Gayana Bot. 74(1): XX-XX, 2017.
Short communication
ISSN 0016-5301

Nuclear DNA content and chromosome number of *Krameria cistoidea* Hook. & Arn. (Krameriaceae)

Contenido de ADN nuclear y número cromosómico de *Krameria cistoidea* Hook. & Arn. (Krameriaceae)

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RESUMEN

Krameria cistoidea Hook. & Arn. tiene un valor 2C de 18,64 ± 1,09 pg con un coeficiente de variación de 5,8%. El número cromosómico 2n = 12 descrito para otras seis especies de *Krameria* está también presente en *K. cistoidea*. Estos datos citológicos de *K. cistoidea* son discutidos en relación a antecedentes disponibles para otras Angiospermas, así como para tres géneros de la familia taxonómicamente relacionada Zygophyllaceae.

Krameria cistoidea Hook. & Arn. (Krameriaceae) is a species endemic to Chile that inhabits coastal and pre-andean slopes (Squeo *et al*. 2001), with a center of distribution located between the rivers Huasco (28ºS) and Limari (30ºS) in the semiarid zone. Along its geographical range *K. cistoidea* shares the habitat with its sister species *K. lappacea* (Dombey) Burdef & Simpson. At present, almost 16 *Krameria* species have been described 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 & 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 & Skvarla 1981). Currently, *Krameria* is classified within the Zygophyllales order together with other genera belonging to the family Zygophyllaceae (Soltis *et al.* 2000, Simpson *et al*. 2004). Cytological studies carried out previously for five *Krameria* species show a haploid chromosome number n = 6 (Turner 1958, Lewis *et al*. 1962). Nevertheless, cytological data such as nuclear DNA content (or 2C-values) have not been estimated for *Krameria* species, nor the chromosome number has been reported previously for the taxa that inhabits Chile. On this respect, 2C-values and/or 2n numbers have been previously described for genera belonging to the related family Zygophyllaceae (*e.g. Larrea*, *Bulnesia, Tribulus*). These genera show cytological characters that differ to *Krameria* species described so far, with diploid species (2n = 26), tetraploids (2n = 52), hexaploids (2n = 78) and octoploids (2n = 104), and all having low 2C-values (ranging between 0.7 and 4.5 pg) (Poggio & Hunziker 1986, Poggio *et al*. 1989, Laport *et al*. 2012).

The goal of this work is to describe the 2C-value and the chromosome number of *K. cistoidea*, thus increasing the cytological information available for the genus. These cytological data, although partial, may contribute to improve the understanding on cyto-evolutionary patterns within the family Krameriaceae, which could also be important characters to corroborate its current classification within the order Zygophyllales.

Plants of *Krameria cistoidea* were collected from a naturally growing population in Chile, Coquimbo Region, Provincia de Elqui, Cuesta El Churque to 35 km south of Vicuña by route D-445, 800m (30º02'S; 70º52'W), 25-III-2010. G. Arancio, GA3686. The voucher specimens were deposited at the Herbarium of the Universidad de La Serena, La Serena - Chile. To count chromosomes, roots of germinated seeds were pre-treated with 8-Hydroxiquinoline 2 mM at 7 ºC for 3 h, fixed in ethanol-glacial acetic acid (3:1 v/v) at 4ºC for 24 h, and stored in ethanol 70% (v/v) at 4ºC until required. The roots were stained with the Feulgen reaction and chromosome preparations were made by squashing the root tips. The chromosome count was made on

ten metaphases obtained from ten individuals. To estimate the nuclear DNA content (2C-values, which is defined as the total amount of DNA contained within the diploid chromosome set of the cell) Feulgen image densitometry of root tip cells were analyzed, using the software Image Pro-Plus 4.0 (Copyright © by Media Cybernetic, 1993-1998). The cells of *K. cistoidea* were squashed on slides, air-dried, fixed in methanol–glacial acetic acid (3:1, v/v) at 4ºC for 24 h and stained with the Feulgen reaction (hydrolysis with 5N HCl for 60 min at room temperature, staining with Schiff's reagent for 60 min, followed by three washes of 5 min each using sulphurous water). The software captures black and white images from the microscope Nikon Eclipse 400 connected to CCD COHU camera, and analyzes the different visible structures on the images. Nuclear optical density (OD) is calculated by the software according to the formula $OD = \log_{10}(1/T) = -\log_{10}T$, where T = intensity of transmitted light/intensity of incident light. From this estimation, the computer integrates the values of OD obtained for each one of the pixels and calculates the integrated optical density (IOD = ΣOD). For *K. cistoidea* IOD values of 50 early prophase nuclei and 50 late telophase nuclei of root tip cells were determined by the software. The IOD values were converted to absolute mass of DNA by simultaneous comparison with nuclei of root tip cells of *Allium cepa* (2C=33.5 pg, Bennett & Leitch 2005). The root tip cells of the standard were included in the same staining runs and IOD estimations with the cells of *K. cistoidea*. The 2C-value was determined using the equation CVu = CVs x (IODu/IODs). In the equation CVu = 2C-value of *K. cistoidea*, CVs = 2C value of standard; IODu = average IOD of *K. cistoidea* IODs = average IOD of standard.

