shown in Figure 1a, and chromosome measurements are presented in Table 1. Both populations of *K. cistoidea* studied here showed a diploid chromosome number of 2n=12, with a

karyotype composed only by metacentric chromosomes. Satellites and secondary constrictions were not observed in these chromosomes. The chromosomes of *K. cistoidea* are large, with a mean chromosome size of 8.68 ± 0.78 µm and a total haploid set length of 51.9 ± 2.3 µm.

The C-banded karyotype of *K. cistoidea* is shown in Figure 1b. *K. cistoidea* exhibited C-bands located in the telomeres of the short and long arms of all chromosomes, except the short arm of pair 1. These large regions of constitutive heterochromatin in the karyotype were concurrent with the presence of many conspicuous and large chromocenters in the interphase nuclei (Figure 2). Homologous chromosomes exhibited similar C-banding patterns and pairing was possible. The centromeres were also evident in all chromosomes. The relative length of the C-bands was equivalent to 23.5% of the total haploid set length. Polymorphism in C-banding pattern was not observed in both studied populations.

**Table 1.** Karyotype characters of *Krameria cistoidea*. SA, short arm (%); LA, long arm (%); TL, total chromosome length (%); CS, absolute chromosome size (µm), CI, centromeric index; SD, standard deviation; m, metacentric.

| Chromosome pair | SA (%) (Mean ± SD) | LA (%) (Mean ± SD) | TL (%) (Mean ± SD) | CS (µm) (Mean ± SD) | CI | Type |
|---|---|---|---|---|---|---|
| 1 | 8.92 ± 0.43 | 10.19 ± 0.67 | 19.11 ± 0.96 | 10.0 ± 0.70 | 0.47 ± 0.03 | m |
| 2 | 8.57 ± 0.25 | 9.40 ± 0.5 | 17.97 ± 0.66 | 9.3 ± 0.45 | 0.48 ± 0.02 | m |
| 3 | 7.56 ± 0.42 | 9.19 ± 0.45 | 16.74 ± 0.65 | 8.6 ± 0.50 | 0.45 ± 0.02 | m |
| 4 | 7.48 ± 0.35 | 8.67 ± 0.64 | 16.15 ± 0.49 | 8.4 ± 0.43 | 0.46 ± 0.01 | m |
| 5 | 6.89 ± 0.6 | 8.56 ± 0.61 | 15.45 ± 0.49 | 8.0 ± 0.40 | 0.45 ± 0.04 | m |
| 6 | 6.51 ± 0.5 | 8.06 ± 0.33 | 14.58 ± 0.53 | 7.6 ± 0.39 | 0.45 ± 0.03 | m |

SA= Short arm; LA= Long arm; TL= Total length; CS= Chromosome size; CI= Centromeric index; m= metacentric.