Krameria cistoidea showed a 2C-value of 18.64 ± 1.09 pg with a coefficient of variation of 5.8% (Table 1). The 2C-value of *K. cistoidea* can be classified as intermediate according to the ranges described for Angiosperms (intermediate range varies between 7.0 and 28 pg). Other Angiosperms may show higher 2C-values (≥ 28 pg) or lower 2C-values (≤7.0 pg) according to the group, being recognized other four ranges (Leitch *et al*. 2005). Besides, *K. cistoidea* had a mean 2C-value higher than the mean of 6.3 pg described for Angiosperms.

The chromosome number 2n = 12 of *K. cistoidea* (Fig. 1) was similar to the previously described for other six species of the genus (Turner 1958). According to qualitative observations made here, the chromosomes of *K. cistoidea* have a length between 8 µm and 10 µm. Nevertheless, quantitative studies on karyotype morphology have to be made in populations of *K. cistoidea* to corroborate these observations.

Within the order Zygophyllales the family Zygophyllaceae is the most studied on the basis of 2C-values and ploidy levels. Thus, some species of the genus *Larrea* and the genus *Bulnesia* show 2C-values lower then *K. cistoidea*. In the genus *Larrea* diploid species (2n = 26) show mean 2C-values between 0.9 to 1.5 pg, whereas the tetraploid (2n = 52) and hexaploid (2n = 78) species show 2C-values between 1.8 to 3.1 pg (Poggio *et al*. 1989, Laport *et al.* 2012). In the case of the genus *Bulnesia*, a wide variation in 2C-values was observed among diploid species (2n = 26) with a range between 0.8 pg and 4.5 pg. Besides, the chromosome size decreases in higher ploidy levels where octoploid species (2n = 104) have 2C-values near to 0.5 pg (Poggio & Hunziker 1986). These differences in 2C-values among *K. cistoidea* and *Larrea-Bulnesia* may be explained by variations in chromosome size, as it was previously described to compare species within the genus *Bulnesia* (Poggio & Hunziker 1986). Another genus cytologically studied within the Zygophyllaceae is *Tribulus*. This genus has a basic chromosome number x = 6 similar to *Krameria*, but only tetraploid and hexaploid species have been described so far, with 2n numbers of 24 and 36, respectively (Hilu 1981). At present, no data are available on 2C-values for *Tribulus*. Finally, all these observations described here show inconsistencies when compared 2C-values, 2n numbers, and ploidy levels among *Krameria* (Krameriaceae), *Larrea* and *Bulnesia* (both belonging to Zygophyllaceae), which could question its classification within Zygophyllales. All these information suggests that the 2C-value and the chromosome number could be robust characters to analyze phylogenetically the controversial taxonomic classification of the family Krameriaceae within the Zygophyllales order. This situation was previously evidenced on the basis of cytogenetic, molecular and morphological characters (Turner 1958, Soltis *et al*. 2000, Simpson *et al*. 2004).

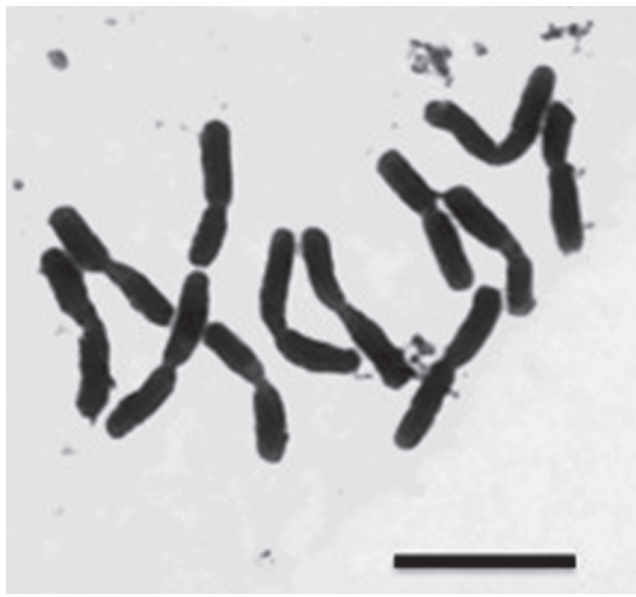

Figure 1. Feulgen stained metaphase of *Krameria cistoidea*, 2n = 12. Bar = 10 µm. / Metafase de *Krameria cistoidea*, teñida con la Reacción de Feulgen, 2n = 12. Barra = 10 µm.

Table 1. IOD values in arbitrary units (au) and nuclear DNA content in picograms (pg) of 50 late telophase (2C) and 50 early prophase (4C) nuclei of the standard *Allium cepa* and of the sample *Krameria cistoidea.* LT, late telophase; EP, early prophase; IOD, integrate optical density in arbitrary units. / Valores de IOD en unidades arbitrarias (au) y contenido de ADN nuclear en picogramos (pg) de 50 núcleos en telofase tardía (2C) y 50 núcleos en profase temprana (4C) del estándar *Allium cepa* y de la muestra *Krameria cistoidea*. LT, telofase tardía, EP, profase temprana; IOD, densidad óptica integrada en unidades arbitrarias.

Species	IOD LT (au) (Mean ± SD)	2C DNA content (pg) LT (Mean ± SD)	IOD EP (au) (Mean ± SD)	4C DNA content (pg) EP (Mean ± SD)	2n
A. cepa L.	26320 ± 1099	33.5 ± 1.40	53390 ± 2451	67.5 ± 3.12	16
K. cistoidea Hook. & Arn.	14643 ± 794	18.64 ± 1.09	29121 ± 1268	37.06 ± 1.61	12

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Recibido: 14.01.2016
Aceptado: 03.10.2016