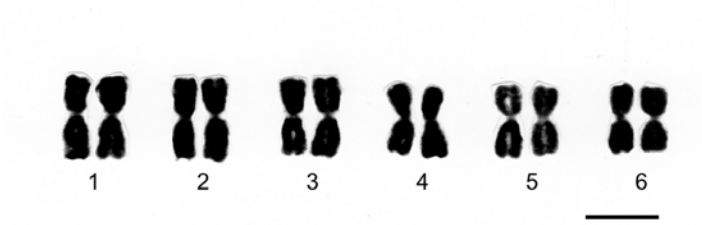


**Figure 1a.** Karyotype of *Krameria cistoidea*, 2n=12. Feulgen stain. Bar=10 µm.

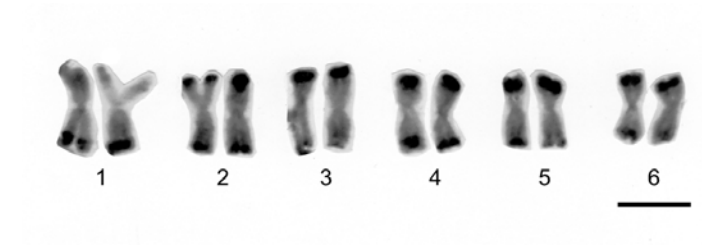


**Figure 1b.** Karyotype of *Krameria cistoidea* with Giemsa C-banding. Bar=10 µm.

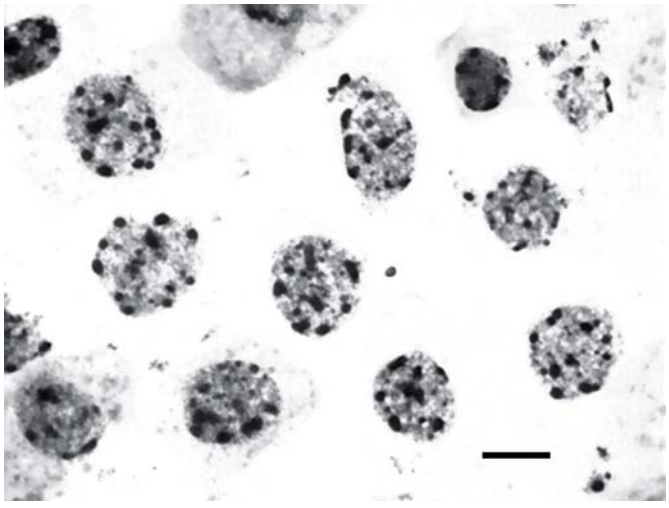

**Figure 2.** Meristematic interphase nuclei of *Krameria cistoidea*, with many chromocenters. Bar=10 µm.

## DISCUSSION

The results of this study corroborate the chromosome number of 2n=12 described previously for *K. cistoidea* (Palma Rojas *et al.*, 2017), which is also similar to other six species of the genus described some decades ago (Turner, 1958; Lewis *et al.*, 1962; Teppner, 1984) (Table 2). In this work, quantitative karyotype morphology of one Chilean species of the genus *Krameria*, *K. cistoidea* (Figure 1a, Table 1) is reported, which is additional to the data on chromosome number and 2C-value previously reported for one population of the same species (Cuesta El Churque population, Chile) (Palma Rojas *et al.*, 2017). The karyotype of *K. cistoidea* was uniform among both populations studied. However, at the interspecific level differences in chromosome morphology and size were observed between the karyotype of *K. cistoidea* and *K. triandra* from Perú. *K. cistoidea*, with a metacentric and unimodal karyotype, had all chromosomes with a centromeric index CI between 0.45 and 0.48 (mean CI= 0.46 ± 0.012), and a chromosome size that varied between 7.63 and 10.0 µm with an average size of 8.68 µm. In the case of *K. triandra* the centromeres are located in median or sub-median region as determined by mean qualitative analysis, and the range of chromosome size varied between 10 µm and 14 µm (Teppner, 1984). Such interspecific differences in chromosome morphology and size among these *Krameria* species may be preliminary evidence on the occurrence of mechanism of chromosome rearrangements (*e.g.*, inversions, duplication, deletions) during the evolution of the genus as it has been described in various other Angiosperm groups (Stebbins, 1971; Levin, 2002). Future comparative karyotype studies in *Krameria* may give more evidence to corroborate this hypothesis.

**Table 2.** Chromosome number for *Krameria* species. n, gametic chromosome number; 2n, somatic chromosome number.

| Species | n | 2n | Reference |
|---|---|---|---|
| *Krameria cistoidea* Hook & Arn | - | 12 | Palma-Rojas et al. (2017) |
| *K. cistoidea* | - | 12 | Present study |
| *K. grayi* Rose & Painter | 6 | - | Weedin & Powel (1978) |
| *K. lanceolata* Torr | 6 | - | Lewis et al. (1962) |
| | - | 12 | Kondo et al. (1981) |
| | 6 | - | Spellenberg (1986) |
| | 6 | - | Freeman & Brooks (1988) |
| *K. parvifolia* Benth | 6 | - | Weedin & Powel (1978) |
| *K. parviflora* var. *glandulosa* | 6 | - | Ward (1983) |
| *K. triandra* Ruiz & Pav | - | 12 | Teppner (1984) |

The chromosome location of constitutive heterochromatin is another additional genome character for the first time studied here for one *Krameria* species. The banding pattern of both populations of *K. cistoidea* exhibited large blocks of constitutive heterochromatin, located only in telomeric regions in the short and long arms of the metacentric chromosomes with a symmetrical banding (Greilhuber, 1984). However, due to the large chromosome size (higher to 5.0 µm according to Guerra, 2000), it is possible that the entire constitutive heterochromatin content of the species has not been revealed through this method, as it has also been described in other plant groups (Schweizer and Loidl, 1987; Buitendijk and Ramanna, 1996; Guerra, 2000). In this way, the information on C-bands in *Krameria* can be a fundamental knowledge for the application of other modern molecular techniques (FISH, GISH, $CMA_3$, and/or DAPI) focused on describing genome organization, as it has been done in other flowering plants in which complex C-banding patterns have been performed (Joachimiak *et al.*, 1997; Guerra, 2000; Zhou *et al.*, 2003; She *et al.*, 2007; Hamon *et al.*, 2009).

Within the Zygophyllales, comparative karyological studies have been made within the genus *Bulnesia* belonging to Zygophyllaceae. *Bulnesia* species with highest (*B. retama* 2C= 4.5 pg, and *B. chilensis* 2C= 2.9 pg) and lowest 2C-values (*B. sarmientoi* 2C= 0.7 pg) possess the most asymmetric karyotype (with metacentric, submetacentric, subtelocentric and telocentric chromosomes), whereas species with intermediate 2C-values (*B. foliosa* and *B. schickendantzii*, both with approximately 2C-Values of 1.1 pg) possess most symmetric karyotypes (with metacentric and submetacentric chromosomes) (Poggio *et al.*, 1986). In addition, the species with the highest 2C-values (*B. retama* and *B. chilensis*) have the highest constitutive heterochromatin content as reveled by C-banding patterns (Poggio and Hunzkiker, 1986). All these karyotype studies in *Bulnesia* have been useful to elucidate interesting evolutionary trends within the genus. In the case of *Krameria*, the quantitative karyotype characters described here for *K. cistoidea*, as well as additional data on C-banding and 2C-values, should be studied in other *Krameria* species from both hemispheres, thus revealing the mechanisms of chromosome evolution that have occurred in this genus along its distribution range. On the other hand, phylogenetic relationships among Krameriaceae and Zygophyllaceae should be carried out including all genome data that could be feasible to obtain (*e.g.*, cytogenetic, molecular), thus providing valuable data to clarify the taxonomical relationship of both families within the order Zygophyllales, which is still confuse.

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